



Antimicrobial Activity of Ginger (*Zingiber Officinale Roscoe*) and Turmeric (*Curcuma Louga*) Extracts Against *Propionibacterium Acnes* Isolates from Human Pimples, Abuja, Nigeria

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ABSTRACT

Propionibacterium are known to cause outbreaks of a variety of skin infections like pimples, chronic blepharitis, inflammation, post-operative shoulder infections, etc., in both rural and urban settlers. This study therefore aimed to investigate the efficacy of ginger (*Zingiber officinale roscoe*) and turmeric *Curcuma louga*) extracts against *Propionibacterium acnes* isolate from pimples on students of Veritas University, Abuja, with a view to suggesting ways of solving the skin disfiguring problems caused by pimples. Fresh ginger and turmeric were obtained from open market in Jos, they were air-dried in the laboratory, processed into powder and stored in airtight bottles to prevent evaporation of the active ingredients in them. Ethanolic extracts of the plants materials were obtain using standard laboratory procedures. Phytochemical analysis was carried out to determine their chemical composition. Result of phytochemical analysis of the ginger extract revealed the presence of Tannins, Saponins, Flavonoids, Terpenes, Steroids and Carbohydrates in Ginger while the Turmeric extract also revealed Saponins, Terpenes, Steroids and Carbohydrates. 100 individuals (50 males and 50 females), were used in this study. Pus samples from facial pimples were taken to the laboratory for further processing. Sensitivity test was carried out using 1ml of each of the extract at 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5} concentrations and at 1:1 mixture of the two. Result of the sensitivity test showed that only 10^{-1} and 10^{-2} inhibited growth of *Propionibacterium acnes* in ginger while in turmeric only 10^{-1} concentration produced zone of growth inhibition. The mixed extract concentrations produced zones of growth inhibition in all concentration (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5}). The overall result showed that *Zingiber officinale roscoe* extract were more efficacious against the test organism

than the *Cucurcuma lougha* while the mixture of the two extracts produced the highest antibacterial activity at all concentrations compared to the extracts used singly. A control using commercial antibiotics (Amoxil, Ciprofloxacin, Ampiclox and Pefloxacin) showed low inhibitory effect against the test organism meaning they are less efficacious than the mixture of two extracts. In conclusion therefore, *Zingiber officinale roscoe* and *Cucurcuma lougha* extracts both showed promise as antibacterial agents against *Propionibacterium acnes* compared with the commercial antibiotics and therefore have a good promise for treatment of *Propionibacterium acnes* infections.

Keywords: *Propionibacterium acnes*, *Zingiber officinale roscoe*, *Curcuma lougha*, Pimples, Ethanolic extracts, Veritas University.

INTRODUCTION

The emergence of different strains of antibiotic-resistant bacteria following their indiscriminate use has since become very worrisome global problem which invariably has spurred the present serious search for new antibacterial agents from natural sources (1, 2). Fortunately, nature is endowed with many resources necessary for the good of man and for the benefit of the society. The different plants in the plant kingdom, have different uses which may either be food, medicine, or use as natural preservatives or additives in food processing (3). Throughout human history medicinal plants have been identified and used as medicaments for treating different kinds of ailments. This has become possible because plants have the great ability to synthesize a wide variety of chemical compounds which are used in performing these important biological and medicinal functions, beside their use as protection against predators such as insect, fungi and herbivorous mammals and also as antimicrobial preservatives or additives (4, 5). The discovery of plants as rich source of natural products for maintaining human health and for pharmaceutical purpose has greatly improved in the last few decades (6).

The medicinal potentials of these plants lie in some chemical substances that produce a definite physiological action on the human body and the most important of these identified bioactive constituents being alkaloids, tanins, quinins, flavonoids, saponins and phenolics (7, 8). Medicinal plant-based drugs are especially explored and exploited because of their associated

advantages of being simple, cheap, effective as well as exhibition of broad spectrum antimicrobial activity. It is the revival of interest in the exploration and exploitation of African medicinal plants by the World Health Organization (WHO) and many countries which has led to the present improved documentation of ethno-medical data of medicinal plants.

