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Full Length Research

Comparative studies of physico-chemical composition and antibacterial activities of essential oil extracted from medicinal plants of scent leaves (O*cimum basilicum* Lamiaceae *and Ocimum gratissimum* Lamiaceae)

Aladekoyi, Gbenga* and Orungbemi O. O.

Food science & Technology Department, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria.

*Corresponding author. Email: gbengu7@yahoo.com. Tel: +2348034651804, +2348036267170

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ABSTRACT: A comparative study was conducted on essential oil extracted from sweet basil (*Ocimum basilicum* L.) and Africa basil (*Ocimum gratissimum* L.) using standard methods (AOAC, 2000, Pelczer and Black, 1993), for their physico-chemical composition and antibacterial activities. The comparison between the two scent leaves was necessary to indicate the one with high potency in terms of their physical, chemical and antibacterial activities for Industrial and medicinal purposes. The experimental result obtained from essential oil extracted from *Ocimum* basilicum L. and *Ocimum gratissimum* L. revealed some physiological properties like a viscosity of 1.342 ± 0.01 and 1.299 ± 0.01, Specific gravity 0.9026 ± 0.02 and 0.9032 ± 0.02, Refractive index 1.672 ± 0.20 and 1.660 ± 0.10 respectively, and the chemical compounds of Saponification(mg/g) 19.074 ± 0.10 and 13.464 ± 0.20, Iodine(g/100g) 34.7706 ± 0.30 and 35.2782 ± 0.30, Free fatty acid(oleic)(g/100g) 0.949 ± 0.10 and 1.267 ± 0.10, Peroxide value(mg/g) 0.923 ± 0.01 and 0.945 ± 0.02 and Acid value(g/100g) 3.366 ± 0.10 and 4.488 ± 0.10 for O*cimum basilicum* L. and *Ocimum gratissimum* L. respectively. The antibacterial activities revealed that the zone of inhibition between the pathogenic organism under test for O*cimum basilicum* L. and O*cimum gratissimum* L. were; *Staphylococcus spp.* 3.00 ± 0.01 mm and 5.00 ± 0.01 mm, *pseudomonas spp.* revealed no zone for both essential oil and E*. coli spp.* had 3.00 ± 0.01 mm and 4.00 ± 0.02 mm respectively. *Klebsiella spp.* had 4.00 ± 0.02 mm and 4.00 ± 0.02 mm, Proteus also indicated no zone for both oil samples. An observable trend was that *Pseudomonas* and *Proteus spp.* showed resistance to both oil samples while *Staphylococcus spp., E. coli spp., Klebsiella spp.* were susceptible with the highest of value of 5.00 ± 0.01 mm Staphylococcus *spp.* essential oil from *Ocimum gratissimum* L.

Keywords: Physic-chemical, antibacterial activity, essential oil, pathogenic bacteria.

INTRODUCTION

The genus of *Ocimum* belonging to family of Lamiaceae is widely distributed in tropical and warm temperate regions of the world wide (Wangner et al., 1999). It is usually named as sweet basil and is an annual plant, with extraordinary medicinal properties and contains several antioxidant compounds. In traditional medicine, *Ocimum basilicum* has been used as an antiseptic, preservative, sedative, digestive regulator and diuretic (Effraim et al., 2003). It has also been recommended for the treatment of headaches, cough, infections of upper respiratory tract, kidney malfunction and to eliminate toxins. Both Ocimum oil and its extracts were shown to exhibit antibacterial activities against gram positive and gram negative bacteria by various experimental researches.

Ocimum basilicum L. commonly called as sweet Basil is a native plant of Indo-Malayan region. It is called the *king of herb* which contains plenty of phytochemicals with significant nutritional value as well as antioxidant capabili-

ties and health benefits. Sweet Basil is cultivated for production of essential oils, dry leaves as a culinary herb, condiment/spice or as an ornamental plant. It is used as an ingredient in various dishes and food preparations, especially in the Mediterranean cuisine. Leaves and flowering parts of *O. basilicum* are traditionally used as antispasmodic, aromatic, carminative, digestive, stomachic, and tonic agents. Due to its insecticidal activity and very pleasant aroma, basil essential oil is widely used in the food, pharmaceutical, cosmetic and aroma-therapy in industries. In addition, now-a-days public prefers natural food additives, hence naturally derived antimicrobial and antioxiadative agents from basil are gaining popularity.

