

Comparative Study of the Antibacterial Quality of Aseptical Prepared [Dawadawa] from Locust Beans and Soybeans

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Abstract

This project work has vividly revealed the comparative study of the antibacterial quality of aseptically prepared dawadawa from locust beans and soya beans sold in Birnin Kebbi, central market. The test organisms used for this research were Escheriachia coli, Staphylococcus aureus, Pseudomonas spp, Salmonella spp. Agar well diffusion method was used to test the antibacterial effect of the two samples this was obtained by dissolving the samples in distilled water. Varying concentration of each of the samples were prepared. 1mg/ml, 2mg/ml, 3mg/ml, 4mg/ml, 5mg/ml, 10mg/ml 20mg/ml 25mg/ml. The test medium use was nutrient agar on each nutrient agar plates four wells dug and filled with the samples. The test bacteria were inoculated and incubated for 24hrs after which the zone of inhibition were observed and was measured in millimeter. Soya beans has the highest zone inhibition [22mm] against Pseudomonas spp and Staphylococcus aureus at [25mg/ml] concentration while the lowest zone of inhibition [1mm] was observed on Pseudomonas spp at concentration of [2mg/ml]. Statically analysis confirms that there is significance between the antibacterial activity of locust and soya beans dawadawa.

1. Introduction

Dawadawa is a condiment used widely across West Africa. It is usually prepared by women over the course of several days, traditionally from locust bean (*Parkia biglobosa*) seeds. It can be made from over kinds of seeds and the use of soya beans for this purpose is increasing due to mainly inadequate supply of locust bean seeds. It serves as an important food condiment in Nigeria and many other West and Central African countries. Dawadawa can also be obtained from fermented soy bean (glycine max) seed. Another fermented condiment of leguminous plant origin common in West Africa is Ogiri a popular strong smelling food condiment from melon seed. The fabrication process involves boiling, cleaning and then packing away to ferment and the fermentation process giving it a pungent smell. Salt can be added to the finished product to facilitate storage life (Latunde-Dada, 2000).

Dawadawa is traditionally sold in balls or patties that can be kept for several months at a time. It is a traditional ingredient used across West Africa, especially in cooking. The traditional production now faces strong competition from low-quality stock cubes due to heavy publicity. Dawadawa is rich in protein and a variety of dietary minerals which are completely absent from these bouillon cubes. In the recent years however good quality commercial production has allowed the product to make a comeback into everyday cuisine (Odufa, 1985).

The production of the condiment is largely on a traditional small scale house hold basis under highly variable condition (Odufa, 1985). Microbial fermentation of dawadawa have been found to involve only bacteria since fungi found have been regarded as incidental and does not play any notable role in its fermentation. Most bacteria implicated are facultative anaerobes while

approximately 10% are aerobic after 36 hours of fermentation. The predominant fermenting organisms are bacillus species Odunfa [1985].

2. Aims and Objectives

- i. To aseptically prepared dawadawa from locust bean seed soya bean.
- ii. To comparatively determine the antibacterial activities of the prepared locust bean seed and soya bean dawadwa.

3. Materials and Methods

The glass were used in this project work were washed with detergent, rinsed with clean water and was sterilized in the hot air oven at 160^oc per 60minutes. All reagent, requirement and glass wares used for this experiment are mention under each procedure.

3.1 Sample Collection

A locust bean (*Parkia biplogosa*) was obtained from Birnin Kebbi central market. Soya bean (*Glycine max*) was also obtained from Birnin Kebbi central market. All the samples were taking to laboratory for experiment.

Isolates use was obtained from Usman Danfodio University, Sokoto, and the Isolates used includes *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas spp.* Biochemical tests were carried out on them to confirm their identity.

3.2 Preparation of Media

The media used in this research was nutrient agar medium, 28g of nutrient agar powder was weighed and dissolved in 1000ml of distilled water it was shake vigorously and dissolved using hot plate then was sterilized in autoclave for 15minutes at 121^oc it was then allowed, to cool after which it was dispensed in petri dishes and allowed to solidity.

3.3 Dawadawa Production

3.3.1 Locust Bean Dawadawa Production

Locust beans were boiled for 24hrs using hot plate to soften the hard testa after which it was dehuled by using hand especially fingertip the separated cotyledon was boiled for another 2hrs to soften them. The cotyledon were poured in a sterile plastic bucket with aluminum foil paper and then it was covered with several aluminum foil paper, it was left to ferment for 3days to yield a dark brown product pound with stirred in a mortar and pestle to form dawadawa paste. (Abdulkadir, 2011).

3.3.2 Soya Bean Dawadawa Production

The raw soya beans were dried and fried for about 15-20mins it was then soaked in water for about 15 minutes then it was dehusked to remove the testa from the soya beans cotyledon. It was then boiled for up to 4-5hrs. Potassium chloride was added to the boiling soybeans seeds it was then allow to cool, the cooked soybeans was then transfer aseptically into a sterile air tight container, and was then allow to ferment for 3days by proteolytic residential microorganism. After fermentation the beans were pound with a sterile mortar and pestle it was made into a soy paste dawadawa then was mold to give it a shape. [Mohammed, 2011].