Studies have proved that there are a variety of diseases and infections caused by *Propionibacterium* and therefore there is need for development of new antibiotics the treatment of such infections and diseases. It is this finding which has necessitated the search for new plant-based therapeutic agents which can produce antimicrobial activities against the pathogenic microorganisms. Singh (9) and Effiom *et al.*, (24) reported that researches which have been conducted so far were limited to pepper, scent leaves, bitter leaf, clove, citrus fruits, garlic, bitter leaves, neem, ginger, cinnamon, and turmeric (9). It is in this vein that this research was carried out to verify the antibacterial efficacy of ginger (*Zingiber officinale roscoe*) and turmeric (*Curcuma louga*) against *Propionibacterium spp.*

Literature has revealed that ginger and turmeric have several medicinal properties and thus offer an array of health benefits. Both ginger (*Zingiber officinale roscoe*) and turmeric (*Curcuma louga*) are of *zingiberaceae* family, which together with other species are known for treating infections (10). These species of plant have been known to have a long history of use in traditional medicine as both the bark and full plant extract are found to have an invitro toxic effect against numerous bacteria. Daily *et al.*, (11) had reported that extracts of ginger and turmeric have served as effective herbal antibiotics which exhibit significant antibacterial, antifungal, anti-inflammatory, anti-carcinogenic, antithrombotic, antioxidants actions and have therefore been used to treat nausea, cold including, colie, asthma, cough, heart palpitation, swellings dyspepsia, loss of appetite, and rheumatism. The phytochemicals in these plants are

said to be rich in gingerols, zingerone, shogaols and other bioactive compounds which belongs to different classes such as tanins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (12). Mabhiza *et al.*, (2016) in their study to verify antibacterial properties of alkaloid extracts from *Callistemon citrinus* and *Vernonia adoensis* found that inhibit bacterial growth by interfering with ATP-dependent transport compounds across the bacterial cell membrane. Another study reported that flavonoids act against bacterial cells by tampering with some cellular activities thereby inhibiting nucleic acid synthesis, cytoplasmic function, energy metabolism, attachment and biofilms formation, alteration of the cell membrane as well as inhibition of the porin on the cell membrane (Cushnie and Lamb, 2016).

Pimples are said to develop when the skin pores get clogged with either too much oil (sebum), dead skin cells or infected with bacteria and acne being a sign of puberty attainment develops when sebaceous glands typically produce more sebum (13). This eventually causes collection of pus in the hair follicle which appears as a pustule or as a popular red bump. It can also be seen as a small swelling which occurs as a result of blockage in the hair follicle by fatty oil and dead skin cells. These local bacteria multiply, causing the immune system to react and form the reddish bumps referred to as pimples (14). Pimples are of different types, acne pimples are the most common, and they usually develop on the face, upper chest, back and shoulders. Their occurrence is not considered as infection because they do not contain harmful or pathogenic bacteria and cannot be spread from one person to another. Hu *et al.*, (15) had explained that pus formation is as a result of the breakdown of sebum into fatty acids by the skin bacteria known as *Propionibacterium acnes* thereby causing inflammation of the hair follicles that secrete sebum. *Propionibacterium spp* can occur due to either changes in hormonal levels in pre-teens, hormonal fluctuation during menstrual cycle and pregnancy in adult, use of oil base cosmetics,

stress and anxiety, humidity and exposure to dirt, physical irritation from occlusive clothing head band, hats helmets (16). Use of certain medications such as phenytoin, isoniazid, phenobarbital, lithium, quinine, rifampin and steroids has been recommended for treatment of pimples. *Propionibacterium* is capable of spreading to other parts of the body when hands used in pressing or bursting a pimple are not washed or when pus from a punctured pimple spill to other parts (16).

Propionibacterium are pathogens found in warm blooded animals, mostly in humans. They cause infectious diseases (Acne vulgaris-inflammatory lesions or non-inflammatory lesions or mixture of both on the face, back, chest, and shoulders)

The prevalence of pimple infection is becoming alarming perhaps as a result of certain predisposing factors such as seasonal changes, application of wrong creams, soaps etc. This study will therefore help to create awareness about pimples and its control using natural plant products such as ginger and turmeric extracts. This is necessary because the manifestation of pimples bring about economic loss, lack of self-confidence, and stigmatization on people that are affected by it.

MATERIALS AND METHODS

Study area and population

This research was conducted among students of Veritas University, Bwari, Bwari Area Council, F.T.C., Abuja, Nigeria. 100 students (i.e. 50 females and 50 males) ages 15 to 27years were used in the study.

Ethical approval

Before embarking on the study, ethical approval was obtained from Ministry of Health, FCT, Abuja, through the Veritas University Ethical Committee. In the letter of request, the rationale and importance of the study were clearly and satisfactorily explained to both Veritas University Management and the Minister for Health to be able to convince them to approve and issue the Ethical Approval.