*Ocimum gratissinum (*scent leaf) is one of the plants of interest in the quest for solving the above mentioned problems militating against the animal production industry in Nigeria (Effraim et al., 2003). It is a perennial plant, woody at the base and widely distributed in the tropics of Africa and Asia. It has average height of 1 to 3 meters. The leaves are broad and narrowly ovate usually 5 to 13 cm long and 3 to 9 cm wide. It is a scent shrub with lime-green fuzzy leaves (Wangner et al., 1999). In Nigeria, the plant is called *Effinrin* by the Yoruba speaking tribe*, Nchonwu* in Igbo, while in the northern part of Nigeria, the Hausas called it *Daidoya* (Effraim et al., 2003).

Studies on the effects of aqueous (water) extracts and carbon tetrachloride (CCl_4) of *O. gratissimum* leaves induced liver damage in albino Wistar rats (Nwinyi et al., 2009). Antibacterial effects of *O. gratissimum* extracts on *Eschericha Coli (E. coli) and staphylococcus aureus (S. aureus)* have been reported (Nwinyi et al., 2009). In an experiment by Chikwendu et al. (1999), the aqueous extract of the leaves of *O. gratissimum* inhibited castor oil induced diarrhea in rats, extracts inhibited the propulsive movement of intestinal contents on the isolated ileum of guinea pig. The benefits of *O. gratissimum* have been used in both human and animal sciences. Other reported benefits include antioxidant properties (Bunrathep et al., 2011), antilesishmanial activity (Tania et al., 2006) and possession of novel cancer, fighting compounds that need to be isolated, purified and characterized (Bunrathep et al., 2011). The plant is also used for the treatment of rheumatism, paralysis, epilepsy, high fever, diarrhea, sunstroke and mental illness (Oliver, 1980). Basil has long been used as an embalming and preserving herb, found in mummies of ancient Egypt. Basil was used also as symbol of mourning in Greece where it was being known as basilikon photon, meaning magnificent, royal or kingly herb. Ancient records from 907AD indicate sweet basil in the Hunan region of china. Many beliefs and rituals are accompanied with basil. In Italy it is a symbol of love, In France it is named as an herb of royalty. Jewish people used it to get strength during fasting while an African legend claims that basil protects against scorpions. However, a European group has considered it to be a symbol of satan (Marwat et al., 2011).

The plant has been used in many parts of the world to treat a wide variety of disorders. Basil is a popular herb in US and Mediterranean diets (Lee and Scagael, 2009). *O. basilicum*, sweet basil is used in Yemeni traditional medicine to treat various ailments: abdominal cramps, gastroenteritis, dysentery, and diarrhea. In northern Oman and Saudi Arabia, juice of leaves or crushed leaves is used in the treatment of wound and acne. It is used also as a deodorant, it is considered to be an aphrodisiac, worn by men when visiting their women (Ghahazanfar, 1994).

In the coastal areas of Nigeria*, O. gratissimum* is used in the treatment of epilepsy, high fever and diarrhea (Effraim et al., 2003). *O. gratissimum* is used by the Ibos of Southeastern Nigeria in umbilical cord management, to keep wound surfaces sterile, as well as the treatment of fungal infections, fever, cold and catarrh (Ijeh et al., 2005).

Essential Oil

The essential oils are compounds made up of several organic volatile substances like alcohols, acetones, ethers, aldehydes, and were produced and stored in the secretion canals of plants. At room temperature they are usually liquid. Given their volatility, they can be extracted using steam distillation, though other methods exist. On the whole, they are responsible for the aromas of plants.

Essential oils are widely distributed in nature and are found in conifers (pine, fir), myrtaceae (eucalyptus), rosacea (*Citrus spp*), compounds (chamomile), although the majority of plants with essential oils are found in the labiate (mint, lavender, thyme, rosemary) and umbelliferous (aniseed) families. They are found in different organs: roots, rhizomes (ginger), wood (camphor), leaf (eucalyptus), flowering parts (Lamiaceae family). Composition depends on place of origin. The habitat where the plant grows (normally warm climates have more essential oils), the moment of harvesting, extraction methods, etc. are also important.

Among the main therapeutic properties of essential oils antiseptics stands out (for many years these spices have been added to foodstuffs not just for flavouring but to help preserve them). Other properties are: antispasmodic, expectorant, carminative and eupeptic. There is need to note that certain essential oils, especially in high doses, may be toxic to the central nervous system in particular. Others, such as rue or juniper have abortive properties. Others may cause skin problems, rashes or allergies. In addition to having therapeutic properties, essential oils are widely used in the pharmaceutical, food, and perfume (especially) industries (Grayer et al., 1996).

