ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF CALABASH SEED OIL ON CLINICAL ISOLATES FROM SKIN

¹Kashari, O., ¹Osesua, B. A., ¹Danjumma, B. J., and ²UDEME, A. M.

¹Department of Science Technology, Waziri Umaru Federal Polytechnic, Birnin Kebbi, Kebbi State, Nigeria. ²Federal Polytechnic, Ekowe, Bayelsa State, Nigeria. E-mail: <u>Osesuaa@yahoo.com</u> Phone: +2347034525762

ABSTRACT

The antibacterial activity and phytochemical screening of calabash seed oil was carried out on clinical isolates obtained from the skin using Agar "well" diffusion techniques. The test bacteria (Bacillus subtilis, Staphylococcus aureus, Serratia marcescens, Pseudomonas aeruginosa, Salmonella species and Bacillus macerans) were isolated from skin of patients with wound infection attending Federal Medical Centre Birnin Kebbi. The result shows that, at 4mg/ml the calabash seed oil was not active on any of the test bacterial while at 8mlg/ml, the calabash seed oil was active on Staphylococcus aureus, Salmonella species and Bacillus macerans with zone of inhibition of 16mm, 14mm and 10mm respectively while at 12mg/ml the calabash seed oil was active on all the test bacteria. The phytochemical screening carried out revealed the presence of some bioactive compounds such as alkaloids, flavonoids, glycoside saponin, terpenes and carbohydrate while tannin and free anthraquinone were absent. Therefore, the result of this research work has provided a clue that the oil can be used for the formulation of any skin hygiene preparation and in the treatment of bacterial skin disease.

KEYWORDS: Antibacterial, phytochemical "Agar well" best bacteria, clinical, test bacteria, bioactive, compounds, formulation, hygiene etc.

INTRODUCTION

The calabash, *Lagenaria siceraria*, also known as opo squash, bottle gourd or long melon is a vine grown for its fruit, which can either be harvested young and used as a vegetable, or harvested mature, dried, and used as a bottle, utensil, or pipe. The fresh fruit has a light green smooth skin and a white flesh. They come in a variety of shapes, they can be huge and rounded, or small and bottle shaped, or slim and serpentine, more than a meter long. Rounder varieties are called calabash gourds. The calabash was one of the first cultivated plants in the world, grown not primarily for food, but for use as a water container. In addition to the young fruits sometimes being boiled as vegetables, the large, strong, hard-shelled, and buoyant fruits have long been used as containers for water and food, musical instruments (drums and flutes), fishing floats, and apparel such as penis sheaths. It is often called Kwarya in Hausa language, Ibale in Igbo language and Igba in Yoruba language all in Nigeria. Along with several wild perennial *Lagenaria* species, the Bottle Gourd has long been believed to be indigenous to Africa. However, until the recent discovery and morphological and genetic characterization of a wild population of *L*, *siceraria* in Zimbabwe, the Bottle Gourd had only ever been well documented as a domesticated plant (Decker-Walters *et al.* 2004).

The bottle gourd may have been carried from Africa to Asia, Europe and the Americas in the course of human migration (Erickson *et al.* 2005), or by seeds floating across the oceans inside the gourd. It has been proven to be in New World prior to the arrival of Columbus (Erickson, *et al.* 2005). It shares its common name with that of the calabash tree *Crescentia cujete* (Erickson, *et al.* 2005). It is a commonly cultivated plant in tropical and subtropical areas of the world, now believed by some to have spread or originated from wild populations in southern Africa (Chandra, 2010).

Experiments have shown that domesticated Bottle Gourds contain still-viable seeds even after floating in sea water for more than 7 months (Thube et al. 2009). A range of data suggests that the Bottle Gourd was present in the Americas as a domesticated plant by 10,000 B.C., which would make it among the earliest domesticated species in the New World. Comparisons of DNA sequences from archaeological bottle gourd specimens and modern Asian and African landraces identify Asia as the source of its introduction to the New World. Erickson *et al.* (2005) suggested that this "utility species" (along with another such species, the Domestic Dog) were domesticated long before any food crops or livestock species and that both were brought to the Americas by Paleo-Indian populations as they colonized the New World. Dixit *et al.* (2008) developed chloroplast and nuclear markers to investigate the origins of Bottle Gourds in Polynesia and suggested that their work also has implications for understanding the complex history of domestication and dispersal of the species as a whole.

