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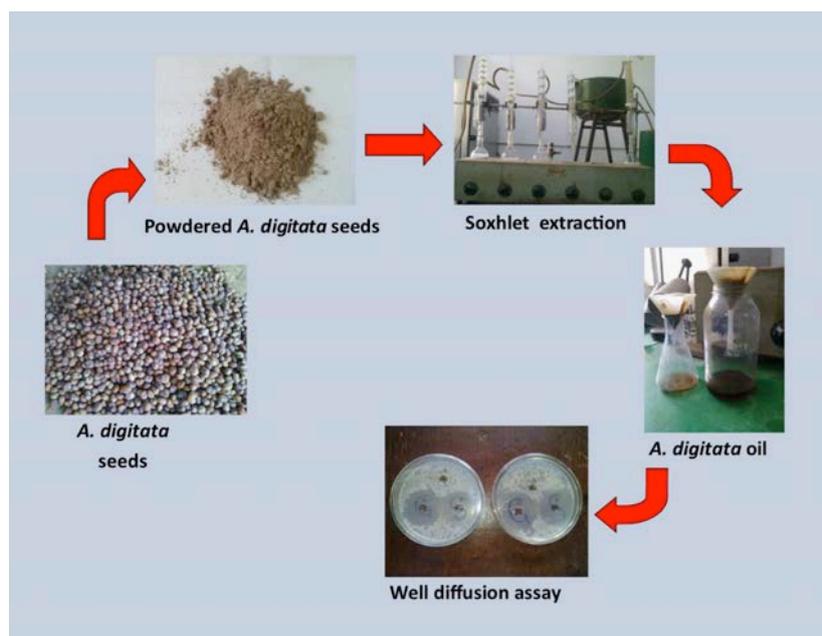
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Evaluation of the Antibacterial Activity of *Adansonia digitata* L. Seed Oil Obtained from Zaria, Kaduna State, Nigeria on Some Strains of Bacteria

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Graphical Abstract



Abstract

The use of essential oils as antibacterial agents have become popular over the years, this is in a bid to search for alternative ways of dealing with strains of bacteria that have become resistant to conventional antibiotics. This study was carried out to evaluate the antibacterial potentials of *Adansonia digitata* seed oil obtained from Zaria, Kaduna state, Nigeria, on the clinical isolates of some bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*). The oil was extracted from the seeds using the soxhlet extraction method with n-hexane as the solvent. The Well diffusion method was used to test the susceptibility of the strains of bacteria to the oil, using Gentamycin and Streptomycin as standard positive controls. Experiments were carried out in duplicates. Data obtained from the experiment was analysed using one-way analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT), with $P < 0.05$ considered significant. The results revealed that *A. digitata* oil was unable to create any inhibition zones in the bacteria cultures. From this research, it can be concluded that *A. digitata* oil had no Antibacterial activity.

Keywords: *Adansonia digitata*; Antibacterial; Bacteria; Essential oils; seed

Introduction

The World health Organisation has reported that diseases caused by pathogenic bacteria are one of the main cause of morbidity and mortality worldwide¹. Drug resistance has become a mitigating factor in the effective treatment of many bacterial diseases, therefore, there is an intense search for alternative methods of treating these diseases^{2,3}.

Many plants are known to contain essential oils (also known as volatile oils) which are aromatic in nature. These oils are by-products of metabolism secreted by special glands and stored up in various parts (such as leaves, flowers, roots, buds, twigs, rhizomes, bark, seeds and fruits etc.), and are aromatic in nature. From time immemorial the antimicrobial properties of essential oils have been known. The 'first-aid' kit containing myrrh essential oil was used by few Greek soldiers in the battlefield to treat wounds and was introduced by physician Galen⁴. Many of them have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases⁵.

Adansonia digitata L. – Baobab (commonly known as “Kuka” in northern Nigeria) is a deciduous tree belonging to the Malvaceae family and is indigenous to arid central Africa^{6,7}. It is a large imposing tree which reaches heights of about 18-25 m and produces a rounded crown showing a stiff branching habit. It has a peculiarly swollen trunk of up to 10 m in diameter, usually tapering or cylindrical and abruptly bottle-shaped; often buttressed. Giant individuals can reach a girth of up to 28 m. It is widely distributed and can be found in most of Sub-Saharan Africa's semi-arid and sub-humid regions as well as in western Madagascar⁸. Different parts of the tree are used as foods and medicines including the back fibres. No part of the tree is a waste^{9,10,11,12}. The seeds have a characteristic bean shape and have been reported to contain essential oil^{11,12}. This research attempts to evaluate the antibacterial efficacy of the seed oil of *Adansonia digitata*.

Materials And Methods

Collection of Plant Material

Dried fruits of *Adansonia digitata* were collected from the Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria, and were verified in the herbarium of the Department, with a voucher number of 2512. The fruits were split open and seeds were mechanically removed, properly washed, dried, pulverized into powder using mortar and pestle,

sieved through a pore size of about 1mm, and stored in airtight containers.

Extraction of Essential Oils

The extraction of essential oil was done using n-hexane in electro-thermal soxhlet extractor (Gallenkamp, England). 30 g of powdered seeds was weighed and put into the thimbles of the soxhlet extractor, the apparatus was mounted and allowed to run for 1 hour, after which the mixture of essential oil and n-hexane was collected in a beaker and evaporated over a hot water bath to collect the oil as residue.

Bacterial Strains

Clinical isolates of *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* were obtained from the Department of Pharmaceutics and pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. Overnight cultures obtained using 10 ml nutrient agar were used for the study.

