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## Evaluation of the activity of *Garcinia kola* seed oil and honey on skin cream formulation

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#### Abstract

The *Garcinia kola* seed oil was extracted by solvent extraction and the yield of the oil was 8.50%. The oil was characterized were based on density, pH, saponification, acid value and iodine value. Elemental analysis of the oil was determined and antimicrobial activities of the oil and honey both singly and in combination were assessed using agar diffusion method and the sensitivity pattern of the bacteria (*E. coli*, *Klasiella pneumonia* and *Staphylococcus aureus*) assessed was varied found to be concentration dependent. Honey at 25% w/w, 50% w/w and 75% w/w concentration inhibited growth of tested organisms except *Staph. Aureus*, while the *G. kola* seed oil, was most active against *E. coli* and *Staph. Aureus* with zone of inhibition ranging from 14.16 to 2.13mm and 14.33 to 5.12mm respectively but less active against *K. pneumonea* with zone of inhibition in the range of 12.24 to 2.01mm. Creams were formulated using the extracted *G. kola* seed oil and honey alone and in combination then evaluated for some physicochemical parameters as: smear, emmollency, spreadability, homogeneity and pH. Combination of the *G. kola* seed oil and honey had synergistic effect and formed a better and stable cream than either of the ingredients used alone for the cream formulation.

**Keywords:** *G. kola* seed oil, honey, cream

#### Introduction

Medicinal plants have been identified and used throughout human history and have recently found its way as a drug source or lead in modern medicine since they are termed to act by stimulating and supplementing the body's healing forces <sup>[1]</sup>. Antimicrobial agents could be derived from these plants and might be essentially useful in reducing the spread of infectious diseases globally since they could act independently or synergistically to boost the efficacy of the antibiotics whose effectiveness is diminishing as resistant pathogens develop thus posing a serious threat to public health <sup>[2]</sup>.

These properties therefore, necessitates the sourcing for an alternative antimicrobial agent from plants and insect based materials such as in bitter kola seed oil and honey respectively. From origin, oils and fats as well as their hydrolytic products (glycerol and fatty acids) have been widely used as raw materials in food, cosmetics and pharmaceutical industries <sup>[3]</sup> Oils possessing anti-microbial activities are useful in treatment of wounds, formulations of anti-microbial creams and lotions for treatment of skin diseases as well as in food preservation.

#### *Garcinia kola*

This is a well branched evergreen and medium sized tree mostly about 12m high in 12 years and sometimes up to 28m height <sup>[4]</sup>. It is divided into different species depending on their geographical distribution and includes *Garcinia kola* Helkel, *G. multiflora* and *G. cambagia* <sup>[5]</sup> *Gracinia kola* Heckel popularly known as bitter kola is one of the useful indigenous tree in Nigeria and in West and Central Africa <sup>[6]</sup>. It is known as "Orogbo", "Namijin-giro" and "Akuilu" respectively among the Yorubas, the Hausas and the Ibo tribes of Nigeria and is popularly known and accepted mostly for its probable antitussive and suspected antimicrobial potential.

## Taxonomy



Fig 1: *Garcinia kola* Heckel

### Seeds of *Garcinia kola* Heckel

The seeds are used mainly in medicine and herbal formulations. Its therapeutic benefits is largely due to the presence of various secondary metabolites such as complex mixtures of phenolic compounds (flavonoids, bi flavonoids and tannin), xanthenes, benzophenones, triterpenes and taponoids [7, 8].

All parts of the *Garcinia kola* are used in traditional system of medicine for the treatment of various ailments. In human the leaves, seeds stems, fruits and roots have significant medicinal properties with the seed traditionally used as sialagogue to stimulate the flow of saliva, and also as an antidote for snake bite, a snake repellent and remedy for cough, throat infection, diarrhea and vomiting [9]. The leaf is used in ethno medicine for the treatment of tuberculosis and as remedy for typhoid fever [10].

## Honey



Fig 2: Honey comb

Two main types of honey termed the apiary and forest honey exist. The apiary type are produced by the honey bees *Apis carana indica* and *Apis mellifera* collected by modern extraction method and free from foreign materials.

In contrast, the forest honeys are produced by rock bees, *Apis dorsata* or from wild nest of *A. carana indica* in forests and collected by crude method of squeezing the comb [11].

The specified composition of the batch of honey depends largely on type of nectar (flower) available to the bee and other content excluding fructose, glucose, water but include antioxidant such as chrysin, ascorbic acid, catalyst and pino cembrin [12].

