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Explorative *in vitro* evaluation of the inhibitory effect of *Vitellaria paradoxa* seed oil extract on *Staphylococcal conjunctivitis*

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Abstract

More exploration on medicinal plants and other natural products in the present era of increase in poverty level and multi-drug resistance has become crucial. The aim of this study is to explore the inhibitory activities of *Vitellaria paradoxa* seed oil extract on isolated staphylococcal conjunctivitis. Cultured sample of *Staphylococcus aureus* isolated from a patient's eye discharge in the Teaching Hospital Laboratory of the Imo State University, Nigeria having been diagnosed with bacterial conjunctivitis at the eye Clinic. After the incubation period, the diameter of zones of inhibition both horizontal and vertical were measured. Concentrations (100, 50 and 25mg/ml) of the ethanolic seed oil extract of *V. paradoxa* were assayed for the antibacterial activity - Minimum Inhibitory Concentration (MIC) using the agar well diffusion method. Ethanolic seed oil extract of *V. paradoxa* at concentration of 100mg /ml exhibited the highest zone of inhibition at 37.4mm for 24hrs followed by 50mg /ml and lowest using 25mg/ml (5.0mm) indicating a concentration-dependent inhibitory effect on *Staphylococcal conjunctivitis*. *S. aureus* isolated from conjunctivitis swab was susceptible to ethanolic seed oil extract of *V. paradoxa* at 100mg/ml, 50mg/ml and 25mg/ml concentrations, suggesting ethanolic extract of *V. paradoxa* oil as possessing antimicrobial property. Further exploration for its use as an ocular anti-bacterial agent is recommended.

Keywords: Inhibitory activity; Shea butter; Dose dependent; Inhibition zone; Oil separation

1. Introduction

Traditional medicine/complementary alternative medicine may suggest new ideas to modern medicine in order to face new challenges however these concepts should be acknowledged based on experimental studies [1]. Herbal plants have played an important role traditional medicine therapy of multiple human illnesses since the existence of man in many parts of the globe. The most common eye diseases include conjunctivitis, cataract, glaucoma, eye allergies, eye inflammation. The problem of adverse drug effects of modern drugs, has led to the increased use nowadays of herbal remedies in the treatment of eye diseases [2]. About two centuries ago in,'Makhzan-O-L Advieh,'a famous Iranian traditional pharmacopoeia, written by Mohammad Hossein Aghili Khorasani Shirazi (d. 1772 AD), one of the most significant Persian physicians, onion was prescribed for the prevention and treatment of a number of eye diseases,

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including; cataract, epiphora and blepharitis [3]. Plant materials are still being prescribed by traditional healers and herbalists for the treatment of these infections [4].

Available studies on use of Traditional Eye Medicine (TEM) in normal subjects are either hospital-based or have mostly been done in the African countries [5]. Interestingly, the reported prevalence of use of Traditional eye medicine in a hospital-based studies in Nigeria was 13.2%. [6]. Use of traditional eye medicine is prevalent in this population. The rampant use of steroid eye drops without prescription along with use of expired or unlabeled eye drops warrants greater emphasis on safe eye care practices in this population. Public awareness and regulatory legislations must be implemented to decrease harmful effects arising due to such practices [7]. Shea butter is a vegetable fat extracted from the kernel of the fruit of the shea tree (*Vitellaria paradoxa*), a tree belonging to the family of sapotaceae. The tree is the main indigenous oil producing wild plant spontaneously growing in Africa [8]. The shea tree (*Vitellaria paradoxa*) grows naturally or as a cultivated tree corp in the dry Savannah belt of West Africa.

The West Africa subspecies is *V. paradoxa* var. *nilotica* and occurs in 19 countries across the African continent, with Benin, Ghana, Burkina Faso and Cote D'Ivoire, as the major producers [9]. It is a perennial tropical tree that grows to the height range of 15-20 m, the fruits fall when fully ripened. The Shea nut butter obtained from the fruits of shell tree is also known as Chamen, Kandayi/Mankade, Osisi/Okwuma and Emi/Orioyo among the Tiv, Hausa, Igbo and Yoruba people of Nigeria, respectively [10] (Julius et al., 2013). [11] Hee, (2011) reported that shea tree begins to bear fruit of commercial quantities after approximately 20 to 50 years. *Vitellaria paradoxa* (Gaertn C. F.), or shea tree, remains one of the most valuable trees for farmers in the Atacora district of northern Benin, where rural communities depend on shea products for both food and income [12]. The shea tree grows extensively in the northern part of Ghana, which is known to be one of the poorest areas in the country [13]. Shea butter is a high–value shea nut fat used as an edible oil, antimicrobial and moisturiser in the food, pharmaceutical and cosmetic industries, respectively. The annual worldwide export of shea nut from Africa is 350,000 MT of kernels with a market value of approximately $120 million to producing countries [14].