Physical properties of essential oils

Essential oils are volatile and become liquid at room

temperature. When distilled, they are at first colourless or slightly yellowish. They are less dense than water (sassafras essence and clove essence being exceptions). They are nearly always rotational and have a high refractory index. They are soluble in alcohol and in the usual organic solvents, such as ether or chloroform, and also in high grade alcohol and they are lipo-soluble and not very soluble in water, but can be dragged using steam (Onajobi, 1986).

Chemical properties of essential oils (terpenoids)

Essential oil components are divided into terpenoids and non-terpenoids (Hussain, 2009).

Uses of essential oils

Food industry

They are used to season or condiment meats, dried and cured meats, soups, ice-cream, cheese. The most commonly used essential oils are cilantrum, orange, and mint. They are also used in the elaboration of alcoholic and soft drinks, especially the latter.

Pharmaceutical industry

They are used in toothpastes (mint and fennel essences), analgesics, and decongestant inhalers (eucalyptus). Eucalyptol is also widely used in dentistry. They are used in many medicines to neutralize unpleasant tastes (essence of orange or mint, for example).

Cosmetic industry

This industry uses essential oils to make cosmetics, soaps, scents, perfumes, and make-up. The most commonly used essences are geranium, lavender, roses and patchouli.

Aims and Objectives

Aims of this research is to analyze the essential Oils from African basil (*O. basilicum) and* Sweet basil (*O.gratissimum)* and compare their Chemical values such as Acid Value, Iodine Value, Saponification Value and Peroxide Value by steam distillation method. The Anti-microbial activity of the two Essential Oils were carried out to know their efficacy against different Organisms like *Escherichia coli, Staphylococcus aureus*, *Bacillus cereus*, *Salmonella tophi,* and *Klebsiella pneumonia.* Though, many work has been done on the extracts (combination of essential oil and chemical component) using aqua and some organic solvents on scent leaves, there is a paucity report on the differences in the potency of the essential oil of the two closely related plants (*O. basilicum and O. gratissimum)* by steam distillation method. This provides pure form of essential oil without some toxic chemicals for analysis. The aromas are the same, but their sensational effect on tongue when chewed revealed their differences in physical, chemical and antibacterial properties when fully compared. The objectives of this research are to indicate wish of the essential Oil from African basil (*O. basilicum)* and Sweet Basil (*O. gratissimum)* has good Values for their chemical characteristics for industrial purposes, to examines their Antimicrobial activities against the pathogenic organisms under studies for their curative properties and to ascertain the best out of the two scent leaves for human consumption.

MATERIALS AND METHODS

Collection of plant samples

Sweet basil (*O. basilicum)* and Africa basil (*O. gratissimum)* plant samples were bought from “Oja Oba” market in Owo town, Owo local government area, in Ondo state.

Preparation of the sample

The scent leaves from Sweet basil (*O. basilicum)* and Africa basil (*O. gratissimum)* bought fresh and the leaves were manually removed from the stem. Also, the brown old leaves were removed and the seeds separated manually. The particles were hand picked off in order to have clean leaves. The leaves were milled manually and stored at room temperature in a clean Jar for extraction process.

Extraction Procedure

The extraction was done by Steam Distillation method. About 100 g of the grounded fresh sample was Steam Distillated for 4 hr. This process was repeated five times until a required quantity of the oil has been collected. The oil was stored below room temperature (-4^0C) in a refrigerator until it is required for analysis.

Source of Microorganism

Escherichia coli. *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhii* and *Klebsiella pneumonia* were collected from Department of Microbiology, Federal

Table 1: Antibacterial activities of essential oil from sweet basil (Ocimum *basilicum*) and Africa basil (*Ocimum gratissium*)

ORGANISM	ZONE OF INIHIBITION (mm)	
	Sweet basil	Africa basil
Escherichia Coli	3.00 ± 0.01	4.00 ± 0.02
Pseudomonas spp	no zone	no zone
Staphylococcus aureus	3.00 ± 0.01	5.00 ± 0.01
Proteus spp	no zone	no zone
Klebsiella spp	4.00 ± 0.02	4.00 ± 0.02

± mean of triplicate results.

Medical Centre Owo, Ondo state of Nigeria.

Sterilization of glass ware

All the glassware used for this study such as Petri dishes, Agar bottle, test tube, conical flask, beakers, pipette and forceps were soaked with detergent and rinsed with water. They were sterilized using hot air oven at a temperature of 120^{o}C for 2 hr. The wire loop was sterilized by heating it in the blue flame of the Bunsen burner until red hot and allowed to cool before use. 95% alcohol was used to swab the work bench area to prevent contamination. The process was carried out aseptically.

