INTERNATIONAL JOURNAL OF APPLIED SCIENCE RESEARCH
FEDERAL POLYTECHNIC OKO, ANAMBRA STATE

EVALUATION OF ANTIBACTERIAL ACTIVITY OF LEMON JUICE ON SOME CLINICAL ISOLATES

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ABSTRACT

This study was carried out to evaluate the antibacterial effectiveness of lemon juice, (ripe and unripe) extracts; using agar disc diffusion method. The test organisms (*Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Bacillis subtilis* were obtained from National Agency for Food, Drug Administration and Cotrol Agulu, Anambra State. A serially diluted 24 hours broth culture of the test organisms was used to inoculate Mullar Hinton agar; and 10mm diameter paper disc impregnated with test organisms lace in it before incubation. The result of the test showed that the ripe lemon juice inhibited *Staphylococcus aureus* (30mm), *Pseudomonas aeruginosa* (28mm), *Bacillus subtilis* (20mm) but no inhibition on *Escherichia coli* while unripe lemon juice inhibited *Staphylococcus aureus* (24mm), *Pseudomonas aeruginosa.* (30mm), *Escherichia coli* (23) but did not inhibit *Bacillus subtilis.*The MIC and MBC carried out using ripe lemon on *Staphylococcus aureus* gave 30%v/v for both; while unripe lemon recorded 30%v/v and 15%v/ MIC and MBC results respectively on *Pseudomonas aeruginosa.* The result of this work has shown that lemon juice (both ripe and unripe) can be used in the treatment of infections caused by these organisms. However, more specific researches are needed for full application in the pharmaceutical industry.

Key Words: Lemon, Antibacterial, Inhibition.

INTRODUCTION

For a long period in history, plants have been valuable and indispensable sources of natural products for the health of human beings and they have a great potential for producing new drugs (Nascimento *et al.,* 2000). Bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Abeysinghe, 2010). Finding new naturally active components from plants or plant-based agricultural products has been of interest to many researchers. Hence, a great deal of attraction has been paid to the antibacterial activity of citrus as a potential and promising source of pharmaceutical agents (Ortuño *et al.,* 2006).

According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Nascimento *et al.,* 2000).

Lemon is an important medicinal plant of the family Rutaceae. It is used mainly for its alkaloids, which are having anticancer activities and the antibacterial potential in crude extracts of different parts (leaves, stem, root, juice, peel and flower) of Lemon against clinically significant bacterial strains has been reported (Kawaii *et al.,* 2000). Citrus flavonoids have a broad spectrum of biological activity including antibacterial, antifungal, anti-diabetic, anticancer and antiviral activities (Burt, 2004).

Antimicrobial activity of the peel extract is directly concerned with the components that they contain. The studies showed that essential oils, protopine and corydaline alkaloids, lactons, polyacetylene, acyclic sesquiterpenes, hypericin and pseudohypericin compounds are effective toward various bacteria (Keles *et al.,* 2001). Furthermore, citrus fruit had been used in traditional Asian medicines for centuries to treat indigestion and to improve bronchial and asthmatic conditions (Kalpa *et al.,* 2012).

Johann *et al.* (2007) have shown that citrus varieties are considered and containing a rich source of secondary metabolites with the ability to produce a broad spectrum of biological activities.

Extracts of citrus fruit (e.g. lemon, orange and grape fruit) are among the most studied natural antimicrobials for food app

Statement of the problem

Recently, microbes have the ability of resisting the effects of medication, because the roles of medicinal plants have been neglected and despite the leading role of medicinal value of lemon and lime juice in pharmaceutical industries, less than 3% have been incorporated into drug manufacture, because this plant contain some bioactive compound that can serve as antibacterial agents, which can be used to prevent and treat bacterial infections, and antibiotics resistance occurs when bacteria change in response to the use of these medicines, therefore this deemed it necessary to compare the antimicrobial activities of lemon juice on some clinical isolates.

Objectives of the study

This study achieved the following specific objectives:

i. To extract juice from lemon
ii. To assess their antimicrobial activities on some clinical isolates.

