ANTIBACTERIAL ACTIVITY OF CRESCENTIA CUJETE SEEDS OIL ON THREE STRAINS OF BACTERIA

Remi-Esan, I.A.*, Shittu, A.I., and Bankole, O.O.
Department of Science Laboratory Technology, Federal Polytechnic Ilaro, Ilaro Ogun State.
*Corresponding E-mail: ifelolu.remiesan@federalpolyilaro.edu.ng; +2348063922854

Abstract
The antibacterial activity and phytochemical screening of calabash seed oil were carried out. The test isolates were *Escherichia coli, Shigelliadysenteriae and Salmonella typhi*. The phytochemical screening was carried out using standard methods while agar-well-diffusion method was used for the antibacterial activity of the oil. The result obtained showed that *Salmonella typhi* and *Escherichia coli* had no zone of inhibition when tested with the oil extract, while *Shigelliadysenteriae*showed inhibition at 2 ml and 4 ml respectively against the oil extract. Phytochemical screening of the extracted oil revealed the presence of some biologically active compounds, such as flavonoids, glycoside, saponin, steroid, alkaloid, diterpenes and tapens, while tannins, phenols, terpenoid and anthraquinoles were absent. Therefore, the results of this study suggest that calabash seed oil can be used to formulate antibiotics which combat gram negative, non-spore forming, and facultative bacteria.

Keywords: Diterpenes, Antibiotics, N-Hexane, Antibacterial, Phytochemical.

INTRODUCTION

The Calabash tree, (*Crescentia cujete*)belongs to the family of Binoniaceae. The plant grows to about 25 feet and produces some unusual kinds of flowers and fruits. It consists of a broad crown and long branches covered with congenital leaves and gourd-like fruit. It is found in African countries (including Nigeria), the Caribbean and tropical America. Calabash fruit shells are used for various purposes, such as food containers, musical instruments, food plates, bowls and pipes. The white sponge pulp inside the shell contains numerous flat seeds. The fruit itself can be mixed with milk, heated and use in the treatment of colds and asthma. Mature fruits are also considered as laxative. Some traditional healers have recommended that the food may be ingested to force menses, birth and after birth (Arvigo and Balick, 1993), or trigger abortions, the latter has been observed in cattle.

There is evidence that microorganisms are resistant to global threats due to the wider use of antibiotics (Elisha, Twaij, Ali, Tarish, Al-omari and Karim, 2000). The recent emergence of antibiotics resistant strains increased the urgency of increasing the range of intractable bacterial infections and the search for new strategies to combat infections. Unlike synthetic drugs, herbal antibiotics are not associated with many side effects and have tremendous therapeutic potential in the treatment of many infectious diseases (Iwu, Duncan and Okunzi,1999; Sieradzki, Roberts, Haber and Tomasz 1999).

Plants are readily abound in our environment and about 80% of the modern medicines come directly or indirectly from plants. Surprisingly, the large quantity of modern drugs originated in less than 15% of plants known to have been pharmacologically tested out of estimated 500,000 high- growth plant species on earth (Farnsworth, 1996). This study evaluated the phytochemical constituents and antibacterial activity of calabash seed oil.

MATERIALS AND METHODS

The study was conducted in the Environmental Biology Research Laboratory, Science Laboratory Department, The Federal Polytechnic Ilaro, Ogun State, Nigeria.

Collection and preparation of seed materials

The fruits were collected from calabash trees growing at Ilaro, Ogun state, Nigeria. The fruits were cut open in order to obtain the seeds. The seeds obtained were air-dried for 16 days; it was then pulverized using sterile

mortar and pestle to powder. The resultant powder was weighed and kept in air- tight containers until further usage.

Preparation of crude extract

Cold extraction was carried out on the seed material using N- Hexane**.** The powdered seed material was subjected to successive cold extraction using N- hexane. A total of 100 g each of dried seed powder was extracted in 500 ml of N-hexane in a conical flask and placed at 37^0C for 72 hours. The extracts obtained was filtered using Whattman filter paper and stored at 0-4 ^{o}C in air- tight container for further use.