Media and Reagent

Nutrient Agar (N. A) and Nutrient broth used were prepared according to manufacturer's instructions and autoclaved at 121^{o}C for 15 min.

Antimicrobial Screening Test and Physical Characteristics of Sweet Basil (*O.basilicum)* and Africa basil (*O.gratissimum)* Essential oils

The extracts were tested for their antibacteria properties using the agar – well technique (Pelczer and Black, 1993). The assay for antibacteria activities was carried out with *E. coli, Staphylococcus aureus, Bacillus cereus, Salmonella typhii, and Klebsiella pneumonia*. Triplicate plates of media for each organism were inoculated with the appropriate suspension of bacteria. Agar well was aseptically made in the media with a sterile 6.0 mm diameter cork borer. The different concentrations of the test solutions of extracts were dispensed (0.5 ml) aseptically into the wells. The plates were kept in sterilized inoculation chambers for 2 hr to facilitate diffusion of the solutions. The plates were then inoculated at 37^{o}C for 24 hr for the bacteria. The diameters of the zones of inhibitions of bacteria growth were measured in the plates and the mean value and standard error for each organism was recorded.

Characterization of the Essential Extracted Oil

Standard method was used to evaluate the quality of the extracted essential oil, saponification values, acid value, iodine value, free fatty acid value, peroxide value AOAC (2000).

RESULTS AND DISCUSSION

The comparative results of antibacterial activities of essential oil from sweet basil (*Ocimum basilicum*) and Africa basil (*Ocimum gratissimum*) were presented in Table 1. The Values showed that they have susceptibility effect on some pathogenic micro-organisms. The diameter of inhibition zones on *E. coli* was 3.00 ± 0.0 mm in sweet basil as against 4.00 ± 0.01 mm in Africa basil. The oil showed antibacterial effect on *E. coli* in *Ocimum gratissi* and O*cimum basliumum* respectively, but these values were lower than the values obtained by Udochukwu et al. (2015) for ethanolic and aqueous extracts (7.5 mm for both) for *Ocimum gratissimum.* They were higher than aqueous extract at 20 mg/l concentration (Nill) and lower than ethanolic extract at 5 mg/l concentration (18.0 mm) as recorded by Ladipo et al. (2010) for *Ocimum gratissimum* respectively. The increase observed in ethanolic extracts was due to toxic chemical components (phytochemicals) in the leaf extracted with the essential oil as predicted in this study. *Staphylococcus aureus* was 3.00 ± 0.01 mm in Sweet basil as against 5.00 ± 0.01 mm in Africa basil. The oil showed antibacterial effect on *Staphylococcus aureus* in *Ocimum gratissi* and O*cimum basliumum* respectively, but lower than the values obtained by Udochukwu et al. (2015) for ethanolic and aqueous extracts (10.0 mm for both) for *Ocimum gratissimum.* But when compared with each other, the results revealed that pure essential oil from *Ocimum gratissimum* has higher susceptibility on both *E. coli* and *Staphylococcus aureus* than *Ocimum basilicum*. The diameter of inhibition of *Klebisella* was 4.00 ± 0.02 mm for both Sweet basil and Africa basil, that is, equal susceptibility. However, this was lower than aqueous extract at 20 mg/l concentration (9.0 mm) and higher than ethanolic extract at 5 mg/l concentration (Nill)

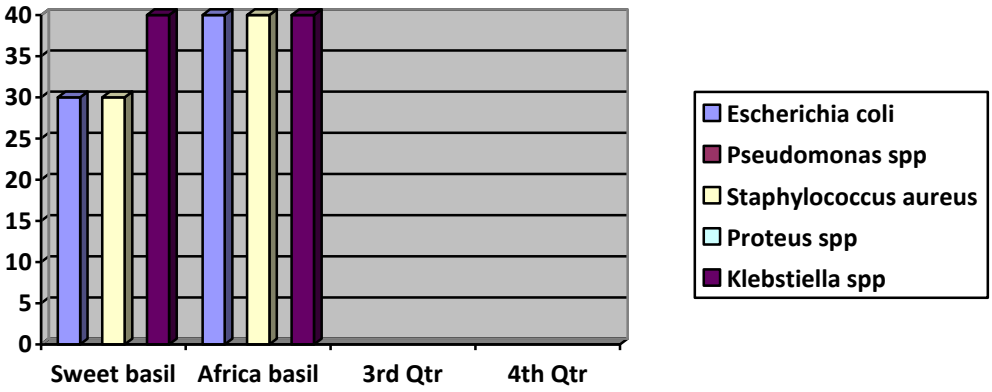

Figure 1. Bar Chart for The Antibacterial Activity of Essential Oil from Sweet basil and Africa basil**.**