LITERATURE REVIEW

An Overview of Herbal Medicine

World Health Organization define Traditional herbal medicines as naturally occurring, plant-derived substances with minimal or no industrial processing that have been used to treat illness within local or regional healing practices. Traditional herbal medicine and their preparations

have been widely used for the thousands of years in developing and developed countries owing to its natural origin and lesser side effects (Tilburt *et al.,* 2008). These medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations. The use of plants for healing purposes predates human history and forms the origin of much modern medicine. Clinical, pharmacological, and chemical studies of these traditional medicines, which were derived predominantly from plants, were the basis of most early medicines such as aspirin (willow bark), digitoxin (from foxglove), morphine (from the opium poppy), quinine (from cinchona bark), and pilocarpine (Jaborandi). Herbal medicine is still the mainstay of about 75 - 80% of the world population, mainly in the developing countries, for primary health care. This is primarily because of the general belief that herbal drugs are without any side effects besides being cheap and locally available. According to the World Health Organization (WHO), the use of herbal remedies throughout the world exceeds that of the conventional drugs by two to three times (Pal *et al.,* 2003).

Indigenous herbal medicines are those which historically used in a local community or region and are very well known through long usage by the local population in terms of its composition, treatment and dosage. It can be used freely by the local community or in the local region. However, if the medicines in this category enter the market or go away from the local community or region, they have to meet the requirements of safety and efficacy as per the national regulations for herbal medicines. Herbal medicines in systems have been used for a long time and are documented with their special theories and concepts, and accepted by the countries. Modified herbal medicines have been modified in shape, or form including dose, dosage form, mode of administration, herbal medicinal ingredients, methods of preparation and medical indications. They have to meet the national regulatory requirements of safety and efficacy of herbal medicines. Imported products with herbal medicine base covers all imported herbal medicines including raw materials and products (Pal *et al.,* 2003).

Plants and natural products were used by humankind over the years as food and medicines to cure and prevent diseases. It is very difficult to point out an exact time when the use of plants was started as medicine, the Carbon dating from ancient Babylon (Iraq) records that plants were cultivated as medicines 60,000 years ago. Written material medical of medicinal herbs go back approximately 5,000 years in India, China and Egypt and at least 2,500 years in Greece and Asia Minor. Neanderthal remains have been found to contain the remnants of medicinal herbs. Sumerians described well-established medicinal uses for plants such as laurel, caraway, and thyme at least 5,000 years ago. Egyptian people where well known to medicine before 2900 BC, these people used papyrus (pithy stem of a water plant for writing or

painting) such as the Ebers papyrus(1500 BC) , the Edwin Smith papyrus (1600 BC), the Berlin papyrus (1200 BC), and the Kahun papyrus (1900 BC), etc but the best known Egyptian pharmaceutical record is the "Ebers Papyrus" contain more than 800 formulae such as gargles, snuffs, poultices, infusions, pills and ointments, with beer, milk, wine and honey being commonly used as vehicles and 700 different drugs like acacia, castor oil and fennel etc with their uses along with apparent reference to some chemical such as iron, sodium chloride and sulphur.

Currently more than 80% of the world population depends on traditional and plant derived medicine because. Plants are important sources of medicines and presently about 25% of pharmaceutical prescriptions in the United States contain at least one plant-derived ingredient. In the last century, roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources. In fact, it is now believed that Nature contributes up to 90% to the new drug molecule. Nature has provided many of the effective agent such as dactinomycin, bleomycin, and doxorubicin, vinblastine, irinotecan, topotecan, etoposide, and paclitaxel (anticancer), Mefloquine chloroquine , amodiaquine artemisinin, dihydro artemisinin, artemether, and arteether (antimalarial) , metformin and eventually the other Biguanide, Harunganin, cryptolepine, maprouneacin (anti diabetic) Calanolide A, cucrcumin, phenethyle isocyanate, phenoxidiol (anti-HIV drugs) etc.

Importance of herbal medicine

Many herbal medicines have been used for hundreds of years and it is assumed in many cases that they must work. For example, about 7000 species of plants are used in China as herbal medicines, but only 230 of the most commonly used ones have been subject to in-depth pharmacological, analytical and clinical studies.

