

Tradomedical Values of Cotton Leaf Plus Lemon Juice Against Clinical Bacterial Isolates

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Abstract

The antibacterial activity of the water and ethanolic extracts of cotton leaf (*Gossypium spp.*) plus lemon juice (*Citrus limon*) were tested against pure clinical isolates of *Salmonella sp.*, *Shigella sp.*, *E. coli* and *Klebsiella sp.* Cotton leaf is normally used in conjunction with lemon juice by the local populace in Nigeria for the treatment of enteric infections. Decoction method was used for the extraction of the active components from the plant in order to simulate the traditional method of extraction. The Minimum Inhibitory Concentration (MIC) and the diameters of zones of inhibition were determined by broth dilution and Agar diffusion methods respectively. The ethanolic extracts are more effective than the water extracts on the test organisms. The MIC of the water and ethanolic extracts ranged between 1.25 -5.0 w/v on the test organisms. Similarly, the average diameter of zones of inhibition of the water extracts on the test organisms ranged between 3.0 to 13.0mm while that of the ethanolic extracts ranged between 12.0 to 21.0mm. The results of this study showed that *E. coli* was the most susceptible followed by *Klebsiella sp.*, then *Salmonella sp.*, and finally *Shigella sp.* at the concentrations used for both water and ethanolic extracts. This observation thereby justifies the traditional uses of these plant extracts among the Nigerian local populace for the treatment of some enteric infections such as dysentery and diarrhoea.

Key words: Cotton leaf plus lemon extracts, antibacterial activity, inhibition, clinical bacterial isolates.

Introduction

Cotton (*Gossypium spp.*) is a tropical plant belonging to the family Malvaceae while lemon (*Citrus limon*) which is a tree crop belongs to the family Rutaceae (Ghazanfar, 1989). Cotton is widely cultivated in the Northern part of Nigeria for its cotton lint production. Lemon is a small citrus fruit which is usually harvested green. In addition to cotton being a fibre crop, its leaves are used traditionally in conjunction with juice from *Citrus limon* (Lemon) in the treatment of diarrhoeal diseases such as Salmonellosis, Shigellosis, amoebic dysentery etc by the local populace in Nigeria. Most of the rural dwellers and people in the urban centre with meagre income still depend heavily on these medicinal plants in the treatment of one form of ailment or the other without really knowing the active components of these plants.

Historical data has shown that many chemotherapeutic drugs or compounds known today were formally

derived from plants (Reiner, 1984). For instance, the first importation of Cinchona to Europe from Peru for treatment of malaria occurred as far back as 1632. Similarly, Emetine, an alkaloid active component obtained from ipecac (*Radix ipecacuanhae*) was known in Brazil for its curative effect against amoebic dysentery since 1871 (Reiner, 1984).

Scientists in most of these third world countries where a large proportion of their population still uses these medicinal plants are saddled with the responsibilities of trying to authenticate the veracity of these supposed curative or medicinal effects. Many Nigerian scientists from the various parts of the country have risen up to these challenges and have done considerable research works on many of these local medicinal plants. For instance, Akujobi *et al.* (2006) worked on the antibacterial activities and phytochemical screening of *Vernonia amygdalina* and *Citrus aurantifolia*. Similarly, Kaufman *et al.* (1989) has shown that *Citrus spp* contains cardiac glycosides, saponins, tannins and alkaloids.

There is little scientific research on the leaves of cotton plant (*Gossypium spp.*) but some investigations have been done on the different varieties of Citrus. However, in the tradomedical practices in Nigeria, the leaf extract of *Gossypium spp.* and juice from *Citrus limon* are often used together in the treatment of some ailments. Therefore, the objective of this study is to investigate the antibacterial activity of cotton leaf plus lemon juice on some clinical bacterial isolates so as to verify the authenticity of its local medicinal usage.

Materials and Methods

Plant Materials collection and Identification

The leaves of cotton plants were obtained from a farm at Osere district in Ilorin, Kwara State of Nigeria. Similarly, lemon fruits were bought in the market situated at Unity Road, Ilorin, Kwara State, Nigeria. The plants were identified with the assistance of the Herbarium section of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria.

Preparation of Plant Extracts

The plucked leaves of cotton plant were washed and crushed by means of sterile mortar and pestle. The aqueous extract was prepared by weighing 10g of the grinded cotton leaf and soaking it in a mixture of 50ml each of the squeezed lemon juice and distilled water.

Similarly, another 10g of the crushed cotton leaf was soaked in a mixture of 50ml each of the squeezed lemon juice

and 70% v/v ethanol. Soaking in both cases were done at room temperature for a period of 24hours. At the end of this period, the extract was shaken and passed through muslin cloth in order to remove the residue. This residue free extract (filtrate) was then further filtered through Whatman No.1 filter paper for better clarity of the solution.