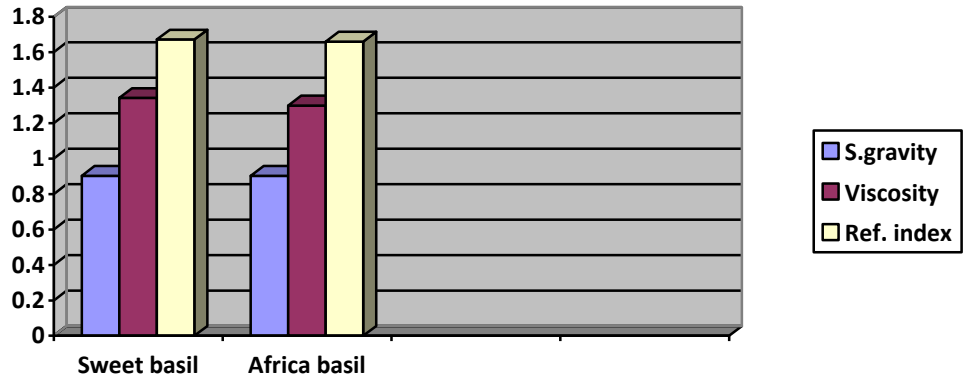

Figure 2. Bar Cart for The Physical Properties of Essential Oil from Sweet basil and Africa basil.

as recorded by Ladipo et al. (2010) for *Ocimum gratissimum* respectively. There were no inhibition zones in *pseudomonas spp.* and *proteus spp.* in O*cimum basilicum* and O*cimum gratissium* respectively. The susceptibility of O*cimum gratissium* to *pseudomonas spp.* as reported by Udochukwu et al. (2015) for ethanolic and aqueous extracts (9.0 mm and 7.5 mm) may not be due to essential oil present but the phytochemicals accompanied it during extraction. They possessed the same susceptibility effect against *klebsiela spp.* There was no effect on *pseudomonas spp* and *proteus spp*. Figure 1 showed the strength of each bacterial compared to one another, Ocimum *basilicum* and *Ocimum gratissium* have the same strength (4.00) against *Klebsiella spp* with maximum peak and *Escherichia Coli (*4.00*)* for *Ocimum gratissium*. The values of the physical parameters from Figure 2, showed no significant difference from each other as observed.

Table 2 showed the results of physic-chemical constituents of both O*cimum basilicum* and O*cimum gratissimum*. The specific gravity of the Sweet basil and Africa basil were 0.9026 ± 0.02 and 0.9032 ± 0.02 respectively, these were in the same range or no significant different. The results of viscosity of Sweet basil and Africa basil were 1.342 ± 0.01 and 1.299 ± 0.01 respectively. It showed that the flow rate of Africa basil is higher than that of Sweet basil. The flow rate depicts lightness and volatile nature of Africa basil over Sweet basil. The result of Refractive index showed that sweet basil had the highest value of 1.672 ± 0.20 to 1.660 ± 0.20 for Africa basil. These values were higher than the refractive index of confectionary oils (1.40 to 1.50) and pure water (1.333). The chemical values also showed the result of Iodine value for Africa basil (35.2782 ± 0.30 g/100g) as the highest value against Sweet basil (34.7706 ± 0.020 g/100g). This revealed the level of saturation in Africa basil than sweet basil. The result of Acidic value of both sweet basil and Africa basil were 3.366 ± 0.10 and 4.488±0.10 in g/100g respectively. The Acid value of Africa basil is higher than that of Sweet basil, which is an indication of level of rancidity in African basil. The free fatty Acid of both Africa basil and Sweet basil were 0.949 ± 0.10 and 1.267 ± 0.010 in g/100g respectively. The result of the Africa basil is higher than

Table 2. Physicochemical of essential oil from sweet basil (ocimum basilicum) and Africa basil (ocimum gratissium)

Parameters	Sweet basil	Africa basil
Specific gravity	0.9026±0.02	0.9032±0.02
Viscosity	1.342±0.01	1.299±0.01
Refractive index	1.672±0.20	1.660±0.10
Iodine value (g/100g)	34.7706±0.30	35.2782±0.20
Acidic value (g/100g)	3.366±0.10	4.488±0.10
Free fatty Acid(g/100g)	0.949±0.10	1.267±0.10
Peroxide value 9mmol/kg)	0.923±0.02	0.945±0.02
Saponification value(mg/g)	19.074±0.10	13.464±0.20

± mean of triplicate results.