Transverse section of *Lagenaria siceraria* leaf shows the following features:- upper epidermis consists of elongated parenchymatous cells, covered by cuticle. Lower epidermiscontains elongated wavy walled parenchymatous cells covered by cuticle. Number of Covering and collapsed trichomes are present, while very few glandular trichomes are also present. Upper epidermis shows few stomata, which are of anisocytic type. Palisade cells are present at upper and lower epidermis. It shows hexagonal to polygonal, large, thin walled colourless cells, and may be water storing. Mesophyll is made up of 3-4 layered chloroplasts containing, compactly arranged, oval to circular cells. It is interrupted by vascular bundles of various sizes. Vascular bundles - Vascular bundles are surrounded by 2-3 layered sclerenchyma. They are conjoint, collateral and closed. Xylem is placed towards upper epidermis and phloem towards lower epidermis (Shah, 2010).

L, *siceraria* is considered to be of African and Asian origin. *L*, *siceraria* is a popular vegetable, grown almost all the year round, particularly in frost free areas. It can be cultivated in all kinds of soil, but thrives best in heavily manured loams. It required warm humid climate or plenty of watering when grown during dry weather. Seeds may be sown in nursery beds and seedlings transplanted when they have put forth 2-3 leaves. They may be also sown directly, 4-5 seeds together, in manured beds or pits 5-6ft. apart; the strongest among the seedlings is retained, while others are removed and transplanted. Seedling transplantation is where an early crop is desired, generally two crop raised in India; the summer crop is sown from the middle of October to the middle of March and the later crop, from the beginning of March to the Middle of July. Round fruit types are usually sown for the early crop and bottle–shaped types for the second crop. Vines are allowed to trail on the ground or trained over walls (The Wealth of India, 2004).

SCIENTIFIC CLASSIFICATION

Scientifically, the plant is classified as follows:

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Cucurbitales
Family:	Cucurbitaceae
Genus:	Lagenaria
Species:	L, siceraria
	(Lakshmi, et al. 2011)

NUTRITIONAL VALUE

Analysis of the nutritional value of *L*, *siceraria* shows that the fruits contain Carbohydrates, amino acids, vitamins, sterols, glycosides e.t.c. as summarized in the table below.

TRADITIONAL USES

L, *siceraria* fruits are traditionally used for its cardioprotective, cardiotonic, general tonic, diuretic, aphrodisiac, antidote to certain poisons, scorpion stings, alternative purgative and cooling effects. It cures pain, ulcers and fever and is also used for pectoral cough, asthma and other bronchial disorders especially syrup prepared from tender fruits (sivarajan, 1996 and Duke, 1992). The pulp of the fruit is considered cool, diuretic, antibilious and useful in coughs and as an antidote to certain poisons (Duke, 1992). Most African tribal communities

use the dry shells of bottle gourd fruits for various purposes. Domestic utensils like bottles, bowls, milk pots, spoons and containers of several types are made out of the dried shells. It is a common sight everywhere in the tribal dominated pockets of khammam district that the ethnic groups are mainly using the dry shells for carrying country liquor, honey and water. In some of the pockets it is being used for making stringed and wind musical instruments and pipes. At few places, the natives use the dried shells as floats on water bodies as well. Though it is nutritionally less calorific, tribals prefer bottle gourd as a vegetable for the preparation of curries and pickles. The koya community in Africa uses the fruits of the wild types for medicinal purposes (Goji et al. 2006). Probably, the bitter constituent of wild bottle gourds is responsible for the medicinal (purgative) property. The Gutti Koya tribals use the bottle gourd as a cure for headache by mixing the seed oil with castor oil and applying it externally. The pulp of the fruit is considered cool and diuretic (Goji, et al., 2006). Leaves of L, siceraria are taken as emetic in the form of leaf juice or decoction. This by adding sugar also used in jaundice. Crushed leaves are used for baldness and applied on the head for headache. Leaves are also used as alternative purgative (Chopra and Chopra, 1992). Flowers are also mentioned as antidote in certain kind of poisons. Stem bark is diuretic, roots are emetic and used in dropsy (Duke, 1992).

