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Phytochemical and Antibacterial Activity of Phoenix Dactylifera (Date Fruit on Selected Clinical Isolates)

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Abstract

Phoenix dactylifera, Date fruit has been used traditionally as medicine for the treatment of ailments such as intestinal disorders, fever bronchitis, and wound. This study was conducted to determine the phytochemical and antibacterial activity of Phoenix dactylifera fruit against two clinical isolates; Escherichia coli and Staphylococcus aureus. The sample of date fruits were collected ,crushed, air dried ,blended into powder and extracted using methanol and water .The phytochemical constituent of the extracts were determined as well as the antibacterial activity of the cruide extract against the test isolates using Agar well diffusion methods. The result of the Phytochemical analysis of the extract revealed the presence of alkaloid, flavonoid, anthraquinone, saponin, terpenoids and tannin while the antibacterial screening of the extract indicates that Date fruit extract are effective on the two isolates. However, Staphlococcus aureus had the higher diameter of zone of inhibition of 24mm while Escherichial coli was less sensitive to the extract with 9mm inhibitry zone at 200mg/ml Conc for cold water extract while for Methanol extract Staph aureus has 17mm,zone of inhibition and E.coli has about 15mm of zone of inhibition both at 200mg/ml Conc .Therefore, the plant extract could be used as potential source of natural product for the treatment of infection.

Keywords: *E.coli, Staphylococcus aureus and Date fruit*.

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Background to the Study

The Date fruit (*Phoenix dactylifera L.*), a tropical and subtropical tree, belonging to the family Palmae (Arecaceae) is one of the humankind's oldest cultivated plants. Date fruit is the most successful and commercially important crop in the hot-arid regions of the world, such as, Saudi Arabia, Emirates, and Egypt. In these countries, date fruit products are commonly used for human and animal consumption, pharmaceuticals, cosmetics, carpentry, and firewood. A large number of date fruit cultivars are known; however, until now, only a few of these cultivars have been evaluated for chemical composition and nutritional quality (Chao, 2007). Date fruits are a good source of vitamins minerals simple carbohydrate and dietary fibers (Sohaimy, 2013). Pulps of dates hold easily digestible sugars (70%), mostly glucose, sucrose, and fructose, dietary fibers, and enclose less proteins and fats (Al Farsi, 2008). Moreover, date fruits possess antioxidant and antimutagenic properties (Vayalil, 2002), attributable to their high levels of polyphenolic compounds and vitamins (Mansouri, *et al.,* 2005).

The date fruit pulp is rich in phytochemicals such as phenolics, sterols, carotenoids, anthocyanins, procyanins, and flavonoids. The ratio and concentrations of these constituents depend on the type of the fruit, stage of the fruit picking, location, and soil conditions; these phytochemicals also contribute to the nutritional and organoleptic properties of the fruits (Abdul, 2008). There was also found a good correlation between the total phenolic content (TPC) and antioxidant activities of the nonvolatile extracts (Al-Diahan, 2012). Considering the nutritional importance of dates, studying their biochemical composition and nutritional quality is increasingly being recognized as a worthy and important task.

Scope and Limitation

The scope of this research work is to obtain a simplified knowledge about the anti-microbial activities of Date's fruit (*Phoenix dactylifera L.*) which tends to be safer or more valuable substitutes than synthetically produced antimicrobial agents. This research work is strictly limited to the determination of the anti-microbial activities and elemental constitutes of the Date fruit against *Staphylococcus aureus and Escherichia. coli.*

Justification

Date fruits are used for prophylaxis and treatment of many human diseases. According to an ethnobotanical study, parts of date fruit are traditionally used to treat anemia and demineralization, in infusion for cold, as a gargle for sore throat, crushed in water to treat hemorrhoids, constipation and jaundice. Green dates are toning, aphrodisiac and can treat intestinal disorders such as diarrhea (Benchelah and Maka, 2006). Date palm syrup is useful in the treatment of Broncho-pulmonary infections and is also used as a sedative. The powdered seeds are used as food supplements, and as coffee substitute (Bellakhdar, 1997). The date fruit pulp is used as a sweetener in the preparation of beer as well as in other industrial processes, especially in the production of syrup and confectionery (Rahman, 2007). Date fruit meal has been marketed to replace coffee powder (Martin-Sanchez, 2013). Some researchers have described the importance of date pollen in traditional medicine. It is widely used to treat male infertility. Indeed, the aphrodisiac effect of date fruit pollen extract may be attributed to the presence of alkaloids, flavonoids and saponins in the extract (Abedi, 2014)