The physical properties of honey vary depending on water content, type of flora, temperature and proportion of the specific sugar contained. The melting point of crystallized honey is within the range of 40 to 50 °C depending on composition and the viscosity is greatly affected by both temperatures and water content however while honey is very viscous, it is rather low in surface tension [13, 14].

Honey contains electrolytes in the form of acid and minerals and therefore exhibits varying degrees of electrical conductivities dependent on the ash content value [14]. Considering the thermal characteristics, honey caramelizes if heated sufficiently to temperature of between 70 to 110 °C especially due to the fructose and acid content where the acid acts as a catalyst. Honey has been used for its medicinal properties since ancient times for the treatment of a wide variety of ailment including wounds dressing, ulcers, chronic rhino sinusitis [15].

### Pharmaceutical Creams

Creams are preparation usually for application to the skin although formulations for application to the mucous membrane such as the rectum or vagina are also used. Creams may be considered a pharmaceutical products as even cosmetic creams are based on technique developed by pharmacy and un- medicated creams are highly used in a variety of skin conditions (dermatoses). Creams are semi synthetic emulsions of oil and water. They are divided into two types: oil in water (o/w) and water in oil (w/o). Creams can be classified based on their functions as cleansing, foundation and massage cream with characteristic properties as in cold and vanishing creams. Creams are more comfortable and cosmetically acceptable as they are less greasy and more easily washed off using water. Pharmaceutical creams help to exert such effects as: provision of barrier (physical or chemical) to protect the skin as with sunscreen, aid in retention of moisture (especially w/o creams, has cleansing and emollient effect, acts as a vehicle for drug administration such as local anesthetics, anti -inflammatory (NSAID), hormones, antibiotics, antifungals or counter irritant [16].

The aim of this study is to extract oil from *G.kola* seed and evaluate the activity and efficacy of the oil and honey on skin cream formulation.

### Material and methods

Materials: n-hexane (Gusang dong Sci tech co ltd, China), alcoholic KOH solution, 0.5mHCl, 0.1m NaoH, di ethyl ether, Wiji's solution, starch, potassium iodide (KI), 0.1M thio sulphite solution, Muller winton agar (Titan biotech ltd, India), peptone water, distilled water, cultures of *Staph aureus*, *Escherchia coli*, *klabsiella pneumonia* (Dept. of med. Microbiology university of Port Harcourt teaching hospital (UPTH) Nigeria, Fresh seeds of *G.kola* purchased at Choba market Port Harcourt, Rivers state, Nigeria. Fresh honey purchased from the local market in Choba, Port Harcourt. Nigeria.

**Methods**

**Preparation of *G. kola* seeds**

The seeds were peeled manually to remove the brown coat then adequately dried in an oven at 40 to 50 °C and milled to powder, packaged in a sterile bottle, sealed and stored at ambient temperature.

**Oil extraction**

A 1.2g of the seeds powder was subjected to extraction by cold maceration using about 2.5 liters of n-hexane. The solvent was evaporated using a vacuum evaporator at 40 °C. The recovered oil was purified by filtration through a glass wool with dark brown sediments discarded while the pure oil was dried at 40 °C in a vacuum oven for 24hrs then introduced into a glass container sealed and stored under a refrigerated environment for subsequent use.

Percentage pure oil content was determined using the relation.

$$\text{Weight of powdered } G. kola \text{ seed} = \frac{\text{Weight equivalent of } G. kola \text{ oil} \times 100}{\text{Weight of powdered } G. kola \text{ seed}} \dots (1)$$

**Oil content analysis**

**Relative density:** A clean dried 25ml capacity density bottle was tared and 1.5ml of *G.kola* seed oil was introduced into it and weight of the oil was recorded. The bottle was emptied, washed, dried and cooled in a desiccator, and then 1.5ml of water was substituted for the oil.

The weight was recorded, and the density of the oil was determined using the relationship

$$\text{Density} = \frac{\text{Mass of oil}}{\text{Mass of equal volume of water}} \dots (2)$$

**Iodine value**

A 0.1g of oil was introduced into a conical flask and 10ml of carbon tetra chloride was added to dissolve it. 20ml of Wiji's solution was added and stoppered and moist potassium iodide solution was used to seal the flask.