Aside its medicinal applications, Calabashes are used to collect and store palm wine in Bandundu Province, Democratic Republic of the Congo and other parts of West African countries. Hollowed out and dried calabashes are a very typical utensil in households across West Africa. They are used to clean rice, carry water, and as food containers. Smaller sizes are used as bowls to drink palm wine. Calabashes are used in making the West African kora (a harp-lute), *xalam/ngoni* (a lute) and the *goje* (a traditional fiddle). They also serve as resonators underneath the *balafon* (West African marimba). The calabash is also used in making the *shegureh* (a Sierra Leonean women's rattle) and *balangi* (a Sierra Leonean type of *balafon*) musical instruments. Sometimes, large calabashes are simply hollowed, dried and used as percussion instruments, especially by Fulani, Songhai, Gur-speaking and Hausa peoples (Elisha, *et al.* 2000). In Nigeria, the calabash has been used to avoid a law requiring the wearing of a helmet on a motorcycle. In South Africa, it is commonly used as a drinking vessel by tribes such as the Zulus. Ebore tribe children in Ethiopia wear hats made from the calabash to protect them from the sun. The table below shows a brief summary of the ethnobotanical uses of the fruits.

SIGNIFICANCE OF THE STUDY

There is a worldwide belief that herbal remedies are safer and less damaging to the human body than synthetic drugs. Therefore laboratories around the world are engaged in screening of plants for biological activities with therapeutics potential. One major criterion for the selection of plant for such a study is traditional healer's claim for its therapeutics usefulness. Skin infections are widely encountered in the tropics with lots of orthodox remedies involving the use of systemic antibiotics. The problem of drug resistance and reported allergies also abound. Oils of various plants have been reported to possess antimicrobial, antiviral and anti-protozoal activities. Over the years, it has become a practice

in many communities in Nigeria and Africa to extract oils from seeds and use them for treatment of skin infections. Scientists have endorsed this ancient practice finding it safer than synthetic creams. The pharmacological activities of *L. siceraria* have been reported earlier by numerous researchers, prompting the present attempt to explore the antibacterial activity of its oil on some selected clinical bacteria skin isolates.

The aim and objectives of this research work is therefore to extract oil from calabash seed, isolate and characterize bacteria from infected skin and test for the activity of the calabash seed oil on the isolates.

MATERIALS AND METHOD

SAMPLE COLLECTION

The calabash seed used for this research were purchased from Birnin –Kebbi central market of Kebbi state. The seeds were peeled to remove the repicarp, after which the seeds were dried under the sun. The seeds were crushed into powder form using laboratory blender.

EXTRACTION OF OIL

Mechanical cold press methods were used for extraction of the oil from calabash seed powder. 200g of the powder was tightened with sterile sieving cloth and fixed into the mechanical vice and sterile container was placed under the mechanical vice to collect the oil aseptically; pressure was then applied by turning the handle until oil begins to seep out of the powder, the pressure was maintained until the extraction was over. 40mls of oil was obtained, from the 200g obtained 4g of the oil was weighed to make or form a concentration of 4mg/ml the same was repeated for 8mg/ml and 12mg/ml respectively.

MEDIA PREPARATION

The media used in this research work was prepared according to the manufacturer`s instructions. The media used were nutrient broth and nutrient agar.

ISOLATION OF BACTERIA

Skin swabs were collected from ten (10) patients with wounds infection attending Federal Medical Center Birnin Kebbi, Kebbi state using sterile swab sticks, which was done by rolling the swab sticks on the skin/wounds and returned back into a sterile caped container; then transported to the laboratory. These were incubated at 37°c for 24hours. Colonies were picked from the plates and sub-cultured in order to obtain a pure culture. The colonies that grow were indentified using specific biochemical tests.

DETERMINATION OF MORPHOLOGICAL CHARACTERISTICS

Based on the differences in chemical and physical properties of gram positive and gram negative bacteria, gram positive bacteria have greater amount of peptidoglycan in their cell wall 50-90% when crystal violet solution [a basic dye] is added to the smear, they retain the primary colour i.e crystal violet which is purple/blue while gram negative bacteria have

less peptidoglycan in their cells wall, 20% which tends to retain the secondary color safranin solution in their cell wall very well and appear pink/red on the microscope.

Procedures

From the colonies that developed on the nutrient agar, a smear was made on a glass slide using sterile wire loop and the smear was dried and heat fixed. The smear was flooded with crystal violet solution for 30 seconds and washed; this was later tripped off and covered with lugols iodine for 30-60 seconds and washed off and decolorized with acid alcohol, the smear was counter stained with safranin solution for 60 seconds followed by rising with distilled water; this was then air dry before viewing under the microscope using oil immersion objective (Oyeleke and Manga 2008).