Sterilization of the Plant Extracts

These aqueous and ethanolic extracts (filtrates) obtained above were rendered sterile by aseptic filtration through Millipore filter of size 0.45 μ m with the aid of Millipore filtration apparatus connected to a vacuum pump (Ronald, 1995).

Sterility Proofing of the Extracts

This was done by introducing 2ml of the sterile filtrate obtained above into 10ml of sterile Tryptone soy broth. Incubation was done at 37⁰C for 24hours. At the end of this period, the broth was observed for the absence of turbidity as an indication of sterility (Atata *et al.*, 2003; Sule and Agbabiaka, 2008).

Collection of test Organisms

Pure clinical isolates of *Salmonella sp.*, *Shigella sp.*, *Escherichia coli* and *Klebsiella sp.* were collected from the Department of Medical Microbiology and Parasitology of the University of Ilorin Teaching Hospital, Ilorin, Nigeria. All the isolates were checked for purity and maintained on Tryptone soy agar slant and kept at 4⁰C in the refrigerator until when required for use.

Standardization of Culture Suspension

Five colonies of each of the test organisms were introduced into 5ml of sterile tryptone soy broth and incubation was done for 24hours at 37⁰C. The turbidity (opacity) standard was prepared as described by Cheesbrough (1984). This was then used to match the turbidity of the cell suspension of an overnight broth culture of each test organism.

Determination of the Minimum Inhibitory Concentration (MIC)

Double fold serial dilutions were made using tryptone soy broth adopted from the method of Ibekwe *et al.*, (2001); Sule and Agbabiaka (2008). The initial concentration of the plant extract was 10% w/v and from it the following concentrations were obtained: 5.0% w/v, 2.5% w/v, 1.25% w/v, 0.625% w/v and finally 0.312% w/v. The different dilutions of the broth above were inoculated with 0.1ml of each pure and standardized bacterial suspension

and incubation was done at 37⁰c for 24 hours. Growth of the inoculated test organism was indicated by turbidity (Cloudiness) of the broth tubes while clearness of the broth tube indicates absence of growth. The broth dilution tube with the least concentration of extract where no growth was observed was taken as the MIC.

The control experiments were also set up as follows: Sterile tryptone soy broth only; sterile tryptone soy broth and sterile extracts (Negative controls) and finally sterile tryptone soy broth inoculated with a test organism (positive control).

Determination of Zones of Inhibition

The diameter of zones of inhibition of the extract on each of the test organism was determined using agar diffusion method as described by Hugo and Russel (1996). 15ml of the sterile molten Tryptone soy Agar was poured into the sterile plate (8cm in diameter) and allowed to solidify. The surface of the sterile, dried and solidified Tryptone soy Agar plate was then streaked with the standardized cell suspension.

Flamed Cork borer (8.0mm in diameter) was used to create hole at the centre of the streaked plate. This hole was then filled with the sterile plant extract and incubation of the plate was done at 37⁰C for 24hours. The experiments were repeated in duplicate and the average diameters in millimetre of zones of inhibition were taken.

Statistical Analysis: t-test was used to determine if there is any significant difference between the inhibition of the test organisms in the water and ethanolic extracts for both the broth dilution tubes(MIC) and agar diffusion plate (Bello and Ajayi,2000).

Results and Discussion

Both the water and ethanolic extracts exhibited antibacterial activities against the test organisms to different extents. However, in most cases the ethanolic extracts showed higher activities than the water extracts. Based on the minimum inhibitory concentration (MIC) tests, both *Salmonella sp.* and *Shigella sp.* had the highest resistant in the water extract (5% w/v) followed by *Klebsiella sp.* (2.5% w/v) and lastly *E. coli* (1.25% w/v) has shown in Table 1. Similarly, in the ethanolic extracts, *Salmonella sp.* and *Shigella sp.* were inhibited to the same extent (5.0% w/v) in the MIC broth tubes and they were the least susceptible organisms. Furthermore, it was observed that *E. coli* and *Klebsiella sp.* has equal resistant, their MIC being 1.25% w/v. They are more susceptible compared to *Salmonella sp.* and *Shigella sp.*

Three of the test organisms viz. *Salmonella sp.*, *Shigella sp.* and *E. coli* had the same zone of inhibition in both the water and ethanolic extracts. However, *Klebsiella sp.* showed shift in susceptibility from 2.5% w/v in the water extract to 1.25% w/v in the ethanolic extract of the MIC broth tubes. In the Agar diffusion plates, the water extract at 10% w/v concentration created a zone of inhibition of 3mm on *Shigella sp.* This indicates the highest resistant. Its resistant was followed by inhibitory zone of 10mm on *Klebsiella sp.*, 13mm on *Salmonella sp.* and the least resistant of 18mm on *E. coli*.