The setup was allowed to stand in the dark for 1 hour, 15ml of 10% potassium iodide solution was added to the mixture followed by 100ml of water. The mixture was titrated with 0.1M thio sulphate solution using starch as indicator. The blank titration was carried out with 10ml carbon tetrachloride and Iodine value calculated by the expression.

$$\text{Iodine value} = \frac{1.269C \times V_1 - V_2}{C = \text{Conc. of Na thioSO}_4 \text{ used for the blank}} \dots (3)$$

- M  $V_1$  = vol. of Na thioSO<sub>4</sub> used for blank
- $V_2$  = vol. of Na thioSO<sub>4</sub> used by sample
- M = mass of sample

**pH determination of the oil:** A clean dried test tube was used to measure out 2.0g equivalent of the *G-kola* seed oil. Then 13.0ml of hot distilled water was added into the tube with continuous stirring in a water bath at 25 °C. pH determination of the mixture was carried out using a pH meter and in triplicate.

**Acid value:** A fat solvent prepared by mixing equal volume of diethyl ether and ethanol 90% w/v in a beaker was made available.

A 0.1g equivalent of the extracted oil accurately weighed was introduced into a conical flask and 50ml of the fat solvent was used to dissolve it. About 3 drops of phenolphthalein as indicator was added and the mixture titrated with 0.1M NaOH with constant shaking until end point was reached and the value calculated and recorded using the formula

$$\text{Acid value} = \frac{\text{Titre volume (ml)} \times 5.61}{\text{Weight of sample (oil) used}} \dots (4)$$

The free fatty acid (FFA) value was determined as

$$\frac{\text{Acid value}}{2} \dots (5)$$

**Saponification value:** A 0.1g equivalent of the extracted oil was introduced into a conical flask and approximately about 25ml of alcohol KOH solution (0.5mol/l) was introduced into the flask. A reflux condenser was attached to the flask which was heated for 1 hour in a water bath with intermittent shaking. The flask was allowed to cool to room temperature then 1ml of 1% phenolphthalein was added.

0.5mol/l HCl was used to titrate against the mixture (a ml), the same was repeated for the blank (carbon tetra chloride) (b ml).

Triplicate observation (readings were made, recorded and calculations made using the formulae

$$\text{Saponification value} = \frac{(b-a) \text{ ml} \times 28.05}{\text{Weight (g) of sample (oil)}} \dots (6)$$

**Elemental analysis**

The oil sample was ashed in a muffle furnace at 630 °C for 3hours. The ash sample was dissolved in 10ml Conc. HCl and heated on an electro-thermal heater/hot plate. The solution was diluted to 50ml with distilled water and analyzed for metal ions (potassium (k), calcium (ca), sodium (Na), iron (Fe), magnesium (mg)) at different wavelength by atomic absorption spectrophotometer.

**Anti-microbial identification of *Garcinia kola***

**Preparation of culture media:** The Mueller Hinton agar media was prepared following manufacturer's directives and guides.

The prepared agar was distributed into 10 of 20ml universal bottles and sterilized using an autoclave at 121 °C for 15minutes.

**Preparation of standard solution:** A 0.5ml of gentamycin solution (80mg/2ml) was measured into a volumetric flask, diluted to obtain 20mg/ml using sterile distilled water. 5ml of the resulting solution was measured and further diluted to 100ml with same solvent to obtain a 10µg/ml solution.

**Sensitivity test determination of *G. kola* seed oil:** A 1.0ml of the oil was used as 100% undiluted stock. Serial dilutions of the stock solution were made to obtain the concentrations of 75, 50, and 25% w/v respectively.

### Identification of pure/original honey

The sample (honey) was identified and confirmed to be honey by the following methods.

A match stick was dipped into the honey and struck with the packet to observe for ignition.

About 5ml volume of the sample was dropped onto the sand and observed for immediate sinking

A finger was dipped into the honey and the honey caused to drop on the floor. Observation was made to see whether the drop will be like a thread or it will break.

About 5ml quantity of the honey was introduced into a cup of water and observed for its sinking to the bottom without mixing with the water except by agitation.

### Preparation of the honey for sensitivity test:

A 0.1g equivalent weight of the honey was used as 100% undiluted stock. Serial dilutions of the solution was made with

freshly prepared distilled water to obtain concentrations of 75, 50 and 25% w/v respectively

### Anti-microbial activities of *G. kola* seed oil and honey used separately and in combination.

The zone of inhibition was measured by the agar-well diffusion method. Sterilized Mueller-Hilton agar, inoculated with the test bacteria was antiseptically poured into the petri-dishes and allowed to set on the bench for 30minutes. Sterile cork borer was used to bore 10mm wells (holes) in the agar. Each of the holes in the petri-dish was inoculated with *G. kola* seed oil and honey solutions used alone and in combination of (1:1) and incubated for 24hours at 37 °C. Standard antibiotic (gentamycin) solution was used as positive control and water as negative control. The zones of inhibition was measured and recorded in millimeters to indicate the degree of sensitivity.