BIOCHEMICAL TESTS

The following biochemical test were carried out as described in (Oyeleke and Manga, cheesbrughi 2008).

Catalase Test: The principle behind this test relies on the ability of some micro organisms to produce the enzymes catalase which split hydrogen perioxide to produce (O_2) and water (H_2O) . In this test, two glass slides were set up and labeled "test" and "control" respectively. On the "test" slide 3 drops of hydrogen perioxide were placed and 3 drops of water were dropped on the "control" slide. A loop full of microbial colony was emulsified on each of the slide and observed for the production of bubbles; the presence of gas bubbles indicate positive result while absent of gas bubbles indicate negative.

Lactose/Gas Test: some micro organisms can utilize lactose to produce CO_2 and lactic acid. The CO_2 is given off as gas which can be trapped in durham tubes as gas bubbles. The colonies were inoculated in sterile lactose broth containing inverted durham tubes in test tubes; plugged with cotton wool and foil paper. The test tubes were incubated at 37°c for 24hours. The presence of gas bubbles in the durham tubes indicate positive result for both lactose and gas production test.

Citrate Test: this test relies on the ability of micro organisms to use citrate as an intermediate of citric acid cycle in the form of sodium citrate as the primary carbon source. The microbial colony was loop inoculated on simmons citrate agar using a sterilized wire loop and incubated for 48hours at 37°c. A deep blue coloration indicates a positive result.

Motility Test: this is used to determine the ability of micro- organisms to move from one point to another (motility) using its flagellum or cilia. The colonies were inoculated using stab techniques through a media containing 0.2-5% agar and incubated at 37°c for 18-24hours. Motile organisms are able to move away from the line of inoculation through the sloppy agar.

Idole Test: the test relies on the ability of micro organisms to convert or utilize the amino acid tryptophan giving off indole as a by-product. The microbial colony were inoculated in nutrient broth containing peptone water $[H_2O]$ and incubated for 24hours at 37°c. after which ten drops of kovac`s indole reagent were added and shaken gently; a positive result is indicated by formation of a red colored ring in the reagent layer just above the broth within one minute, in the negative reaction the indole reagent retain its yellow coloration.

Urease Test: this relies on the ability of some micro organisms to produce the enzymes urease which split urea into ammonia and carbondioxide. The ammonia combines with CO_2 and H_2O to form ammonium carbonate, turning the medium to alkaline medium, thus the indicator phenyl red presence in the medium changes from its original orange color to bright pink. The inoculums were inoculated into prepared urea medium and incubated at $37^{\circ}c$ to 48hours, a bright pink color indicate a positive result.

Methyl Red Test: this relies on the ability of micro organisms to produce and maintain acidic by-product from the fermentation of glucose. The acidity of the medium is indicated by the indicator methyl red which remains red in color for positive result.

Vagues Proskuer Test: the test relies on the ability of micro organism produce butylenes glycol when inoculated and incubated in methyl red vagues proskuer medium in the presence of potassium hydroxide (KOH) and Naphthol. The colonies were inoculated in MRVP medium and incubated at 37°c for 24 hours; after which 5 drops and 15 drops of 40% potassium hydroxide (KOH) and 5% naphthol solution were added respectively. The medium was shaken, the cap was loosen and placed in a slanting or sloping position. Development of red coloration indicate VP position test while no change in color indicate negative result.

Spore Detection: some micro organisms have the ability to produce non-vegetative spore which makes them resistant to numerous sterilization techniques, when smear of this micro organisms is stained with malachite green and safranin the spore retains the green color. A smear of each colony was prepared and flooded with malachite green and heated until it steams. After allowing to standing for 2-3 minutes the smear was washed under tap water and stained with safranin (Nutrial red) solution for 30 seconds. After washing, the slide was air dried and viewed under oil immersion object. The spore retains a green color against a red background.

Coagulase Test: This is based on enzymes (coagulase) produced by *Staphylococcus aureus* that convert soluble fibrinogen in plasma to insoluble fibrin. Staphylococcus aureus produces two forms of coagulase, bound and free coagulase reacting factor (CRF) in plasma to form a complex, which is thrombin. This converts fibrinogen to fibrin resulting in clotting of plasma. Based on this, three test tubes were taken and labeled "test negative control" and "positive test control". Each tube was filled with 1ml of 1 in 10 diluted blood plasma, to the tube labeled "test". 0.2mls of overnight broth cultured test bacteria were added, then all the

tubes were incubated at 37°c and the suspension was observed at half hourly intervals for a period of four hours. Positive result was indicated by gelling of the plasma which remains in place even after inverting the tube while absence of gelling indicates negative.