3.4 Preparation of Cultures for Sensitivity Test

Pure cultures of *Staphylococcus aureus*, *Salmonella spp*, *Pseudomonas spp* and *Escherichia coli* that were obtained from the Microbiology Department of Usmanu Danfodiyo University, Sokoto, Sokoto State. Biochemical tests were conducted on the isolates to find out their identity. The pure isolates were then subculture into petri dishes containing nutrient agar by streaking method to cover the whole surface of the plate using a sterile wire loop. The plates containing inoculated organisms were punched using a cork borer. Four "wells" were "dug" on each plate (2 plates per Microorganism).

3.5 Biochemical Test

3.5.1 Gram Staining

A drop of normal saline was placed on a clean grease free slide after which a colony from the pure culture was picked using a sterile wire loop and emulsified in the drop of normal saline until a thin smear was obtained. The smear was then air dried and then heat fixed by passing the underside of the slide over a flame after which it was allowed to cool. The smear was then flooded with the primary stain (crystal violet) and was allowed to stand for 10 minute. It was then washed with running tap water. After that, Iugol's iodine was added on the slide for 30second and then washed with clean water and then 75% alcohol was added to decolorize until the iodine is totally washed away. Finally the slide was counter stained using safranin for 5 minutes and the slide was rinsed with running tap water and then stand on a slide rack to air dry before examine with 100 objective lens under the microscope (Cheesbrough, 2000).

Other biochemical tests ran on the isolates to confirm their identity includes; catalase test, coagulase test, indole test, sugar fermentation, etc. (Manga and Oyeleke, 2008).

4. Results

The results of the antibacterial effect of soybeans and locust beans after 24hrs incubation were presented in table 1 and table 2.

Table 1: Shows the zone of inhibition of locust beans dawadawa of the test organisms.

Table 2: Shows the zone of inhibition of locus beans dawadawa on the test organisms.

Table 1: Zone of Inhibition in Millimeter (mm) of Soya Beans Dawadawa.

| Conc. | Isolates | | | |
|---------|--------------------|----------------|-------------------|-----------------------|
| | <i>Pseudomonas</i> | <i>E. coli</i> | <i>Salmonella</i> | <i>Staphylococcus</i> |
| 1mg/ml | - | 10mm | - | - |
| 2mg/ml | 1mm | 11mm | - | - |
| 3mg/ml | 7mm | 12mm | 11mm | - |
| 4mg/ml | 8mm | 13mm | 13mm | - |
| 5mg/ml | 12mm | 13mm | 17mm | 11mm |
| 10mg/ml | 18mm | 14mm | 18mm | 13mm |
| 20mg/ml | 15mm | 14mm | 19mm | 14mm |
| 25mg/ml | 22mm | - | 21mm | 22mm |

Table 2: Zone Inhibition in Millimeter (Mm) Of Locust Beans Dawadawa

| Conc. | <i>Pseudomonas</i> | <i>E. coli</i> | <i>Samonella</i> | <i>Staphylococcus</i> |
|---------|--------------------|----------------|------------------|-----------------------|
| 1mg/ml | - | - | 9mm | - |
| 2mg/ml | - | - | 11mm | - |
| 3mg/ml | - | 12mm | 12mm | - |
| 4mg/ml | - | 13mm | 12mm | 12mm |
| 5mg/ml | 12mm | 16mm | 14mm | 14mm |
| 10mg/ml | - | 16mm | 17mm | 14mm |
| 20mg/ml | - | 17mm | - | 19mm |
| 25mg/ml | - | - | 21mm | 21mm |

5. Discussion

The inhibitory effect of some members of the foods microbiota on other microorganisms in well established. Some food borne organisms produce substances that are either inhibitory or lethal to others. This includes antibiotics, bacteriocins, hydrogen peroxides and organic acids such as lactic acid, acetic acid (Abdulkadir, 2011).

From the above result obtained soya beans [dawadawa] has the highest zone of inhibition [22mm] against *Pseudomonas spp* and *Staphylococcus aureus* at [25mg/ml] concentration while the lowest zone of inhibition [1mm] was observed on *Pseudomonas spp* at concentration of [2mg/ml].

For the locust beans [dawadawa] the highest zone of inhibition [21mm] against *Salmonella spp* and *Staphylococcus aureus* at concentration of [25mg/ml] while the lowest zone of inhibition [9mm] was observed on same *Salmonella spp* at concentration [1mg/ml] *Pseudomonas spp* prove resistance to the samples of locust beans at most of the concentration except [5mg/ml] [12mm]. This may be related to the work of Nester, (2001) confirm that, *Staphylococcus* and *Pseudomonas spp* easily resistance to most antibacterial drug.

6. Conclusion

Base on the results obtained it was found out that soya and locust beans dawadawa were active against *Staphylococcus aureus* *Escherichia coli*, *Salmonella spp* used but not really active on *Pseudomonas spp*. Therefore it is concluded that both soya and locust beans dawadawa should continue to be used as food spice since they can control diseases from these common opportunistic pathogenic bacteria.

7. Recommendation

From the findings the following are recommended:

1. Aseptic technique is recommended in Preparation of soya and locust beans dawadawa to reduce contamination.
2. Further research work should be carried out an improveantibacterial study of soybeans and locust beans dawadawa in vivo.

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