Phytochemical screening

The phytochemical constituents of the extracts were determined according to standard method of Harbone (1998). Phytochemicals screened includes: flavonoids, glycoside, tannins, phenols, saponin, steroid, alkaloids, terpenoid, anthraquinoles, diterpenes and tapens.

Test organisms

The test organisms; *Salmonella typhi, Shigella dysenteriae* and *Escherichia coli* were obtained from Microbiology Laboratory, University College Hospital, Ibadan. They were sub-cultured and incubated at 37 ^{o}C for 24 hours on nutrient agar slant and stored at 0-4 ^{o}C till use.

Nutrient agar preparation

The nutrient agar (5.04 g) was weighted using top-loading weighing balance, into conical flask and 180 ml of distilled water was added. The conical flask was cotton plugged and then covered with foil paper; it was sterilized using an autoclave at 121 ^{o}C for 15 minutes. After sterilization, it was allowed to cool to about 40 ^{o}C and then poured into the Petri dishes (approximately 20 ml each) and allowed to solidify.

In-vitro antibacterial activity and determination

Antibacterial activity of Calabash seed extracts was carried out using the agar well diffusion method as reported by Hassan, Sirat, Yagi, Koko, and Abdulwahab (2011). The test organisms were sub-cultured on freshly prepared nutrient agar plates, in order to obtain 18-24 hours old organisms. In order to determine the antibacterial activity of the extract (oil), nutrient agar plate was seeded with test organism (18-24 hours old) by streaking little inoculum of the test organism on the entire surface of the plate.
Furthermore, a sterile cork-borer (6.0 mm) was used to make wells in each seeded plate and 2ml (plate 1) and 4ml (plate 2) of the extract was introduced into the different wells. The plates were allowed to stand for 6 hours after which they were incubated for 12 to 24 hours at 37^{o}C. After incubation, the zones of inhibition were measured using a veneer caliper and recorded accordingly.

Statistical Analyses

Statistical analyses were carried out using the SPSS statistical software Version 23. The comparison of means was done using the One-Way Analysis of Variance (ANOVA). All experimental setups were in triplicate and separated using Tukey's test.

RESULTS

Phytochemical constituents of the oil extract

Table 1 shows the qualitative analysis of the crude extract of calabash seed. Bioactive compounds absent included anthraquinoles, terpenoid, phenols and tannins. The presence of flavonoids, glycoside, and saponin, steroids, alkaloid, diterpenes and tapens was recorded in the extract.

Table 1: Phytochemical constituents of calabash seed oil extract.

Phytochemicals	Level of presence
Flavonoids	+
Glycoside	+
Tannins	-
Phenols	-
Saponin	+
Steroid	+
Alkaloid	+
Terpenoid	-
Anthraquinoles	-
Diterpenes	+
Tapens	+

Key: (-) not present, (+) present

Antimicrobial activity of the seed extract

Figure 1, shows the three strains of bacteria isolates were tested against 2 ml of the oil extract for its inhibitory capability and *Shigella dysenteria* at 24 hours of incubation showed 2 mm zone of inhibition.The extract showed no visible activity against *E. coli* and *S. typhi* at 2 ml concentration after 24 h incubation. Figure 2, further illustrates the inhibitory capacity of the seed oil extract at 4 ml, which was 22 mm in diameter at 24 hours of incubation.