**Table 1:** Working formula for cream formulation

Ingredient	Quantity (g)		
	F1	F2	F3
Stearic acid	8.0	8.0	8.0
Potassium hydroxide	0.2	0.2	0.2
Sodium carbonate	0.2	0.2	0.2
<i>Garcinia kola</i> seed oil	-	2.0	2.0
Glycerin	6.0	6.0	6.0
Honey	2.0	-	2.0
Propyl paraben	0.02	0.02	0.02
Methyl paraben	0.02	0.02	0.02
Water qs	50ml	50ml	50ml

F1= formulation with honey, F2=formulation with *G. kola* seed oil, F3= formulation with honey and *G. kola* seed oil

The *G. kola* seed oil was dissolved in the oil phase (stearic acid) and heated to 70 °C. The preservatives and other water soluble components and honey were dissolved in the aqueous phase and then heated to 70 °C. After heating the aqueous phase was added in aliquot to the oil phase with continuous stirring until emulsion was formed.

The cream was then removed from the heat and cooled with continuous homogenization upon addition of baline perfume then transferred to suitable container. The formed cream was then evaluated for various physical parameters such as appearance, emollient, dye test, homogeneity, type of smear, removal and irritation tests.

### Evaluation of creams

**Appearance-** the appearance of the cream was ascertained by its colour, pearlsence, roughness and grade.

**pH:** The pH meter was calibrated using the standard buffer solution. About 0.5g of the cream was weighed dissolved in 50ml of distilled water and the pH determined in triplicate.

**Emollient:** Emolliency, slipperiness and amount of cream was checked.

**Dye test:** Scarlet red dye was mixed with the creams. A drop of the cream was placed on a microscopic slide and covered with a cover slip then examined under a microscope. Observations for the appearance of the dispersed globules and the background were made.

**Homogeneity:** The formed creams were tested for the homogeneity by visual appearance and feel.

**Type of smear:** After application of the cream, the type of film or smear formed on the skin was checked.

**Removal:** The ease of removal of the cream applied was examined by washing the parts applied with running water.

**Irritation test:** An area was marked (1sq. cm) on the left hand side or the dorsal surface of study laboratory animal (rat of wistar strain) after shaving part of the hair. The rats were divided into 4 groups of 4 rats per group and numbered A to D.

Group A, served as the control while the others of the Group B, C and D were administered with cream B for F1, C for F2 and D for F3.

The cream was applied twice daily at the specified area and time applied was noted. Irritancy, erythema and edema were checked at regular intervals of 3 hours per day up to 14 days.

### Results

The yield of the extracted *G. kola* oil from powdered seed is 8.5% w/v

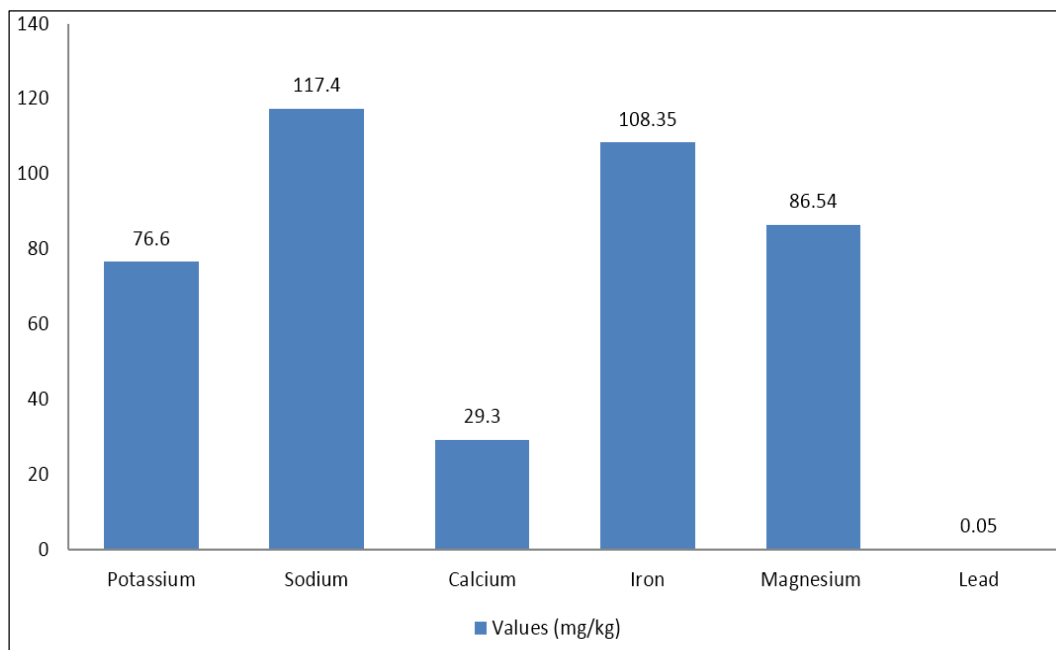
**Table 2:** Physicochemical properties of extracted *G. kola* seed oil