Aim of the Study

The aim of the study is to investigate antimicrobial activity of extracts of Date fruit (*Phoenix dactylifera L.*) on *Staphylococcus aureus* and *Escherichia coli.*

Objectives of the Study

i. To determine phytochemical analysis of *date fruit* (*Phoenix dactylifera L*)
ii. To test the antimicrobial effect of date fruit on selected clinically obtained Staphylococcus *aureus* and *E. coli.*
iii. To determine minimum inhibitory concentration of the date fruit (*Phoenix dactylifera L.*) extracts

Literature Review

Antimicrobials

In an attempt to combat the various forms of disease that have continued to plague humans from time immemorial to this day, different types of antimicrobials have been developed to fight the pathogens responsible for these diseases. Antimicrobials, which are substances that kill or inhibit the growth of microorganisms, could be in the form of antibiotics, which are products of microorganisms or synthesised derivatives (Cowan, 2009), antimicrobial peptides produced by complex organisms as well as some microbes (Palada and Changl, 2003) and medicinal plants, which appear to be the focus of mainstream medicine today (Cowan, 2009).

Types and Sources of Antimicrobials

Different types of antimicrobials exist: antibiotics, anti-viral, anti-fungal, anti-protozoan etc. Antibiotics are used in the treatment of bacterial infections and can be obtained from either natural or synthetic sources. Examples of those with a natural origin are phenyl propanoids (chloramphenicol), polyketides (tetracycline), aminoglycosides (streptomycin, gentamycin, Amoxillin), macrolides (erythromycin), glycopeptides (vancomycin) and second-generation-lactams (cephalosporins)β. Those from synthetic sources are sulphonamides, quinolones and oxazolidinones. Most antibiotics exert their action either by inhibition of the bacterial cell wall or protein synthesis. Exceptions are the quinolones that inhibit DNA synthesis, and the sulphonamides that inhibit the synthesis of metabolites used for the synthesis of deoxyribonucleic acid (DNA) (Singh and Barrett, 2006). Most anti-viral, anti-fungal, anti-protozoa and anti-cancer drugs however are obtained from synthetic sources.

Because of the re-occurring resistance of pathogenic microorganisms to antibiotics, as well as the side effects presented by these antibiotics, investigation of other sources of antimicrobials, such as medicinal plants, for their antimicrobial properties is gaining ground. The date fruit produce secondary metabolites (phytochemicals), which have demonstrated their potential as antibacterials when used alone and as synergists or potentiators of other antibacterial agents. Phytochemicals frequently act through different mechanisms than conventional antibiotics and could therefore be of use in the treatment of resistant bacteria (Abreu *et al.,* 2012).

Medicinal Plants

General Uses of Medicinal Plants

Medicinal plants (otherwise referred to as herbs, herbal medicines, pharmacologically active plants or phytomedicinals) remain the dominant form of medicine in most countries. Over three end four primarily on raw plant products to meet their daily health care needs (Barrett and Kieffer, 2001). Most of the plant materials collected are used fresh in order to obtain the extract from the whole plant or parts of it, which could be leaves, roots, flowers or fruit. In case of woody forms, mostly the bark, roots and other parts are used. Carminatives such as ginger, cloves and coriander are also usually added as fresh or dried materials (Rao and Arora, 2004). For example, bearberry (*Arctostaphylosuva-ursi*) and cranberry juice (*Vaccinium macrocarpon*) have been reported indifferent manuals of phytotherapy to treat urinary tract infections while species such as lemon balm (*Melissa officinalis*), garlic (*Allium sativum*) and tea tree (*Melaleucaalternifolia*) are described as broad-spectrum antimicrobial agents (Heinrich *et al.,*2004).