Well Diffusion Assays

Mueller Hinton's agar was prepared according to manufacturer's instructions, poured into Petri dishes, and allowed to set. The surface of the agar was flooded with 2 ml of isolate and the excess was drained into a disinfectant container. A cork borer was flamed and used to bore three wells in each agar. 100 µml each of undiluted *Adansonia digitata* oil, 1 mg/ml of Gentamycin and 0.1 mg/ml of Streptomycin (which served as standard positive controls), were put separately in the three wells and allowed to stand for 1 h to diffuse properly. The plates were incubated for 24 h at 37°C and inhibition zones were measured¹³. The experiments were done in duplicates.

Statistical Analysis

One way Analysis of Variance (ANOVA) was used to compare the mean inhibition zones of the different treatment groups and Duncan Multiple Range Test (DMRT) was used to separate means where significant. $P < 0.05$ was considered significant.

Results

At the end of the experiment, it was observed that *A. digitata* oil had no inhibitory effect on all the bacteria isolates, this was confirmed by its failure to cause any inhibition zone around the wells containing the oil,

unlike the standard antibiotics used (Table 1 and Figure 1 – 4).

Table 1: Mean Inhibition zones of the different treatments on bacterial isolates

Bacteria	Mean Inhibition Zones (mm)		
	Gentamycin (1 mg/ml)	Streptomycin (0.1 mg/ml)	<i>A. digitata</i> oil
<i>B. subtilis</i>	37.00 ± 1.00 ^a	39.00 ± 1.00 ^a	0.00 ± 0.00 ^b
<i>S. aureus</i>	36.00 ± 0.05 ^b	37.00 ± 0.05 ^a	0.00 ± 0.00 ^c
<i>P. aeruginosa</i>	34.50 ± 2.50 ^a	33.50 ± 1.50 ^a	0.00 ± 0.00 ^b
<i>E. coli</i>	34.50 ± 0.50 ^a	29.50 ± 0.50 ^b	0.00 ± 0.00 ^c

Mean ± S.E.M; n = 2; means in the same row with different superscripts are significantly different (P < 0.05).

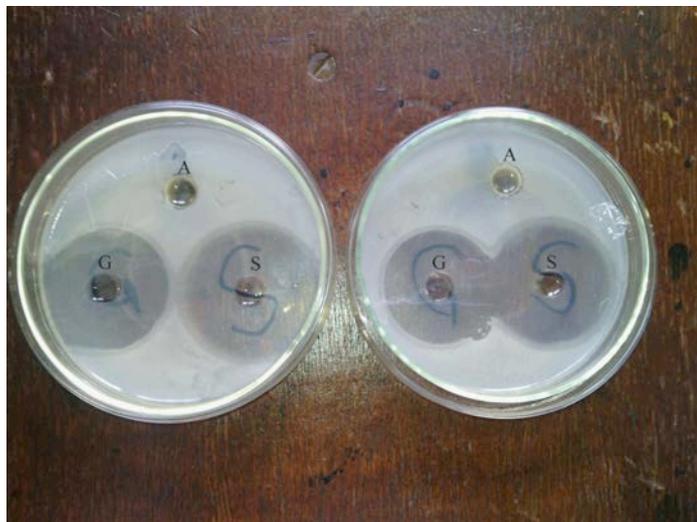


Figure 1: Inhibitory effect of *A. digitata* oil (A), Gentamycin (G) and Streptomycin (S) on *B. subtilis*.



Figure 2: Inhibitory effect of *A. digitata* oil (A), Gentamycin (G) and Streptomycin (S) on *S. aureus*



Figure 3: Inhibitory effect of *A. digitata* oil (A), Gentamycin (G) and Streptomycin (S) on *P. aeruginosa*



Figure 4: Inhibitory effect of *A. digitata* oil (A), Gentamycin (G) and Streptomycin (S) on *E. coli*

Discussion

The findings of this research were not in agreement with a similar work of Samie *et al.* (2012)¹⁴ which reported that *A. digitata* oil was able to weakly inhibit the growth of a number of bacterial organisms, of which *P. aeruginosa* and *S. aureus* were amongst them. Although Samie *et al.* (2012)¹⁴ also acknowledged that even though the oil showed inhibitory effect, it, however, had little bactericidal activity. The failure of *A. digitata* oil to cause an inhibitory effect on the bacterial organisms used in this study may be due to the fact that it is a weak antibacterial agent, and the strains of bacteria were resistant to it. Djouahri *et al.* (2013)¹⁵ reported that methods used in the extraction of essential oils causes variation in their chemical composition of the active compounds, and this may also be responsible for the discrepancy in results. Samie *et al.* (2012)¹⁴ used the hydrodistillation method while the soxhlet extraction method was used in this research. Ozcan and Chalchat (2005)¹⁶ reported that location causes a variation in the chemical composition of essential oils and this may be another possible factor that affected the results. Samie *et al.* (2010)¹⁴ used baobab seeds obtained from the South African region, while the seeds used for this research were obtained from the Central African region (Zaria, Kaduna state, Nigeria).

It has also been reported that the hydrocarbon monoterpenes compounds found in essential oils show low antibacterial activity while oxygenated compounds possess high potential, especially phenol type compounds as thymol and carvacrol. Oxygenated monoterpenes, exhibit strong antimicrobial activity, especially pronounced on whole cells, while hydrocarbon derivatives possess lower antimicrobial

properties, as their low water solubility limits their diffusion through the medium^{17, 18}. The limitation of *A. digitata* oil's antibacterial activity may be due to the fact that it contains more hydrocarbon monoterpenes than it does oxygenated compounds, although this has not been verified. However other parts of *A. digitata* have been reported to possess high antibacterial activity such as the root and stem barks¹⁹, fruit pulp²⁰, and leaves²¹.

Conclusion

From this research, it can be concluded that *A. digitata* oil had no antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. Further research could be done using pure strains of these bacteria and a different method of extraction of oil.

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