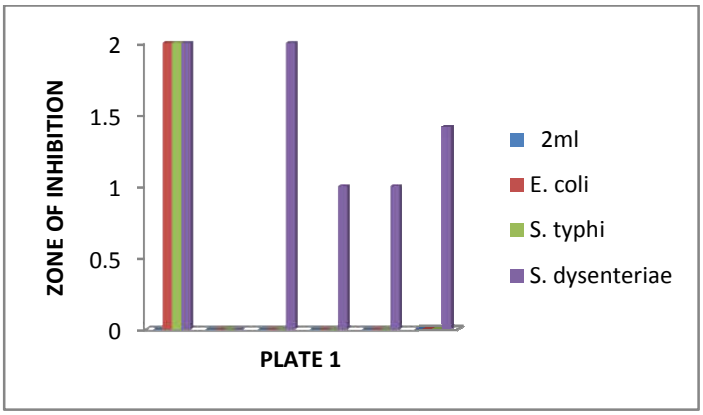

Figure 1: Diameter zone of inhibition of 2 ml concentration of calabash seed oil

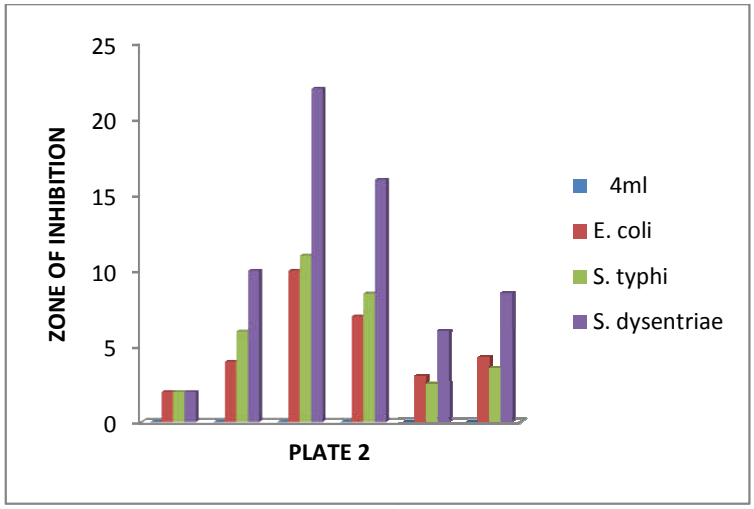

Figure 2: Mean diameter zone of inhibition of 4 ml concentration of calabash seed oil

Table 2: Mean ± standard error of the percentage inhibition of the organisms

	Zone of Inhibition	
ORGANISM	2 ml	4 ml
Escherichia coli	0.00 ±0.00	7.00 ± 4.24[b]
Salmonella typhi	0.00 ± 0.00	8.50 ± 3.54[c]
Shigella dysenteriae	1.00 ± 1.41	16.00 ± 8.49[a]

Each value is a mean ± SEM of three replicates. Means within the same column followed by the same letter(s) are not significantly different at P>0.05 using Tukey's test.

DISCUSSION

The results showed that calabash seed oil contains many bioactive compounds, which makes them more effective in drug development. Herbs have become the basis for their use in therapeutic in developing countries, but in developed countries the use of herbs has increased in recent years (Fagiwara*et al.,* 1982). The Phytochemical screening of calabash seed oil revealed the presence of some bioactive compounds, this justifies the use of plants in fork-medicine and current attention that has been shifted to plants as source active drugs without side effects like the synthetic ones.

The flavonoids found in calabash seed oil act as anti-oxidants to protect the body's cells from damage by free radicals. Free radicals are reputed to damage cell and contribute to various health problems (Arthur, 1992). The presence of Alkaloid explores the effective roles, based on the fact that they act as the main active agent in analgesic, anti-spasmodic and bactericidal effects (Frantisek, 1998). The presence of Saponin is known to act as a natural antibiotics and energy enhancer (Muhammad, Abdulateef, Jin, Jun, Min and Jong, 2018); it also helps to reduce inflammation of the upper respiratory tract and also used as a foaming detergent and emulsifier (Frantisek, 1998).

The results of this study show that calabash seed oil had different inhibitory potentials against the test bacteria, at 2ml the oil was inactive against *Escherichia coli and salmonella typhi*, but showed inhibitory property on *Shigella dysenteriae*. This is consistent with the fact that Rao (2005) found that he suppresses the oils which are often used for therapeutic purposes, because they suppress various type of pathogenic bacteria. In 4 ml, the oil was active against all tested bacteria, in accordance with Olajuyigbe and Afolayan (2013).