Similarly, in the ethanolic extracts using Agar diffusion method *Klebsiella sp.* was the most susceptible organism with diameter of zone of inhibition of 21mm. The next susceptible organism was *E. coli* with an inhibitory diameter of 19mm and this was followed by *Salmonella sp.* that was inhibited up to 13mm diameter. *Shigella sp.* was the least susceptible among the test organisms with inhibitory diameter of 12mm. The result of the students' t-test statistic showed that there was no significant difference between the inhibition of the test organisms in both water and ethanolic extract at the concentrations of 0.312 – 5.0% w/v used in broth dilution tubes since the calculated value 3.2084 is less than the critical table value 4.541. However, in the agar diffusion plates at concentration of 10% w/v in both extracts there is significant difference between the inhibition of the test organisms in the water and ethanolic extracts (calculated value 5.5468 greater than the table value 4.541).

The results from this study showed that the leaf extract of *Gossypium spp* (Cotton plant) in conjunction with the juice of *Citrus limon* (lemon fruit) have significant antibacterial effects on all the clinical bacterial isolates used for this investigation at the concentrations used. However, these antibacterial activities were to different extents (Tables 1 to 3). Ethanol has a higher volatility than water. Thus, it tends to extract more active compounds from the leaves of the plants than water (Dutta, 1993). This observation correlates with the works of Ibekwe *et al.* (2001); Akujobi *et al.* (2004); Atata *et al.* (2003); Sule and Agbabiaka (2008).

E. coli was the most susceptible to the water extract among the test organisms (Table 1). It was also observed that both *E. coli* and *Klebsiella sp.* were the most susceptible in the ethanolic extract (Table 2). The observation that *Klebsiella sp.* which was less susceptible than *E. coli* in the broth dilution tubes of the water extract having the same susceptibility with it in the ethanolic extract could be explained by the fact that *Klebsiella sp.* are capsulated organism. Hence, the outer capsule of such organism would offer a higher degree of being less soluble in water but are easily dissolved or solubilised in ethanol (Tables 1 and 2). *Salmonella sp.* and *Shigella sp.* were the most resistant among the test organisms in the broth dilution tube in both the water and ethanolic extracts.

In the Agar diffusion tests, the concentration of the plant extract used was 10% w/v (Table 3). *Salmonella sp.* was inhibited by both the water and ethanolic extracts to the same extent (diameter of zones of inhibition being 13.0mm). Similarly, *E. coli* showed almost the same inhibition in both extracts. Furthermore, in the water extract the most resistant among the test organisms was *Shigella sp.* followed by *Klebsiella sp.* *Salmonella sp.* and finally *E.coli* (most susceptible). The most resistant among the test organisms in the ethanolic extract was still *Shigella sp.* followed by *Salmonella sp.*, *E. coli* and *Klebsiella sp.* (most susceptible).

In this study, Decoction method was used to extract the active components from the plant materials rather than other efficient methods such as Soxhlet apparatus. This was done in order to simulate the extraction's method of the local populace who use these plants for curing ailments.

In conclusion, this study suggests that the extracts of cotton leaf plus lemon juice have potential inhibitory effects against the test organisms at the concentrations used. Both the water and ethanolic extracts showed significant inhibitory effects on all the test organisms thereby providing alternative for those who dislike the use of ethanol for religious or health reasons. However, it was observed that the ethanolic extract exert more or on a few occasion equal inhibitory effect on all the test organisms. Hence, it is suggested that ethanol should be used for extraction as much as possible.

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Table 1. Minimum inhibitory Concentration (MIC) of the water extract on the clinical bacterial isolates.

Test Organisms	Concentration of extracts (% w/v)				
	5.0	2.5	1.25	0.625	0.312
<i>Salmonella sp.</i>	-	+	+	+	+
<i>Shigella sp.</i>	-	+	+	+	+
<i>E. coli</i>	-	-	-	+	+
<i>Klebsiella sp.</i>	-	-	+	+	+

- = No growth; + = growth

Table 2. Minimum inhibitory concentration (MIC) of the ethanolic extract on the clinical bacterial isolates.

Test Organisms	Concentration of extracts (% w/v)				
	5.0	2.5	1.25	0.625	0.312
<i>Salmonella sp.</i>	-	+	+	+	+
<i>Shigella sp.</i>	-	+	+	+	+
<i>E. coli</i>	-	-	-	+	+
<i>Klebsiella sp.</i>	-	-	-	+	+

-, No growth; +, Growth

Table 3. Diameters of zones of inhibition (mm) of the extracts at 10% w/v on the clinical bacterial isolates.

Test Organisms	Diameter of zone of inhibition (mm)	
	Water extract	Ethanolic extract
<i>Salmonella sp.</i>	13.0	13.0
<i>Shigella sp.</i>	3.0	12.0
<i>E. coli</i>	18.0	19.0
<i>Klebsiella sp.</i>	10.0	21.0