The consumption of herbal medicines is increasing steadily throughout the world as an alternative treatment for alleviating a number of health problems including heart diseases, diabetes, high blood pressure and even certain types of cancer. In India use of herbal drugs is much more because of their easy accessibility. Unlike drugs, herbal products are not regulated for purity and potency .There are neither studies on their effectiveness nor control over the quality and safety of these preparations. As per Food and Drug Administration mandates, only medicines have to be proven to be safe before being released into market. Herbal products do not fall under the category of medicine as long as they are not marketed for the prevention of any disease. Herbal drugs are considered as 'food integrators and readily available

in the market without prescription. The major driving force for the use of herbal drugs is the perception that 'they are safe because they are natural and have fewer side effects than prescription drugs'. However, various studies and researchers have high lightened their possible side effects, if taken irregularly, in excessive amounts or in combination with some medicines (Stickel *et al.,* 2005).

A common problem with herb use is that people do not take into consideration how they may interact with any prescription drug they are taking, or with each other. Interaction between drugs and herbs can result in unexpected concentration of drugs and also cause undesired effects. Sometimes the use of commonly used herbs with prescription medicines become big barrier for the diagnosis of certain diseases as people do not inform their physicians about their consumption. The aim of this paper is to highlight the uses and side effects of some selected below mentioned herbal drugs so that these may be used safely (Stickel *et al.,* 2005).

Distribution and taxonomy of Citrus species

According to statistics of FAOSTAT, Citrus species are grown all over the world in more than 140 countries, with more than 8.7 million hectares and about 131 million tons of fruits produced in 2012. And China, Brazil, the U.S.A., India, Mexico, and Spain are the world's leading Citrus fruit-producing countries, representing close to two-thirds of global production. In China, citriculture has existed traditionally, and the Citrus varieties have been naturally selected: (1) *C. aurantifolia* (Christm.) Swingle, (2) *C. aurantium* L., (3) *C. hongheensis* Ye *et al.,* (4) *C. hystrix* DC., (5) *C. ichangensis* Swingle, (6) *C. junos* Sieb. ex Tanaka, (7) *C. limon* (L.) Burm. f., (8) *C. limonia* Osb., (9) *C. macroptera* Montrous., (10) *C. maxima* (Burm.) Merr., (11) C. medica L., (12) *C. paradisi* Macf., (13) *C. reticulata* Blanco, (14) *C. sinensis* (L.) Osb (Caristi *et al.,* 2003).

The genus Citrus belongs to the subtribeCitrinae, tribe Citreae, subfamily Aurantioideae of the family Rutaceae. However, continual taxonomic study appears to be very complicated and controversial, mainly due to sexual compatibility between Citrus species and related genera, the high frequency of bud mutations, apomixis (e.g., adventitious embryony). Consequently, there has been no consensus among taxonomists as to the actual number of Citrus species. Later, phylogenetic analysis indicated only three true species within the cultivated Citrus, i.e., *C. medica* L. (citron), C. reticulata Blanco (mandarin) and C. maxima (Burm.) Merr. (pummelo). In order to be convenient, the existing taxonomic systems are combined currently. Because morphological characters are of limited use, studies have mainly focused on new taxonomy methods, i.e., chemotaxonomy. 66 Citrus species and near-Citrus relatives can be cited in accordance with Tanaka's classification system with 24 flavonoids. Flavanones were used as chemotaxonomic markers to distinguish 77 Zhishi (traditional Chinese

medicine) samples from three Citrus species. Another study suggested that the content of certain monoterpenes could be as taxonomic markers between *C. sinensis* and *C. junos*(Liu *et al.,* 2014).

Active secondary metabolites

Plentiful active natural metabolites including flavonoids, alkaloids, coumarins, limonoids, carotenoids, phenolic acids and essential oils, have been found in Citrus fruits. Tables in additional files have summarized these secondary metabolites isolated from peel, pulp, seed, pressed oil, juice or whole fruit from 31 common species to give a systematical profile. By these at least, the types of Citrus-derived secondary metabolites vary among different Citrus species and different fruit parts. Moreover, flavanones, synephrine, auraptene and limonin are the most dominants among the flavonoids, alkaloids, coumarins and limonoids groups, respectively.