The Nigerian agricultural industries have the potentials to contribute significantly to the economic and industrial development of the nation, especially with the wide range of the nation's agro-produce like Shea trees [15]. Chen et al., (2019) suggested that SheaFlex75 may be an effective management strategy for symptom relief and cartilage protection in patients with both acute and chronic OA [16]. Olasunkanmi et al., (2017) and Kalgo et al., (2019) also reported that shea butter has antioxidant constituents with antibacterial and antifungal activities as well as immunomodulatory properties [17, 18].

The vascularized nature of the conjunctiva coupled with its exposure to dust, wind, heat and radiation makes it to easily get infected [19]. Ocular tissues are vulnerable to Staphylococcus aureus infection due to the exposed nature of the eyes. Among the pathogenic bacteria, Staphylococcus aureus is the most common cause of ocular infection by Gram-positive bacteria second only to Pseudomonas aeruginosa, causing ocular diseases such as conjunctivitis, blepharitis, blepharoconjunctivitis, corneal ulcer, dacryocystitis, orbital cellulitis and dacryoadenitis [20]. Staphylococcus aureus is the most common cause of bacterial conjunctivitis, blepharoconjunctivitis and keratoconjunctivitis [21]. Medical practitioners consider drug resistant micro-organisms a major problem in the treatment of sick individuals (Lepape *et al.,* 2019) [22].

The aim of this study is to explore the inhibitory activities of *Vitellaria paradoxa* seed oil extract on isolated staphylococcal conjunctivitis.

Figure 1 Shea butter tree [23]

Figure 2 Shea butter seed [24]

2. Material and methods

2.1. Study site

The research was carried out KENTEC Medical Laboratory, Owerri, Imo State Nigeria.

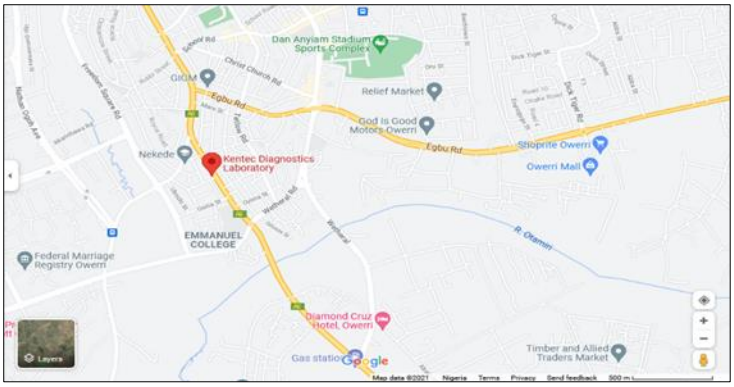

Figure 3 Google map showing KENTEC Medical Laboratory [25]

2.2. Plant material procurement, preparation and extraction

Fully matured fruits (nuts) of shea butter (*Vitellaria paradoxa)* were purchased from Douglas market in Owerri LGA of Imo state, Nigeria in mid-July, 2019. The plant material was identified and authenticated at the Department of plant science and Biotechnology, Imo State University, Owerri, Nigeria.

The seeds were cracked in order to remove the kernels after which 400g was weighed, oven-dried, crushed, ground and kneaded to obtain a paste. Subsequently, 250g of the paste was weighed into a 500ml beaker with the addition of 120ml H_2O 85°C and 230 ml of ethanol and allowed to stand for 24 hours in order to allow for oil separation followed by decantation (Julius *et al*., 2013) [26]. This was stored in an airtight glass container.

2.3. Extract concentration preparation

Three different concentrations (100, 50 and 25mg/ml) of the ethanolic seed oil extract of *V. paradoxa* were prepared using 10% Dimethyl Sulphur Oxide (DMSO) as a diluent according to the method of Julius et al., 2013[26]. The plant extracts assayed for the antibacterial activity, Minimum Inhibitory Concentration (MIC) using the agar well diffusion method were also employed.

2.3.1. Antibacterial assay

Cultured sample *Staphylococcus aureus* isolated from a patient's eye discharge in the Teaching Hospital Laboratory of the Imo State University, Nigeria having been diagnosed with bacterial conjunctivitis at the eye Clinic. This was transported to KENTEC Medical Laboratory, Owerri, Imo State Nigeria for analysis.

Wells were made using a sterile cock borer (6 mm), filled with 50 μL of each concentration (100, 50 and 25mg/ml) of the ethanolic seed oil extract of *V. paradoxa.* Bacterial suspension was added into each of the wells containing the crude extract. The inoculated plates were then left at room temperature for 30 min for the extract to evenly diffuse and later incubated at 37°C for 24 hours. Thereafter, the diameter of zones of inhibition both in horizontal and vertical meridians were measured in millimeters (mm) using a calibrated ruler, while results were interpreted according to N. Gurnani *et* al., 2016 guidelines [27].