Ethical consent

All the volunteer participants were sufficiently briefed about the essence of the study and were given the opportunity to declare their willingness to participate or opt out. They were also made to know that the information gathered from them were purely for research purposes and would therefore be treated with maximum confidentiality.

Plant materials, their sources and processing

The plant materials used for this study were fresh *Zingiber officinale roscoe* and turmeric (*Curcuma longa*) bought from Faringada market in Plateau State. They were packed separately in black polyethylene bags and transported to the Microbiology Laboratory of Veritas University, Abuja, for processing. The *Zingiber officinale* and *Curcuma longa* were thoroughly washed in distilled water, peeled, sliced and air-dried at room temperature and then packaged in envelopes and sealed and stored at 4°C until use. The dried ginger and turmeric were macerated in a sterile ceramic mortar and 700g each of ginger and turmeric were weighed and soaked with 2250mls of ethanol solvent for each of the ginger and turmeric for 3 days at room temperature. Two different extracts were then made from them by filtering first with an almost transparent fine cotton cloth and then with filter papers for an effective filtration. The extracts so made with

ethanol solvent were further concentrated using rotatory evaporator at a temperature of 60°C for 120rpm and there after the extracts were collected and stocked in sterile sample bottles.

Source of Test organisms

The test organisms used for this study were isolated from the pimple pus found on the forehead, cheek and chin of student victims. The pus was collected after swabbing the face of the victim with methylated spirit to sterilize it.

Media preparation

The media used were Brain Heart Infusion Broth which is a general purpose liquid culture medium containing peptones and other nutrients necessary for growth of fastidious and non-fastidious microorganisms and for isolation of obligate anaerobes (such as *Clostridium* spp, *Propionibacterium acnes*, etc.). The media were prepared according to the manufacturer's instruction and sterilized in the autoclave at 121°C for 15minutes. After sterilization, Brain Heart Infusion broth and the Blood agar were aseptically poured into separate petri dishes and labeled appropriately. The pure culture of the isolates was made into agar slant using nutrient agar. Simon's citrate agar and the Triple sugar iron agar were dispensed into bijou bottles and test tubes, respectively, and allowed to solidify in a slant position. Similarly, nutrient broth and peptone water were dispensed into test tubes and McCartney bottles, respectively.

Characterization and identification of the bacterial isolates from pimples

Pure cultures of the bacterial isolates were characterized and identified on the basis of their cultural, morphological and biochemical properties in accordance with the Bergey's Manual for Determinative Bacteriology. The bacterial isolates were subjected several characterization tests,

gram staining, motility test, biochemical tests (such as indole, catalase, citrate, Triple sugar iron, coagulase, methyl red, urease, voges-proskauer tests).

Determination of phytochemical characterization of ginger and turmeric extracts

The phytochemical parameters of ginger and turmeric extracts were determined in the Phytochemistry laboratory of MPR and TM NIPRD. This was achieved using standard phytochemical characterization methods as described by Harborne (17) for alkaloids and phenolics, Van Burden and Robinson (18) for tannins, Obadoni and Ochuko (19) for saponins, and Boham and Kocipai (20) for flavonoids.

Preparation of extracts dilutions

Serial dilutions of the stock extracts were made using sterile water. 1ml of each stock extract was diluted into 9ml of sterile water to give 1 in 10ml dilutions (10^{-1}). 1ml from the first tube was transferred to the second tube containing 9mls of distilled water. This was repeated through the 5 tubes until the initial 1ml was completely discarded from the last tube. The dilutions were 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} .

Determination of efficacy of the different extract concentrations against the test organism

Paper disc diffusion method was used to test the sensitivity of the organisms to the different concentrations of the extracts. Nutrient agar plates were prepared and inoculated with the organisms using streaking plate method. From the dilutions made (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5}), the paper discs were aseptically labelled according to the different dilutions and carefully placed on the fresh cultured nutrient agar plates (i.e., test organism and nutrient agar). After placing the sterile paper discs on the plates, the plates were incubated at 37°C for 24 hours after

which they were observed for zone of growth inhibition at the spots with different concentrations of ginger and turmeric extracts. A control experiment was set up using four broads of commercial antibiotics (Amoxil, Ciprofloxacin, Ampliclox, Pefloxacin) against the same organism. The zones of growth inhibition produced by the extract concentrations were matched with those of the control commercial antibiotics.

RESULTS

Result of morphological identification of the bacterial isolate

Morphological characterization proved that the isolates were both gram positive cocci and gram positive bacilli, with creamy colonies that produce propionic acid in MacConkey agar, and are white, red and yellow colonies in blood agar (Table 1).