Sucrose Test: Sucrose is fermented to produce acidic end product, the PH of the medium will drop. A PH indicator in the medium changes color to indicate acid production. Inoculums from the pure culture were transferred aseptically to a sterile tube of phenol red sucrose broth, the inoculated tubes were incubated at 35-37°c for 24 hours and the result was determined. A positive test consists of a color change from red to yellow indicating a PH change to acidic.

Hydrogen Sulfide (H_2S) **Production Test:** This test determines whether the microbes reduce sulfur-containing compounds to sulfide during the process of metabolism; sulfide combines with iron compounds to produce iron sulfide (**FeS**), a black precipitate. Based on this, inoculums from the pure culture were transferred aseptically to a sterile triple sugar iron agar (TSIA) slant. The inoculated tubes were incubated at 35-37°c for 24hours and the result was determined. The irons sulfide (**FeS**) has a high affinity (strong attraction) for sulfide ions. The result was H_2S combined with iron to make iron sulfide (**FeS**), a black compound in tubes of triple sugar iron agar (TSIA) containing bacteria-producing hydrogen sulfide; the agar turns black from the FeS.

Preparation of Test Medium: The test medium (nutrient agar) was prepared using standard, procedures described in Barrow and Felthan (**1993**). A sterile syringe was obtained, cut using a sterile razor blade; the syringe (12mm) in diameter was used to dig the "wells" on the media. The varying concentrations of the calabash seed oil were then used to fill the "wells". After inoculating the test bacteria on each of the plates, they were incubated at 37°C for 24 hours.

PHYTOCHEMICAL SCREENING

The following are some phytochemical screening tests carried out. The tests were carried out in accordance to standards described by Oyeleke and Mangas (2008).

Test for Alkaloid: 1mls of the oil was stirred in 10mls of 1% aqueous hydrochloric acid on a steam bath. After which 2mls of the resultant solution was treated separately with few drops of dangedorff's reaction, mayer's reagent and wanger's reagent. A deep brown creamy precipitate indicates a positive result.

Test for Flavonoide: 1mls of the oil was stirred in 5mls of sodium hydroxide solution and treated with hydrochloric acid. The presence of flavonoids is indicated by disappearance of oil in the set up.

Test for Tannis: 1m/s of the oil was treated with 2 drops of 10% ferric chloride solution. Blue or green coloration indicate the presence of tannins.

Test for Glycoside (killer-killiani test): 1ml of the oil was mixed with 5mls of ferric chloride in glacial acetic acid left for 1minute, after which 2mls of hydrogen tetraoxosulphate (VI) acid (H_2SO_4) was dispensed drop-wise down the slide of the test tube, a positive test is indicated by a clear interphase with a blue layer.

Test for Saponin: 1mls of the oil was added into 20mls of distilled water in a test tube, the content was vigorously shaken in the test tube for 2 minutes, the presence of bubbles indicates the presence of saponin.

Test for Steriod: 5 drops of concentrated hydrogen tetraoxosulphate (VI) acid (H_2SO_4) was added into 1ml of the oil and mixed properly; a redish color indicates the presence of steroid.

Test for Carbohydrates: 5 drops of molisch reagent was added into 2mls of the oil and mixed properly, after which 3 drops of sulphuric acid were added, mixed and allowed to form a lower layer. The presence of purple ring at the interphase of the liquid indicates the presence of carbohydrate.

Test For Free Anthraquinoles (Borntrager`s test): 1ml of the oil was dispensed in a test tubes and 10mls of chloroform was added into the test tubes and shaken for 5 minutes. A bright pink coloration in the aqueous layer indicates the presence of anthraquinones.

Test for Tapens (Lieber burhad test): A chloroform solution of the oil was prepared by mixing 2mls of the oil with 5mls of chloroform. After which 0.5ml of the chloroform solution was mixed with acetic and hydrite and 1ml of concentrated sulphuric acid was added down to the wall of the test tube to form a layer underneath, then formation of redish violet color indicates the presence of tapenes.

FINDINGS AND DISCUSSION

The results of this research works are presented on tables.