CONCLUSION

The world is blessed with a rich wealth of medicinal plants that play important roles in the lives of rural dwellers in underdeveloped countries with little or few access to good health services and facilities. Results obtained from this study demonstrates the inhibitory potentials of the oil extract on strains of bacteria isolates. This antimicrobial effect makes it an attractive option for pharmaceutical industries to inculcate in their formulation in the manufacturing of soaps and disinfectants due to the response of the seed oil extract on the test isolates. The presence of bioactive compounds provides assertions that the oil can be used in the treatment and prevention of skin infections, hence further research is needed in this area to serve as a confirmation.

ACKNOWLEDGMENT

Authors wishes to acknowledge Dr. P.O. Bankole of Zoology Department, Federal University of Agriculture Abeokuta and Blessing Ayo for their support on this study.

REFERENCES

Arvigo, R., &Balick, M. (1993). Rainforest Remedies: One Hundred Healing Herbs of Belize, 2nd Edition, Lotus Press, TwinLakes, Wisconsin, USA, 1993, 60-61.

Arthur, M. (1992). Literature Review of an Under- utilized legume, Canadian Glandiata plant sources, *Human Nutrition,* 55, 355-321.

Elisha, E.E., Twaij, H.A., Ali, N.M., Tarish, J.H., Al-omari, M.M., & Karim, S. (2000). The anthelmintic activity of some Iraqi plants of the cucurbitaceae. *Journal of Pharmaceutical Biology,* 25, 153–7.

Fagiwara, T., Takeka, T., Gihara, O., &Shimimumin, Y. (1982). Studies on the structure of polysaccharides from the bark of medicinal Ziziphur. *Chemistry,* 30,425-430.

Farnsworth, N.R. (1996). Biological and Phytochemical screening of plants. *Journal of Pharmaceutical Science*, *55(3),* 226-227.

Frantisek. (1998). The Natural Guide to medicinal Herb plants. Tiger book International Plc. Twickers. 8: 20.

Gonzalez, F., & Silva, M. (1987) Survey of plants with antifertility properties described in the South America folk medicine. In First Princess Chulabhorn Science Congress International Congress on Natural Products, Bangkok (Thailand), 10-13.

Harbourne, J.B. (1998). Phytochemical Methods: A Guide to Modern Technique of Plant Analysis. 2nd edition London: Chapman and Hall Ltd. pp: 282.

Hassan, L.E.A, Sirat, H.M, Yagi, S.M.A., Koko, W.S., &Abdelwahab, S.I. (2011). In vitro antimicrobial activities of chloroformic, hexane and ethanolic extracts of *Citrullus lanatus* var. citroides (watermelon). *Joint Meeting of Pesticide Residue,* 5(8), 1338-1344.

Iwu, M.W., Dancun, A.R., &Okunzi, C.O. (1999): New Antimicrobials of Plant Origin. In: *Janick Journal*. (ed.): Perspectives on new crops and New uses. ASHS Press, Alexandaria, VA: 457:462.

Muhammad, I.K, Abdulatef A., Jin, H.S., Jun, S.B, Min, Y.K, & Jong D. (2018). Green Tea Seed Isolated Saponins Exerts Antibacterial Effects against Various Strains of Gram Positive and Gram Negative Bacteria, a Comprehensive Study In Vitro and In Vivo, Evidence-Based Complementary and Alternative Medicine, vol. 2018, Article ID 3486106, 12 pages, 2018. https://doi.org/10.1155/2018/3486106.

Olajuyigbe, O. O., &Afolayan, A. J. (2013). Evaluation of combination effects of ethanolic extract of Ziziphus mucronataWilld. subsp. mucronataWilld. and antibiotics against clinically important bacteria. *The Scientific World Journal*, 769594. doi:10.1155/2013/769594

Oyeleke, S.B. & Manga, B.S. (2008). Essentials of laboratory practical in microbiology. First Edition. Tobest Publisher. pp: 93-98.

Rao, V. (2005): The effect of Calabash seed oil in acute Plasmodium Malaria. *Journal of Medicine,22*, 258-263.

Sieradzki, K., Roberts, R.B., Haber, S.W& Tomasz, A. (1999): The development of vancomycin resistance in a patient with methicillin- resistant *staphylococcus aureus* infection. *England Journal of Medicine*. 340, 517-523.