The diluted standardized bacterial suspension was added into each of the wells containing the serially diluted crude extract. This was mixed to homogeneity to give a final inoculum of 5×10^5 CFU/ml S. Vijayarathna *et al.,* 2012 [28].

2.4. Statistical analysis

The data were presented in bar charts and subjected to one-way analysis of variance using SPSS version 20.0 (Statistical Package for the Social Sciences, Inc., Chicago, IL, United States). Differences between means were considered significant at p-value < 0.05.

3. Results and discussion

Our study results revealed that the zone of inhibition on *Staphylococcus aureus* with 100mg/ml concentration of ethanolic seed oil extract of *Vitellaria paradoxa* increased with increase in time – *Figure 4*.

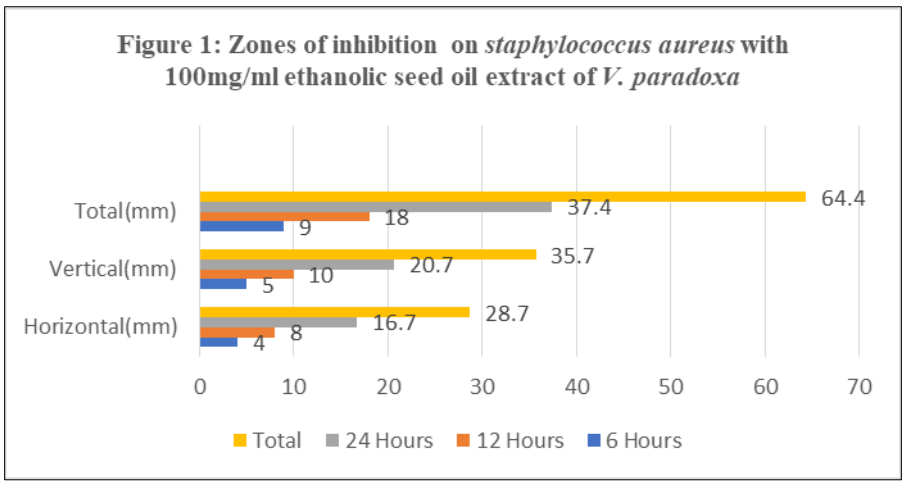

Figure 4 Zones of inhibition on *S. aureus* with 100mg/ml ethanolic seed oil extract of *V. paradoxa*

At 6hours, the zone of inhibition increased to 9.0mm, at 12hours, the zone of inhibition increased further to 18.0mm and at 24hours, the zone of inhibition got to 37.4mm – *Figure 4*.

Same trend of increase in zones of inhibition with time was also observed in *staphylococcus aureus* with 50mg/ml shea butter concentration having 7.0mm, 14.8mm and 30.6mm at 6, 12 and 24hours respectively - *Figure 5*.

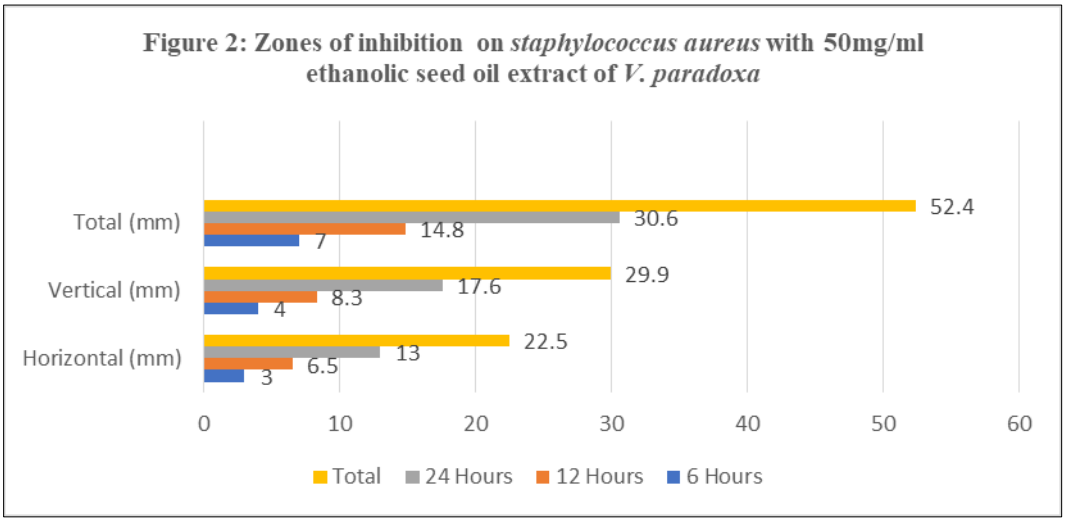

Figure 5 Zones of inhibition on *S. aureus* with 50mg/ml ethanolic seed oil extract of *V. paradoxa*

Figure 6 showed zones of inhibition on staphylococcus aureus with 25mg/ml shea butter concentration. At 6hours, the zone of inhibition increased to 5.0mm while at 12 and 24hours, it was further increased to 11.0mm and 23.9mm respectively. This also depicts increase in zone of inhibition as time increase.