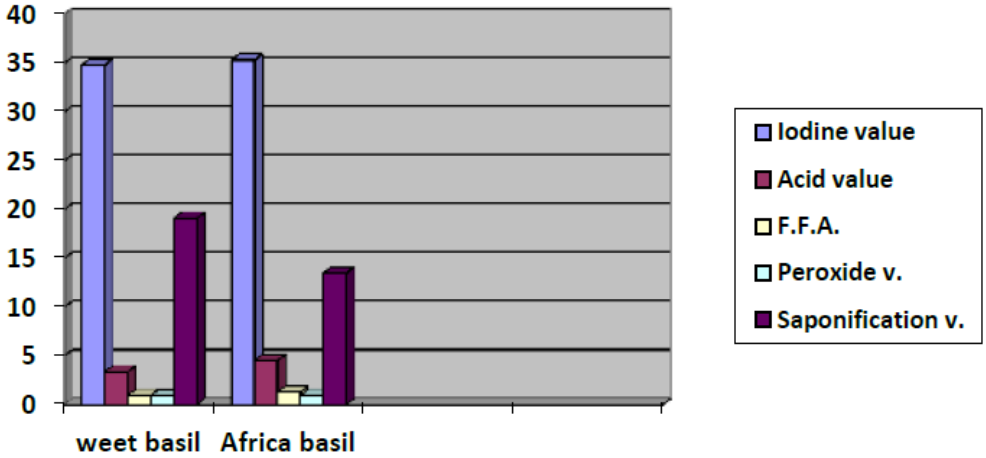

Figure 3. Chemical properties of essential oil from Sweet basil and Africa basil.

the sweet basil. The peroxide Value of Africa basil (0.945 ± 0.02 mmol/kg) was higher than the value in Sweet basil (0.923 ± 0.02 mmol/kg). These values were in the same range as these revealed the level of their oxidative rancidity. The Saponification value showed that Sweet basil value (19.074 ± 0.10 mg/g) was higher than that of Africa basil (13.464±0.20 mg/g). Due to low quantity in production, their physicochemical characteristics for industrial purposes for their high iodine and saponification value cannot be ascertained except for curative purpose. Figure 3 revealed high saponification value with high peak from the chart for the two samples respectively.

Conclusion

The results obtained from essential oil from O*cimum basilicum* and O*cimum gratissimum* revealed reasonable qualities in both physico-chemical and antibacterial activities of the essential oil extracted from them. A significant increase and high value in the zone of inhibition of 50.00 ± 0.01 mm against *Staphylococcus aureus* revealed its susceptibility to O*cimum gratissimum* and may be the reason while *Ocimum gratissimum* have been popular for its traditional medicine preparation in Africa. These may also be as a result of its high level of some chemicals embedded in the oil for its significant anti-oxidant capabilities and health benefits. The antimicrobial effect of *O. gratissimum* can be administered against *Escherichia Coli, Staphylococcus aureus* and *Klebsiella* treatment in human than O*cimum basilicum*. The values observed from pure essential oil by steam distillation method were lower than some values obtained in aqueous and ethanolic extract, but may assumed safer for consumption medically because they are free from chemicals and contaminants present in aqueous and ethanolic extract. As stipulated in the discussion above, their low quantity in production affect their physicochemical characteristics for industrial purposes despite their high iodine value in *Ocimum gratissimum* (35.2782 ± 0.20) and high saponification in *Ocimum basilicum*l(19.074 ± 0.10), which can be employed in soap making for skin treatment and curative purpose.

Reccommendation

Under clinical supervision, *Ocimum gratissimum* can be used for the treatment of chronic ailments associated *Staphylococcus aureus, Klebisella* and *E. coli*, mild and high fever and diarrhea. Also, *Ociomum basilicum* be tested for the treatment of mild headache, cough and infections of upper respiratory tract and prevent food poisoning by microbes when used as preservatives. Moreover, oil from Africa basil can be employed industrially in a small proportion to enhance normal vegetable oil used in soap making due to its high saponification value and the aroma. Proportion measures should be taken into consideration medically to check overdose for human consumption and it should be consumed in its pure state.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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