In Additional file 1, 48 types of flavonoids from 22 common Citrus species of different fruit parts (peel, pulp, seed, pressed oil, juice or whole fruit) have been summarized. These flavonoids belong to the five classes: flavones, flavonols, flavanones, flavanonols and polymethoxylated flavones. Anthocyanins, an uncommon class of flavonoid, only appears in blood oranges of limited data in different fruit parts. Among Citrusderived flavonoids, flavanones comprise approximately 95 % of the total flavonoids (Peterson *et al.,* 2006). And flavones, flavonols and polymethoxylated flavones present in lower concentration. In addition, some of flavonoids are unique to Citrus plants. Citrus-derived flavonoids are present in glycoside or aglycone forms, and usually do not occur naturally as aglycones but rather as glycosides, in which the aglycones are linked to a sugar moiety. Among the aglycone forms, naringenin, hesperetin, apigenin, nobiletin, tangeretin and quercetin are widely detected. For glycoside forms, O-glycosides, C-glycosides, rutinosides, glucosides and neohesperidosides are common. Naringin (neohesperidoside), neohesperidin (neohesperidoside), narirutin (rutinoside), and hesperidin (rutinoside) are commonly present in major quantities. Sinensetin, isosinensetin, nobiletin, tangeretin, which all belong to polymethoxylated flavones, exist only as aglycones because the binding sites for sugar moieties are not occupied by hydroxyl moieties (Nielson *et al.,* 2000).

Bioactivities of Citrus fruits

Owing to these metabolites, Citrus fruits exhibit plentiful bioactivities including anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial and anti-allergy activities, as well as cardiovascular effect, neuroprotective effect, hepatoprotective effect, obesity control, etc. Note that

flavonoids (especially flavanone, flavanonol and methoxylated flavones) are more active compared to other secondary metabolites in Citrus for their remarkable various bioactivities.

Flavonoids The juices from green and ripe chinotto (*C. myrtifolia*), which were full of flavonoid, was tested by DPPH· radical bleaching and superoxide-anion scavenging, and it was shown that immature chinotto fruits, in particular, yield a juice with a remarkable anti-oxidant power. The anti-oxidant activity of the flavonoid mixture isolated from the Citrus peel was determined in terms of the DPPH· and ABTS· + scavenging and the reducing power assay in a concentration range from 25 to 500 mg/L, and its anti-oxidant activity increased in a dose-dependent manner the results of the anti-oxidant capacities and total phenolic acids contents of the Tarhana samples (Kilci and Gocmen, 2014).The anti-oxidant potency composite index showed wide variations, ranging from 58.84 to 98.89 in the 14 studied wild mandarin genotypes native to China, due to different phenolic compounds' levels, including phenolic acids.

Ogiwara *et al.* (2003) found that caffeic, chlorogenic, and ferulic acids scavenged various radicals, such as superoxide anions and hydroxy radicals. Citric acids from Citrus have been found to show anti-oxidant activity in lipopolysaccharide (LPS)-treated mice. Korani *et al.* (2014) demonstrated that gallic acid has a beneficial activity against 2VO-induced cognitive deficits via enhancement of the cerebral anti-oxidant defense. Among the phenolic acid group, gallic acid with three hydroxyl groups on the aromatic ring was the strongest anti-oxidant (Karamac *et al.,* 2005). In contrast, the monosubstitutedphenolic acids (p-coumaric acid, o-coumaric acid, and 4-OH-phenylacetic acid) showed very low activity. In addition, the radical-scavenging activities of phenolic acids are related to their hydroxyl group characteristics in the order: gallic > gentisic > syringic > caffeic > protocatechuic > sinapic > ferulic > isoferulic > vanillic > p-coumaric > o-coumaric > m-coumaric > salicylic ≫ p-hydroxybenzoic (Jabri Karoul and Marzouk, 2013).

Antimicrobial action of Citrus fruits

Fruit juice of *C. limon* inhibited clinical isolates of *Staphylococcus aureus, Escherichia coli, Klebsiellaaerogenes, and Klebsiella pneumonia* (Pradeep *et al.,* 2007). Peel extracts of *C. sinensis* has marked antibacterial action while C. aurantium revealed higher antifungal activity against Colletotrichumcapsici in comparison with each other (Madhuri *et al.,* 2014). Aqueous extracts of peel and juice from fresh and dried Citrus and sweet lemon reported antimicrobial action against six Gram-positive and eight Gram-negative bacterial and one yeast isolates (Nada and Zainab, 2013).