Phytochemicals	Level of presence
Alkaloid	++
Flavonoid	+
Tannin	-
Glucoside	+
Saponin	+++
Steroids	+++
Terpenes	++
Carbohydrates	++
free anthraquinone	-

|--|

Note:

+++	Highly present
++	Moderately present
+	Slightly present
-	Not present

Phytochemical characteristics of calabash seed oil were determined as shown in Table 1 above. Saponin and steroids were highly present, alkaloids, terpenes and carbohydrate were moderately present. Glycosides and flavonoids were slightly present, whereas tannin and free anthraquinones were absent.

Т	TABLE 2:	Shows	s Bacteria	al Isolate o	btained fr	rom Adu	lt Skin				-						
Gram rXn	Shape	Cat	Coag	Lact	Glu	Suc	Citrate	Motility	Indole	Urease	Gas	H_2S	Mr	Vp	Spore	Sh	Species
+	Rod	+	-	-	+	+	+	+	-	-	-	-	+	+	-	+	Bacillus subtilis
+	Cocci	+	+	+	+	+	+	-	-	-	+	-	-	-	-	+	Staphylococc us aureus
+	Rod	+	-	-	+	+	+	-	-	-	-	-	-	-	-	+	Serratia marcescens
-	Rod	+	-	-	+	+	+	+	-	-	-	-	-	-	+	-	P seudomonas aeruginosa
-	Rod	+	-	-	+	+	+	+	-	-	-	+	-	+	+	-	Salmonella species
+	Rod	+	-	-	+	+	-	+	-	-	-	+	-	-	+	+	Bacillus macerans

Concentration	Mean zone of inhibition (mm)/ isolates					
(mg/ml)	BS	SA	SM	PA	S.SP	BM
-						
4	-	-	-	-	-	-
8	-	16	-	-	14	10
12	10	19	16	10	16	16

Table 3: shows the mean zone of inhibition of calabash seed oil on bacterial isolates from the skin.

NOTE:

BS:	Bacillus subtilis
SA:	Staphylococcus aureus
PA:	Serratia marcescens
PA:	Pseudomonas aeruginosa
S.SP:	Salmannella species
BM:	Bacillus macerans.

Table 3 shows the mean zone of inhibition of the isolated bacteria species. At 4mg/ml none of the specie was inhibited while at 8mg/ml *Staphylococcus aureus, Salmonella spp.* and *Bacillus macerans* were present were as at 12mg/ml all the microorganisms were inhibited.

DISCUSSION

Results obtained show that calabash seed oil contains many biologically active compounds which make it potential for development of medicinal agent. Herbal medicines already form the basis for therapeutic use in developing countries, but of recent, there has been an increase in the use of herbal medicines in the developed world (Fugiwara, 1982).

Phytochemical screening of calabash seed oil revealed the presence of some bioactive compounds such as Alkaloid, flavonoid, glycoside, saponin, steroids, terpenes and carbohydrates, while Tannin and free anthraquinone were absent.

Determination of the morphological characteristics of some organisms isolated from adult skin of patients with wounds infection from Federal Medical Centre Birnin Kebbi showed the presence of *Bacillus subtilis, Staphylococcus aureus, Serratia marcescens, Pseudomonas aeruginosa, Salmonella species and Bacillus marcerans.*

Results obtained from this research work show that, the calabash seed oil has varying inhibition potential on the test bacteria. At 4mg/ml the oil was not active on any bacteria, while at 8mg/ml the oil was active on *Staphylococcus aureus, Salmonella species and Bacillus macerans*. This agrees with the finding of Rao, (2005) which says that oil inhibit serratia marceseen and that of Tewari, (1992) which says that oil are commonly used for therapeutic purposes-because it suppresses several species of pathogenic bacteria. At 12mg/ml the calabash seed oil was active on all the bacteria. Also according to Biswa (2002), calabash seed oil is used in industries in the production of pharmaceuticals, soaps, cosmetics, disinfectant, shampoo, face creams and medicated soaps.

CONCLUSION

Medicinal plants are the local heritage with the global importance. The world is endowed with a rich wealth of medicinal plants. Medicinal plants also play an important role in the lives of rural people particularly in remote parts of developing countries with few health facilities. This research showed that calabash seed oil has antibacterial activities against bacteria present on the infected skin; calabash seed oil can be used in the formulation of any skin hygiene preparation and in the treatment of bacterial skin disease.

The response of the calabash seed oil on the bacteria isolates and the bioactive compounds presence in the calabash seed oil have provided a clue that the oil can be used in treatment and prevention of skin infections. Further research is needed in this area which will serve as a confirmation.