Some plant extracts with great medicinal value are the stem bark decoction of *Albiziagummifera,* which is used in the management of venereal diseases (Buwa and VanStaden, 2006), leaves of *Glyphaea brevis*, which are macerated in water and are used to treat intestinal diseases and hepatitis (Noumi and Yomi, 2001) and oil extracts of the roots, seeds and stem barks of *Monodora myristica,* which are used to cure scabies, helminthiasis, malaria and dysenteric syndromes (Palada and Changl, 2003).

Active Components of Plant Extracts

The beneficial medicinal effects of plant materials typically result from the combination of secondary products present in plants. These compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, which are synthesised and deposited in specific parts or in all parts of the plant (Joseph and Raj, 2010). Generally, leaves are the favourable storage site for desired compounds. Fruits also contain a substantial amount of active ingredients, and thus are often consumed as juice via oral administration to obtain the desired compounds. Other parts of plants that can be extracted for therapeutic compounds are roots, aerial parts, flowers, seeds, stem barks, etc. (Chan *et al.,* 2012). Plant secondary metabolites are used as the basis for the production of valuable synthetic compounds such as pharmaceuticals, cosmetics, or more recently nutraceuticals (Bourgaud *et al.,* 2001). These secondary metabolites are largely viewed as potential sources of new drugs, antibiotics, insecticides and herbicides (Crozier *et al.,* 2006). This is because of their biological significance and potential health effects, such as antioxidant, anticancer, anti-aging, anti-atherosclerotic, antimicrobial and anti-inflammatory activities.

Significance of Antimicrobial Susceptibility Testing

In screening new antimicrobials or antibiotics, evaluation of biological activity is essential for the assessment of susceptibility of pathogens to the antimicrobial agent. Antimicrobial susceptibility testing is used in pathology to determine the resistance of certain microbial strains to different antimicrobials and in pharmacology research it is used to determine the efficacy of novel antimicrobials from biological extracts against different microorganisms

(Das *et al.,* 2010). Microbial growth or its inhibition can be measured in a number of ways, e.g. viable counts, direct microscopic counts, turbidity measurement, bioluminescence and fluorimetry (Grare *et al.,* 2008). Of the various antimicrobial susceptibility methods employed, the disk diffusion method and the broth microdilution method are commonly used to evaluate the effect of the plant extracts or any other antimicrobial on disease-causing pathogens.

The disk diffusion method is used in determining the zones of inhibition exhibited by the plant extracts, while the broth micro-dilution method, which has been recommended by the Clinical and Laboratory Standards Institute (2003), is used in determining the minimum inhibitory concentration (MIC) of plant extracts. This method is less cumbersome, less expensive and quite reproducible when compared with the disk diffusion method. The use of microplates allows large amounts of data to be generated quickly. Bacterial growth could be assessed either visually by grading turbidity or better spectrophotometrically by measuring optical density (Grare *et al.,* 2008). The disadvantage of visual assessment of bacterial growth is that it lacks objectivity and precision; whereas the accuracy of spectrophotometric readings may be hampered by (i) additives or antibacterial compounds that affect the spectral characteristics of growth media, (ii) the aggregation of bacteria, or (iii) bacterial pigments (Eloff 2008). Colorimetric methods therefore could represent an alternative approach, using tetrazolium salts as indicators, since bacteria convert them to coloured formazan derivatives that can be quantified (Grare *et al.,* 2008).

History of Date Fruit

Phoenix dactylifera, also known as dates fruits, have a chewy texture and candy-like sweetness. Throughout history they have been recognized as an energizing staple food and are sometimes referred to as the "bread of the dessert" or "cake of the poor." The origins of dates can be traced back to the Middle East and culinary experts estimate there are more than 3,000 varieties. In the US we are most familiar with deglet noor and medjool varieties. Considered the oldest cultivated fruit in the world, fossil evidence indicates that dates go back at least 50 million years ago (Taleb *et al.,* 2016).

The scientific name, *phoenix dactylifera*, comes from ancient Greece. The coastal region between the Jordan Valley and Mediterranean Sea is known as Phoenicia, Greek for *purple land*, a nod to the purple dye made from poisonous sea snails that made the area famous. Because the date palm grew abundantly in Phoenicia, it was believed to be the trees homeland. It became known as Phoenix, or tree of Phoenicia, and was a symbol of the region and was featured on Phoenician coins. Dactylifera translates to "finger bearing", a reference to the oblong shaped dates and the clusters they grow in (Eloff 2008).