Table 1: Morphology and cultural characteristics

Organism	Gram stain	MacConkey agar	Blood agar	BIB
<i>Propionibacterium acnes</i>	Gram positive cocci and Gram Positive Bacilli	Creamy colonies, producing propionic acid.	White, Red and yellow colonies	Nil

Key: BIB= Brain Infusion Broth.

Result of Biochemical test

Similarly, the biochemical test conducted on the bacterial isolates (*P. acnes*) revealed that the organisms were non-motile, indole negative, citrate positive and fermented only three sugars

(fructose, glycerol and glucose), but did not ferment lactose and maltose. They were also catalase positive and good organic acid producers in a positive methyl red test. *P. acnes* also reduce nitrate to nitrite, urease and coagulase negative. (Table 2)

Table 2: Biochemical characterization of the test microorganisms

Organism	Motil	Indo	Citra	TSI					Ur	VP	Cata	Coagu	MR
				Gl	Fr	Gy	La	H ₂ S					
<i>Propionibacterium acnes</i>	-	-	+	+	+	+	-	-	-	+	-	-	+

Key: TSI= Tripple sugar iron, Motil = Motility, Indo = Indole, Citra = Citrate, Gl= Glucose, Fr = Fructose, Gy= Glycerol, La = Lactose, H₂S= Hydrogen sulphide, Ur = Ureas, MR= Methyl red, VP= Voges-Proskauer test, Cata = Catalase, Coagu= Coagulase
 + = positive, - = negative

Result of Phytochemical determination

The extracts revealed the presence of tannins, saponins, flavonoids, terpenes, steroids, and carbohydrates for Ginger, while Turmeric contained only saponins, terpenes, steroids and carbohydrates (Table 3).

Table 3: Qualitative determination of phytochemicals aqueous present in ginger and turmeric

Parameters	Ginger	Turmeric
1. Tannins	+	-
2. Saponins	+	+
3. Alkaloids	-	-
4. Flavonoids	+	-
5. Glycosides	-	-
6. Terpenes	+	+
7. Steroids	+	+
8. Phenols	-	-
9. Resins	-	-
10. Carbohydrates	+	+

Key: +ve= Positive/present; -ve=Negative/absent

Result of test of efficacy of extract concentrations against test organism

Only 10^{-1} and 10^{-2} concentrations showed zones of growth inhibition, while in 10^{-3} to 10^{-5} concentrations, test organism showed resistance and thus did not produce zone of growth inhibition (Table 4).

Only 10^{-1} concentration of turmeric extract produced zone of growth inhibition while in other concentrations 10^{-2} to 10^{-5} the test organism showed resistance and thus did not produce zone of growth inhibition (Tables 5).

Table 4: Zones of inhibition of Ginger extract against *Propionibacterium acnes*

Extracts dilutions	Zones of growth inhibition
10^{-1}	5
10^{-2}	2
10^{-3}	-
10^{-4}	-
10^{-5}	-

Key: +ve= zone of growth inhibition produced; -ve=No zone of growth inhibition.

Table 5: Zones of inhibition of Turmeric Extract against *Propionibacterium acnes*.

Extracts dilutions	Zones of inhibition
10^{-1}	5
10^{-2}	-
10^{-3}	-
10^{-4}	-
10^{-5}	-

Key: +ve= -ve=No zone of growth inhibition; zone of inhibition in millimetres (mm)

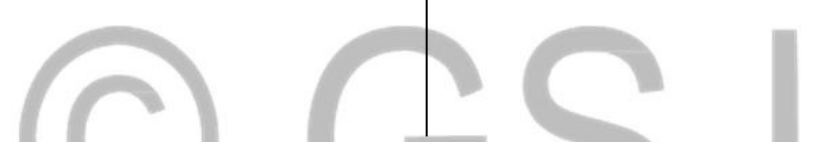
Result of the effect of combined extract concentrations of ginger and turmeric on the test organism

The result of the combined concentrations of ginger and turmeric extracts showed that all concentrations produced zones of growth inhibition. This indicates that the mixture of the two extract produced enhanced inhibitory effect (Table 6).

Table 6: Zones of inhibition of ginger and turmeric Extract against *Propionibacterium acnes*.