Properties	Value obtained
Specific density g/ml	0.924
Acid value	28.05 ± 0.01
Saponification value	168.30 ± 0.4
Free acid value	14.025 ± 0.01
<b>Iodine value</b>	
Using wiji's soluton	75.60 ± 0.02
Mean triplicate pH	6.0 ± 0.09
Stable color	Reddish-brown

**Table 3:** Susceptibility testing by zone of inhibition (mm) using gentamycin (antibiotic) and water

Test organism	Positive control gentamycin (10µg/ml)	Negative control water
<i>E. coli</i>	36.00mm	0.00
<i>K. pneumoniae</i>	33.00mm	0.00
<i>S. aureus</i>	36.00mm	0.00

To show the presence of trace elements and ions contained in garcinia kola seed oil a plot of elements and the amount contained is made as in fig 3 below



**Fig 3:** Elemental analysis of *Garcinia kola* seed oil

**Table 4:** Anti-microbial activity (zone of inhibition (mm) of *G. kola* seeds oil

Test organisms	Concentration (mg)			
	25% w/v	50% w/v	75% w/v	100% w/v
<i>E. coli.</i>	2.13	4.22	8.91	14.16
<i>K. pneumoniae</i>	0.00	2.01	5.32	12.24
<i>S. aureus</i>	0.00	5.12	10.83	14.33

**Table 5:** Anti -microbial activity (zone of inhibition (mm) of honey)

Test organism	Concentration			
	25% w/v	50% w/v	75% w/v	100% w/v
<i>E. coli.</i>	3.12	8.00	16.00	19.00
<i>K. pneumoniae</i>	2.33	4.18	9.23	15.00
<i>S. aureaus</i>	0.00	0.00	0.00	8.00

**Table 6:** Anti-microbial activity (zone of inhibition (mm) of *G. kola* seed oil and honey

Test organism	Concentration			
	25% w/v	50% w/v	75% w/v	100% w/v
<i>E. coli.</i>	8.00	15.03	20.33	28.87
<i>K. pneumoniae</i>	5.36	10.11	18.53	21.13
<i>S. aureaus</i>	0.00	8.13	12.94	17.00

**Table 7:** Result of the nature of formulated cream and its application

Physical parameters	Observations		
	F1	F2	F3
Appearance	White colour	Cream Colour	Cream colour
pH	6.2	6.4	6.8
Homogeneity	Homogeneous smooth and consistent	Homogeneous smooth and consistent	Homogeneous smooth and consistent
Dye test	Dispersed globules red and background colorless o/w cream formed	Dispersed globules red and background colorless o/w cream formed	Dispersed globules red and background colorless o/w cream formed
Removal	Easily removed	Easily removed	Easily removed
Types of smear	Non greasy	Non greasy	Non greasy
Emollient	No residue left	No residue left	No residue left
Irritancy test	No reaction or induration observed	No reaction or induration observed	No reaction or induration observed

F1= formulation with honey F2= formulation with *G. kola* oil F3= formulation with honey and *G. kola* oil.