Greek mythology connects the date fruit to the immortal Phoenix. In Natural History (1 AD), Pliny the Elder describes the phoenix bird, who would build its next at the top of a date palm. After 500 years, the bird would catch fire from the flames of the sun and would be reborn from its own ashes. Some legends say that the date palm would die and come back to life along with the famous bird (Das *et al.,* 2010). The date fruit is native to deserts of North Africa and the

Middle East. During the late 1800s, settlers from Southwestern America saw similar conditions suitable for growing dates. High heat, access to water, and an arid climate in late summer and autumn is exactly what date palms require to thrive. The first attempt at growing date palms from seed failed, but cultivation was a success when nurseries and members of the USDA brought back offshoots from trees in the Middle East. The first two decades of the 1900s showed great promise for commercial cultivation (Das *et al.,* 2010). Now most dates in America are grown in the Coachella Valley and the Bard Valley. Combined, these two areas have over 7500 acres of land for growing date palms. An annual date festival has been held in Indio, California since 1921.An estimated 8.5 million metric tons of dates are produced annually, with countries in the Middle East and North Africa still being the largest producers (Al-Diahan, 2012).

Taxonomic Classification

The date fruit is of the Kingdom Plantae, Division Magnoliophyta, Class Liliopsida, Order Arecales, Family Arecaceae and Genus Phoenix, which contains 12-19 species of date palms. Their binomial name is Phoenix dactylifera. Date palms which produce dates fruit reach a height of approximately 69 – 75 feet. The fruit isa short cylindrical shape about 1-3 inches in length and about an inch in diameter. The color ranges from bright red to bright yellow, to deep purple (Al-Diahan, 2012).

Taxonomic Tree

Domain: Eukaryota
Kingdom: Plantae
Phylum: Spermatophyta
Subphylum: Angiospermae
Class: Monocotyledonae
Order: Arecales
Family: Arecaceae
Genus: Phoenix
Species: Phoenix dactylifera

Ethnomedical Uses

The date fruitis a widely cultivated palm fruit, both for its ornamental value and for the tasty fruit it yields. Not only are the fruit used for a variety of culinary purposes, but the leaves have also been used to weave baskets. Various parts of this plant have been used medicinally to treat a variety of ailments. The fruit of the date palm contains tannin, which makes it an effective astringent. The fruit from this tree has been used to treat sore throats, colds, bronchial catarrh, fevers, gonorrhea, edema and abdominal problems. The seeds from the tree have been ground into a paste that is effective in treating ague. Toothaches have been relieved by date palm roots. Finally, gum extracted from the trunk of this tree has effectively been used to treat diarrhea and urinary ailments (Shomer *et al.,* 2008).

The seeds produced by the date fruit have been used as food for horses, cattle, camels, sheep and goats. Once ground, they can be used to feed chickens as well. The oils contained within

the seeds make them a useful ingredient in cosmetics and soaps. The seeds can also be burned to make charcoal. The chemical composition of the seeds allows them to be used to create oxalic acid. Dates, despite being very sweet, have a high nutritional value. They are rich in carbohydrates, dietary fibers, proteins, minerals and vitamin B complex. They are made up of about 70% carbohydrates (equal ratios of glucose and fructose) and contain calcium, iron, magnesium, and potassium (Al-Diahan, 2012).

Recently there has been increased interest in the health promoting properties of dates. Pharmacological studies have shown that dates are highly nourishing and may have numerous potential health benefits. They are believed to contain phytochemicals that may help to low cholesterol and decrease risk of diabetes, cancer, and cardiovascular diseases. Dates are most often picked when they are the dry stage we are familiar with; however, they are unique in that they experience several stages of ripeness. In the first stage of ripeness, known as the *khalal*stage, dates have a firm and crunchy texture that is juicy and only slightly sweet. When left to ripen, the date becomes sweeter and less firm and is often sold when it reaches the *tamar*stage. Most date lovers prefer them at the *rutab*stage, when they are very soft and sweet. The sweetness of dates is a great accompaniment to many different savory dishes, including cheese, meat and vegetable courses. They also make a great dessert option on their own. Throughout history and in many parts of the world, dates are considered an affordable source of nutrition, comparable to rice, wheat and potatoes. Because they are dried, easily preserved and easily transportable they have an extended shelf life, which is ideal for long periods of travel.