Extract dilutions	Zones of growth inhibition
10^{-1}	12
10^{-2}	8
10^{-3}	5
10^{-4}	3
10^{-5}	1

Key: Inhibition zone in Millimeters



(mm)

Results of growth inhibition zones of commercial antibiotics (control) against the test organism

The result from the commercially prepared antibiotics showed that all the antibiotics disc produced zones of inhibition. This proves that the disc enhanced inhibitory effect and when compared to the mixture extracts, the zones of inhibition were similar but the extract mixture has proven to have greater inhibitory effect against the commercially produced antibiotics (Table 7).

Table 7: Zones of growth inhibition of commercial antibiotics (control) against the test organism

Amoxil (mm)	Ciprofloxacin (mm)	Ampiclox (mm)	Pefloxacin (mm)
15	10	8	4

Key: mm = Millimeter

DISCUSSION

The ginger and turmeric extracts used for this study showed growth inhibition activity only at high concentrations: of 10^{-1} to 10^{-2} and 10^{-1} , respectively, against the test organism while lower concentrations of both ginger and turmeric extracts did not inhibit growth of test organism.

The application of mixture of ginger and turmeric extracts produced double effects against the test organism. Similarly, as the concentration increased, the growth inhibition of *P. acnes* increases showing that while ginger and turmeric individual extract proved to be more effective at high concentrations, ginger-turmeric extracts mixture are effective at all concentrations. However, in comparing the individual effects of the extracts, each seemed to be less effective against *P. acnes* than the ginger-turmeric extracts mixture which showed high inhibitory activity at all concentrations.

These results have confirmed the reports of previous studies that used various plant extracts. Suzan., (21) reported that Turmeric extract of the Curcumin consists of antioxidant,

anti-inflammatory and lipophilic properties and as a result of these associated properties, turmeric consumption has lower incidence of Alzheimer's disease in Indian and Asian population 4.4 times, meaning invariably that curcumin consumption can help reduce Alzheimer's disease as well as dementia (22). Another study to investigate the association of curry form of turmeric powder in 1,010 Asians, aged between 60 and 93 years, turmeric powder was observed to be associated with body inflammation. The result of the qualitative analysis of Ginger extract proved the presence of Tannins, Saponins, Flavonoids, Terpenes in addition to Steroids and Carbohydrates while that of Turmeric extract revealed the presence of only Saponins, Terpenes, Steroids and Carbohydrates. These phytochemicals in plants play many functions including pigmentation in flowers, antimicrobial activity against viruses, bacteria, fungi beside anti-inflammatory activity and protection against insect attacks. Various previous studies have proved that phytochemicals in plant extracts generally exhibit a variety of antimicrobial activity even at least concentrations when used against microbes. This diverse antimicrobial effect can be attributed to their different mechanisms of action against microbes. For alkaloids, it is suspected that interference with ATP-dependent transport compounds across bacterial cell membrane helps to retard the growth of the *Propionibacterium acne* cells. It could also be suspected that the toxicity of flavonoids is what disrupts certain cellular activity such as nucleic acid synthesis, cytoplasmic membrane function, energy metabolism, etc. (Mabhiza *et al.*, 2016). The saponins possessing a detergent property may have acted against *Propionibacterium acne* by increasing their cell membrane permeability and thus promote water influx which eventually destroy the bacterial cell. Earlier studies conducted on phytochemical extracts by Harikrishnan *et al.*, (23) had confirmed this fact. Also fascinating is the finding that the test

organism *Propionibacterium acnes* showed almost equal degrees of susceptibility to the two extracts.

Conclusion

Today, a person's skin can be said to be a paramount reflection of his/her overall health, and radiant, glowing skin is seen as a product of proper dieting, hydration and other lifestyle choices. On the other hand, a skin ridden with pimples is conversely said to be due to factors such as oxidative damage, bacteria infection and poor dietary intake. This study to verify the antimicrobial activity of extracts from ginger and turmeric against *Propionibacterium acnes* isolates from pimples, has demonstrated further that beside being more efficacious antimicrobial agents, these plant products are more relatively safe, inexpensive and readily available, and thus can be used in sustainable and effective treatment and control of pimples which have tended to disfigure the faces of a great number of people the world over. With the rich bioactive phytochemical contents of these extracts, ginger and turmeric therefore offer good alternative in the control of this unlimited global infection.

Recommendation

- Ginger and turmeric extracts should be developed in commercial scale for sustainable control of pimples and used as major constituents in the production of facial soaps, creams and tablets.
- Experiments should be carried out immediately the extracts are prepared so that its potency will not reduce before usage, as poor and prolonged storage could affect its potency.

- Further research should be conducted to investigate the best means of application (systemically or typically).

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