**Discussion**

The yield of oil obtained from garcinia kola seed was 8.50% and this is low compared to some vegetable oil, such as: olive oil hence the oil might not be economically viable to be

utilized in diverse pharmaceutical and cosmetic formulations. The extracted *G. kola* seed oil was subjected to physico chemical characterizations as summarized in Table 1. From the analysis the Iodine value which determines the degree of



unsaturation of fats and oils that is presence of double bond which could readily cause addition of iodine to themselves<sup>[17]</sup>, has a value of 75.60 Wiji's and according to the reference standard the maximum acceptable level of iodine value is 80-100g Iodine/100g of sample and this indicates that the oil have low degree of unsaturation and could be classified as a saturated and non-drying oil. Acid value is the mass of alkaline (potassium hydroxide (KOH) in mg that is required to neutralize 1g of acidic substance. The free acid present in an oil or fat impacts sharp unpleasant flavor to edible oil, and the acid value obtained was 28.05mg/KOH/g which gives an indication that it is edible although this is dependent on the process mechanisms which the oil is subjected to. Saponification value is an indication of number of mg of alkali (KOH or NaOH) required to saponify 1g of fat and it is a measure of the average mol. Weight or chain length of all the fatty acids present. The saponification values are highly significant in making of soaps and the values obtained from *G. kola* seed oil was 168.3mg/KOH/g. This result falls within the range of oil with strong cleansing ability and the value obtained was in an intermediate range between very high and low value because very high value depicts content of high alkali and may lead to reaction with the skin while in low saponification value fatty acid salts may not be sufficient enough to remove fats or oils. The pH of the *G. kola* seed oil was 6.0 this indicates that the oil is slightly acidic inferring good nutritional quality. The seed oils could be a good source of some nutritional /medicinal valuable elemental component such as iron, magnesium, potassium, sodium and calcium as shown in Fig.3 but no composition of deleterious element such as lead or arsenic was reported. From tables 4, 5 and 6, the result of the study reveal that *G. kola* seed oil and honey possess anti-microbial activity (a measure of the diameter/zone of inhibition of species growth in mm) at different oil/honey concentration. The data obtained showed that the inhibitory effect of the samples from the test organisms was dose and species dependent and this is in agreement with findings of<sup>[18, 19]</sup>. [Honey at concentrations of 25%, 50% and 75% w/w, inhibited the growth of tested micro-organisms except *Saphylococcus aureus*. The anti- microbial activity of honey may be due to the presence of some components such as sugar acting in a number of varied mechanisms and influenced by some factors as: low pH, peroxide content, protein component and other un identified materials that could be present<sup>[20]</sup>. The *G. Kola* seed oil was most active against *E. coli* and *S. aureus* with a zone of inhibition ranging from 14.66 to 2.13mm and 14.3 to 5.12mm respectively but less active against *K. pneumoniae* with zone of inhibition ranging from 12.2 to 2.01mm. The anti-microbial and possible anti-viral properties of the *G. ola* seed oil may be attributed to the presence of benzophenone and flavone<sup>[21]</sup>. The mixture of honey and *G. kola* seed oil produces higher zone of inhibition against test organism than either *G. kola* seed oil or honey used alone as shown in table 6 hence combination of *G. kola* seed oil and honey imparts synergistic activity against the growth of some microorganisms. Compared with standard drugs such as gentamicyn, as shown in table 3, the overall anti-microbial activity of honey and *G. Kola* seed oil is lower and this is so probably because gentamycin is used as a pure synthetic substance as compared to the crude oil and honey which might contain minor substances dissolved as secondary metabolites. The prepared creams using *G. Kola* seed oil or honey and combination of both, was found to be of the o/w

emulsion type. This attributes the cream to be easily washable and hence of better acceptance to patients or other potential end users. The creams were found to be of the pH range of 6.2 to 6.9, homogenous and stable over a long period, emollient but non-greasy and easily washed after application. The cream upon application also showed no redness, edema, inflammation and irritation to animal skin used for skin reaction test hence the formulated creams using the extracted *G. Kola* seed oil and honey used alone and in combination is adjudged to be safe and free from irritation or allergic sensitization to the skin.

### Conclusion

The *G. kola* seed oil extracted though had low yield was found to be free from deleterious materials such as lead and arsenic but rich in some elements useful in human physiological activities.

The extracted *G. kola* seed oil was observed to exert a dose dependent anti-microbial activity against such organisms as: *E coli* and *Staph. Aureus* but less active against *K. pneumonea* while honey is less active against *S. aureus* but active against *E. coli* and *K. pneumonea*. This anti- microbial activity was enhanced by synergistic effect of combined *G. kola* seed oil and honey though less active when compared with synthetic drug (gentamicin), due to the unpurified nature of the extracted seed oil and honey. Considering the outcome of this result therefore, effort should be geared towards the purification and elaboration of the activities of the naturally extracted components to enhance their medicinal activities as this will help to reduce the cost and effect (both in human and material) of synthetic anti- microbial agents especially in cream/cosmetic formulation and applications.

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