C. sinensis seed oil is highly effective as an antifungal agent against Lentinussajor-caju, which caused white rot in wood hence, it can be used as a preservative agent in the management of wood infected with white rot fungi (Yekeen *et al.,* 2014).Propionibacterium acnes, bacterial species that causes Acne vulgaris is reported very sensitive to C. limon (Shinkafi and Ndanusa, 2013). Methanolic extract of C. sinensis fruit peel was able to inhibit all the bacteria (*Bacillus subtilis, S. aureus, E. coli*) and fungi (*Candida. albicans* and *Aspergillusniger*) with different degrees of inhibition (Dhiman *et al.,* 2012). The high biological potential of essential oil of *C. limon*, against multidrug resistant *Acinetobacter spp*. was reported (Guerra *et al.,* 2013).

Estrogenic activity

Significantly decrease sperm count was found after administration of alcoholic extract and its fractions of *C. limonum* seeds for 60 days but the count returned to normal after 90 days. Sperm count reduction and atrophic changes in testis and epididymis suggest reduction in male sex hormone that is androgen as the mechanism of anti-fertility effect (Kulkarni *et al.,* 2012). The petroleum ether extract proved to retain high estrogenic activity in immature female rats (El-Alfy *et al.,* 2012). Petroleum ether extract of *C. medica* seeds exhibited estrogenic effects in rats which include an increase in uterine weight and vaginal epithelial cell cornification. The opening of vagina on the 5th day and cornification of vaginal epithelial cells was found in 30-day-old immature rats. Alcohol and chloroform extract of Citrus hystrix fruit peels exhibited postcoital anti-fertility activity in pregnant rats. The ethyl acetate fraction of alcoholic extract of *C. limonum* seeds showed reversible anti-fertility effect in mice by virtue of its anti-zygotic action. *C. medica* Linn. (peel) was used as the traditional/folkloric medicine for anti-fertility activity (Kulkarni *et al.,* 2005).

Description of lemon

Lemon constitutes an important fresh fruit group even though it is not eaten fresh as mandarins and oranges. They usually have high acid content although acidless cultivars also exist (Ortiz, 2002). It is used primarily for drinks and fresh juice or lemonade, cooking and flavouring, especially in the making of lemon pies, lemon cakes, candies, jams and marmalades, and also for medicinal purposes due to its high content of vitamins. The fruit is generally oval to elliptical with characteristic necks and nipples. The peel is yellow at maturity and has prominent oil glands. The flesh is pale yellow in colour and very sour. There are three major groups of lemons: the Femminello, the Verna and the Sicilian groups (Ortiz, 2002).

Scientific classification of lemon

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledonae

Order: Rutales

Family: Rutaceae

Genus: Citrus

Species: *Citrus limon*

Materials and Methods

Autoclave, incubator, petri dishes, test tubes, conical flask, colony counter, syringes, knife , Agar slant bottles, slides, microscope, filter paper, paper tape, bijou bottles, test tube rack, wire loop, lemon (ripe and unripe).

Sample Collection and Extraction

The samples (lemon fruits) were purchased from total market Oko in Anambra state, Nigeria. None of this sample had spoilt at the time of this investigation.

Sample preparation

10 lemon fruits (ripe and unripe) were aseptically squeezed into active sterile bijou bottles to obtain its juice.

Test Organisms

The test organisms that were used for this work were collected from Nation Agency for Food and Drug Administration and Control in Agulu. The organisms include:*Staphylococcus aureus, Bacillus subtilis. Escherichia coli and Pseudomonas aeruginosa.*

Antimicrobial assay.

The antimicrobial activity was performed by agar disc diffusion method. The bacterial strains were grown in nutrient broth, Nutrient agar was the media used to study the bacteria susceptibility. The broth culture were grown 24hours and serially diluted in the same broth c sterilized at 121^0C for 15minutes. The 24hours broth culture containing approximately $1.7x10^7$cfu/ml, $2.6x10^7$cfu/ml, $2.6x10^6$cfu/ml and $5.0x10^7$cfu/ml. for *Escherichia coli, Staphylococcus aureus, Bacillus subtilis and Pseudomonas aeruginosa*. respectively as determined by plate count method sterile swab stick was used.