REFERENCES

- Baranoswka, K. M. and Cisowki, W. (1994): "*HPLC* determination of flavones –*C* glycosides in some species of Cucurbitaceae family"*J. chromatogram A*, 675:240-243
- Barrow G.C. and Feltham R.K.A. (1993): "Cowan and Steels Manual for identification of Medicinal Bacteria" Cambridge University Press, Great Britian 3rd ed. Pp.50-126.
- Biswas K. Chattopadhyay R., and L. Bangaree (2002): "Biological Activities and Medical properties of Ziziphur mucronata" *Curriculum of Science Vol. 82 Pp 1336-1343*
- Chandra N. (2010): "Toxin in lauki kills diabetic city scientist". India TodayLiving Media.
- Chang, S. C., Lee M.S., Li C.H and M. L. Chen, (1995): "Dietary fibre content and composition of vegetable in Taiwan are" *Asian Pacific J. Clinical Nitr.*, *4*:204-210
- Cheesebrough M. (2008): "District Laboratory Manual on Tropical Countries" Cambridge University press.Part 2 Pp. 45-56.
- Chopra B.N. and I. C. Chopra (1992): "Second Glossary of Indian Medicinal plants" Publication and Information Directorate, New Delhi, India Pp414-418
- Decker-Walter D. S., Wilkins-Ellert, S., Chung M., and Staub J.E. (2004): "Discovery and genetic assessment of wild bottle gourd[Lagenaria siceraria (Molina) standley, cucurbitaceae] from Zimbabwe" *Eco. Bot.*, 58: 501-508
- Deshpande, J.R., Mishra, M.R., Meghre V.S., Wadodkar S.G and Dorle, A.K. (2007): "Free radical scavenging activity of Lagenaria siceraria (Mol.)" *Stabdl. Fruit Nat. Prod. Radiance*, 6:127-130
- Deshpande, J.R., Choudhari A.A., Mishra, M.R., Meghre V.S., Wadodkar S.G and Dorle A.K., (2008): "Beneficial Effects of Lagenaria siceraria (Mol.) Standley fruit epicarp in animal models" *Indian Journal Exp. Biol.*, 46:234-242
- Duke, J. A. (1992): "handbook of biologically active phytochemicals and their activities" CRC Press, Boco Raton FL.
- Duke J.A. (1999): "Handbook of Phytochemical and Constituents of GRASS herbs and other economic Plants", CRC Press, FL.
- Dixit Y, Panda S. and Anand K.(2008): *Lagenaria siceraria* peel extract in the regulation of hyperthyroidism, hyperglycemia and lipid peroxidation in mice. *Int J Biomed Pharm Sci.*;2:79–83

- Elisha E.E., Twaij H.A. A., Ali N.M., Tarish J.H., Al-omari M.M and Karim S. (2000): "The anthelmintic activity of some Iraqi plants of the cucurbitaceae".*Pharm Biol*.25:153–7.
- Erickson, D., Smith L., Bruce D., Clarke C., Andrew C.; Sandweiss M., Daniel H.; and Tuross, N. (2005): "An Asian origin for a 10,000-year-old domesticated plant in the Americas PNAS".102 (51):. Retrieved 17 November 2009. 18315–18320.
- Fagiwara T., Takeka T., Gihara O., and Shimimumin Y.,(1982): "Studies on the structure of polysaccharides from the bark of medicinal Ziziphur"*Chem.* Vol. 30 *Pp* 425-430
- Fard, M.H., Bodhankar S.L. and Dikshit M. (2008): "Cardioprotective activity of fruit of lagenaria siceraria (Monila)Standley on doxorubicin induced cardiotoxicity in rats" *Int. J. Pharmacology* 4:466-471
- Fran, Z.S. and Wilf S. (2008): "Minor oil Crops" retrieved on the 21st January, 2013 from http://www.fao.org/docrop
- Goji M, Asres K, Gemeda N, and Yirsaw K.(2006): Screening of the antimicrobial activities of some plants used traditionally in ethiopia for the treatment of skin disorders.*Ethio Pharma J* ;24:130–5.
- Goyal B.R. (2007): "Phyto-pharmacology of Achyranthes aspera: A Review" Pharmacognosy Reviews 1(1):143-150
- Gangwal, A., Parmar S.K., Gupta G.L., Rana A.C and Sheth N.R. (2008): "Immunomodulatory effects of Lagenaria siceraria fruits in rats "*Pharmacognosy Mag.*, 4:S234-S238
- Ghule BV, Ghante M.H., Uparganlawar A.B., and Yeole O.G.,(2006): Analgesic and antiinflammatory activities of *Lagenaria siceraria* (Mol.) stand, fruit juice extract in rats and mice. *Pharmacogn Mag.*;2:232–8.
- Ghule B.V., Ghante M.H., Saoji A.N., and Yeole O.G., (2006): Hypolipidemic and antihyperlipidemic effects of *Lagenaria siceraria* (Mol.) fruit extracts. *Indian J Exp Biol.* ;44:905–9.
- Ghule B.V., Ghante M.H., Saoji A.N., and Yeole O.G.,(2007): Diuretic activity of *Lagenaria siceraria* fruit extract in rats.*Int J Pharm* Sci. ;69:817–9.