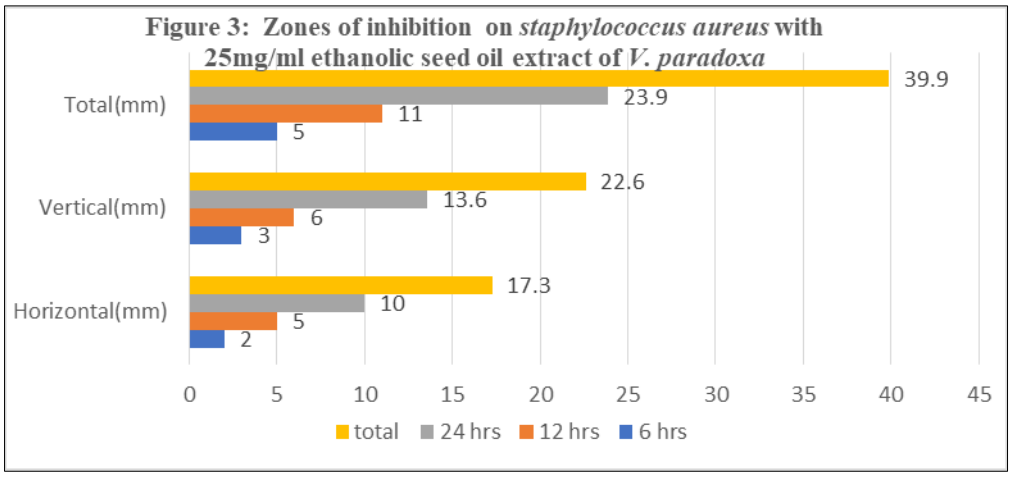

Figure 6 Zones of inhibition on *S. aureus* with 25mg/ml ethanolic seed oil extract of *V. paradoxa*

The zone of inhibition by the ethanolic seed oil extract of *V. paradoxa* with highest diameter of 100mg /ml (37.4mm) at 24hrs time interval for both horizontal and vertical diameters (*Figure 4*) and lowest as shown using 25mg/ml (5.0mm) also for both horizontal and vertical at 6hrs time interval (*Figure 6*) indicated that the higher the concentration of *V. paradoxa,* the greater its inhibitory effect on *Staphylococcal conjunctivitis*, thus, its inhibitory effect was concentration dependent. In other words, decrease in different shea butter concentration led to a direct reduction of the zone of inhibition around the *staphylococcus aureus*. Similar observation by Adamu *et al., (*2013) [29] and Arekemase, *et al.,* (2013) [30] had suggested that higher concentration of antimicrobial compounds gave appreciable antimicrobial efficacy.

The pattern of antimicrobial activities of the plant extracts and the zone of inhibition of the bacteria are in consonance with Ekhuemelo *et al.,* 2021 [31], that asserted that the zones of inhibition of *V. paradoxa* fractions (18 - 24 mm) were at the same range of 25 mm – 31 mm recorded for three control antibiotics. The plant extract *of V. paradoxa* seed oil had profound activities against staphylococcus with higher concentration as varying degrees of inhibition against gram positive bacteria was observed. The marked difference in the effect of the extracts on the organism therefore is suggestive of the activity against cell wall components of the organism. The antimicrobial substance appears to exert antimicrobial activity by increasingly inhibiting the growth of ethanolic seed oil extract of *V. paradoxa* at 100mg/ml, 50mg/ml and 25mg/ml respectively.

4. Conclusion

Staphylococcus aureus isolated from conjunctivitis swab was susceptible to ethanolic seed oil extract of *V. paradoxa* at 100mg/ml, 50mg/ml and 25mg/ml concentrations as it inhibited the growth of *Staphylococcal conjunctivitis*, thus, ethanolic extract of *V. paradoxa* oil possess antimicrobial property. Further exploration for its use as an ocular anti-bacterial agent is recommended.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors hereby declare no conflicting interest.

Statement of ethical approval

This was obtained from the Department of optometry, Imo State University, Owerri, Nigeria.

Statement of informed consent

Study does not require consent; hence not applicable.

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