Minimal Inhibitory Concentration (MIC)

The extract that gave up to 30mm zone of inhibition were used to determine the MIC on the respective organisms they inhibited *(Staphylococcus aureus and Pseudomonas auriginosa)*

The extracts were diluted with sterile distilled water to give the following concentrations (v/v) 50%, 30%, 20%, 15%, 10%, and 5%, corresponding to 2.5ml, 1.5ml, 1ml, 0.75ml, 0.5ml and 0.25ml of the extracts in 5ml of broth. These were inoculated with 0.5ml of 10^{-3} dilution of 24hours broth culture of the test organisms; *Staphylococcus aureus* and *Pseudomonas auriginosa*. Tubes without extract and that without culture were also prepared to serve as positive and negative control respectively. These were prepared for 24hours at 37^0C and the MIC determined as the lowest concentration with no visible growth.

Minimum Bactericidal Concentration (MBC)

Test tube of culture showing no visible growth were gently streaked on NA plates and incubated for 24hours to determine the MBC which is the lowest concentration of the extract that did not allow growth in NA after 24hours incubation.

RESULTS

The result of the antimicrobial activity test showed that the juice extracts (riped and unriped)lemon, were effective to some of the organisms such as; *Staphylococcus aureus* and *Pseudomonas aeruginosa* as shown in tableland plates 1-5.

Minimal Inhibitory Concentration (MIC)

The MIC is the lowest concentration that did not show visible growth in dilution tube. The result of the MIC is as shown in table2.

Minimum Bactericidal Concentration (MBC)

The MBC is the concentration of the extracts that did not allow the growth of the organism after 24hour incubation on Agar. The result of the MBC is as shown in table 3.

Table 1: Antimicrobial activity of the juice extract against some bacteria strains tested.

Test Organisms	Zone of inhibition (mm)		
	Control	Ripe lemon	Unripe lemon
S. aureus	30	30	24
Pseud. aeruginosa.	33	28	30
Bacillus subtilis	38	20	Nil
E. coli	25	Nil	23

Table 2: The result for the MIC of the juice extracts on *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Extracts	Test Organisms	MIC
Ripe Lemon	*Staphylococcus aureus*	30% v/v
Unripe Lemon	*Pseudomonas auriginosa*	30% v/v

Table 3: The result for the MBC of the juice extracts on *Pseudomonas spp.* and *Staphylococcus aureus*

Extracts	Test Organisms	MBC
Ripe Lemon	*Staphylococcus aureus*	30% v/v

Unripe Lemon	*Pseudomonas auriginosa*	30% v/v

DISCUSSION

This study was carried out to investigate the possible antimicrobial activity of lemon (*Citrus limon*)on selected microorganisms (*Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Bacillus subtilis* .

The result of the test showed antimicrobial activities of ripe lemon juice on *Staphylococcus aureus* (30mm), *Pseudomonas aeruginosa* (28mm), *Bacillus subtilis* (20mm) and no zone of inhibition in *Escherichia coli, w*hile in unripe lemon juice showed S*taphylococcus aureus* (24mm), *Pseudomonas aeruginosa.* (30mm), *Escherichia coli* (23) and no zone of inhibition in *Bacillus subtilis.*

This result partially agrees with the finding of Ammara *et al.* (2009) on the zone of inhibition showed on *Staphylococcus aureus* (30mm) but varied with the inhibition showed on *Escherichia coli* (8mm), *Pseudomonas spp* (16mm), *Bacillus spp* (20mm)

This difference could be as a result of specie of lemon fruits used, the laboratory environment, or the method of extraction of the juice. The antimicrobial effect of these fruits could be attributed to the phytochemical constituents present in it, which are known to confer certain health such as antimicrobials.

The result of this work has shown that lemon juice (ripe and unripe) can be used in the treatment of urinary tract infections, diarrhea, wound, virginal discharge, cholera and dysentery.

CONCLUSION

This study shows that lemon (ripe and unripe) juice contains valuable antimicrobials. It has been investigated to inhibit the growth of *Staphylococcus aureus, Pseudomonas, Bacillus* and *Escherichia coli*. Therefore lemon can provide treatment and remedies against human infection caused by these organisms.

RECOMMENDATION

In view of the above results, the natural and traditional use of lemon and lime (ripe and unripe) should be continued, because it contains many antimicrobials. It is also recommended for human consumption, pharmacology and herbal medicines for the treatment of bacterial infections and diseases.

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