Kirtikar K.R (2001): "Indian Medicinal. Oriental Enterprises, Dehradun, India: " Pp. 722-723

Khare C. P. (2004): "Encyclopedia of Indian Medicineal Plants" Springer Berlin Heidelberg: Pp. 278-280

Kochar, S. L. (1993); "Tropical Crops": 2nd edition. Harriman Publishers Nigeria. Pp. 102-115

- Lakshmi B.V.S., Kumar P.U., Neelima N., Umarani V. and Sudhakar M., (2011): Hepatoprotective activity of *Lagenaria siceraria* fruit extracts against carbontetrachloride-induced hepatic damage in rats. *Res. J. Pharma. Biol. Chem. Sci.*, 2: 130-137
- Li N.H. (1994): "Chinese Medicinal Herbs of Hong Kong": Commercial Press (Hong Kong) Limited: Pp. 68-9
- Mahurkar, N., mumtaz, m. and Ifthekar S (2012): protective effect of Aqueous and Methanolic extracts of Lagenaria siceraria seeds in Gentamincin Induced Nephrotoxicity, *International Journal of Research in Ayurveda and Pharmacy*. Vol. 3 Pp 443
- Mirlosawa K.B, and Cisowski W.(1995): Isolation and identification of C-glycosides flavone from *Lagenaria siceraria L*. L. D Res. 52:137–9.
- Oyeleke, S. B. and Manga B.S., (2008): "Essential laboratory Practical in Microbiological" Tobest Publishers Minna 1st Edition Pp.23-66
- Prased L.V. (2002): In: Indian System of Medicine and Homoeopathy Traditional Medicine in Asia. New Delhi: HO- Regional Office for South East Asia; pp. 283–6.
- Pullaiah T. (2006): The Encyclopedia of World Medicinal Plants. Regency publishers;Vol 3:1206–7.
- Rahman A.S.H. (2003): "Bottle Gourd (Lagenaria siceraria) a vegetable for good health"*Natural Product Radiance* 2(5): 249-250.
- Rao V. (2005): "The effect of Calabash seed oil in acute Plasmodium Malaria" *Med. Journal* Vol 7 Pp: 258-263
- Shah B. N. (2010): "Pharmacognostic studies of the Lagenaria siceraria (molina) standley"*International Journal of PharmTech Research*. 2(1):121-124.
- Shirwaikar A. (1996): "Chemical Investigation and antihepatotoxic activity of The fruit of Lagenaria siceraria" *Indian J. of Pharmaceutical Sciences* 58(5):197-202

Shivarajan V.V. (1996): "Ayurvedic drugs and their Plant source". Oxford and IBH Publishers, New Delhi Pp.176-177.

Tewari, H. (1992): "Microbiology" 5th ed. McGraw Hill Inc. Pp 39

- The Wealth of India (2004): A Dictionary of Indian raw materials & industrial products, CSIR, New Delhi III; 16-19.
- Thube S., Tambe R., Patel MF and Patel SD. (2009): In-vitro anthelmintic activity of seed extract of *Lagenaria siceraria* (Molina.) Standley Fruit.*J Pharm Res*: 2:1194–5.
- Wang H.X. (2000): "Lagenin, a novel ribosome inactivating protein with ribonucleatic activity from bottle gourd (Lagenaria siceraria) seeds", *Life Sciences* 67(21): 2631-2638.
- Williamson E. M (1996): "Selection, Preparation and Pharmacological Evaluation of Plant Material. John Wiley and Sons." Pp. 1-3.