As one of the Seven Species mentioned in the Torah, dates are referred to as "d'vash" which directly translates to honey. This has led many scholars to believe that the Torah's mention of honey is actually date honey, or date syrup, rather than traditional honey produced by bees. Dates are particularly popular in Great Britain during the winter holidays where they are eaten whole or added to traditional desserts like Christmas pudding, sticky toffee pudding, and fruit cake. As for the remaining parts of the date, the pits are sometimes are sometimes ground into animal feed or used in bread and the oil is safe for use in cosmetics and soap (Al-Diahan, 2012).

Pharmacological Properties

The date fruit (*Phornix dactylifera L.*) is considered the most important source of food for both human in arid and semiarid (Taleb*et el al.,* 2016). Dates contain a high percentage of sugars reaching 88% in some varieties. Dates are also rich in mineral salts and vitamins (Juice, 2018). For the date pit, the percentage of non-reducing sugars is3.82% and in glucose and fructose is 1.68 and 1.53, respectively. In local medicinal practices, dates are considered a tonic. Some consider it to be an aphrodisiac. The flower of the plant is used as a purgative (Hong*et el al.,*2016). Experimentally, date extracts have been shown to increase sperm count in guinea pigs and to enhance spermatogenesis and increase the concentration of testosterone, follicle stimulating hormone, and luteinizing hormone in rats (Baliga *et al.,*2011). Date fruit pollen (DFP) cure-male infertility by improving the quality of sperm parameters. Date pits have been included in animal feed to enhance growth, an action that has been ascribed to anincrease in the plasma level of testosterone (Shomer *et al.,* 2008).

The pits of Phoenix dactylifera contains different chemical compounds such as saturated and unsaturated fatty acids, Zinc (Zn), Cadmium (Cd), Calcium (Ca), and potassium (K). Saturated fatty acids include satiric and plasmatic acid and unsaturated fatty Acids contain linoleum and oleic acids which could inhibit 5 -α reeducates enzyme (Ghnimi*et el al.,* 2017). Also, dates contain at least six vitamins including a small amount of vitamin C, and vitamins B1 (thiamine), B2 (riboflavin), nicotinic acid (niacin) and vitamin A (Tengberg, 2012). Studies indicate that the aqueous extracts of dates have potent antioxidant activity (Sohaimy, 2013). The antioxidant activity is attributed to the wide range of phenolic compounds in datesincluding p-coumaric, ferulic and sinapic acids, flavonoids and procyanidins.

In recent years, it has been suggested that estrogen, may be involved in the regulating there new of spermatogonial stem cells and male reproductive tissues with estrogen receptors (Shomer *et al.,* 2008). Investigations have revealed that palm kernels and date pollen grains extracts contain estrogenic materials as gonad-stimulating compounds that improve male infertility. Reports have also pointed that isolation of micro elements from DPP has estrogen, sterols, and other agents that may influence male fertility. With regard to these components, snack foods have been supplemented with date pollen to improve male infertility (Taleb *et al.,* 2016). The present work was carried out to study the impact of date palm pits as antibacterial activitieson two species of pathogenic bacteria (klebsiella pneumonia and Escherichia coli) and its role in reducing side effect of methylprednisolone on some neurotransmitter content in the brain, level of hormone testosterone and testclture in male albino rats.

Dates (*Phoenixdectylifera L.*) are an important nutritional source for many countries of the world, because the dates containing different nutrients such as carbohydrates, vitamins and minerals. From pollination to final date-fruit ripening takes 200 days. Date palm flowering and fruiting were recognized to have distinct stages over the ripening period. Hababauk, female flowers and immediate post-pollination period when the very young fruits are creamy white in color; Biser, sometimes named Kimri, green fruit undergoing rapid growth; Khalal, fruit grows slowly to full size, sugar content increasing while moisture content decreases, hard, glossy, red or yellow in color; Rutab, fruits ripening to a soft stage, brown in color; Tamar, fruits fully ripened, wrinkled, brown or black in color. All of the dates in different ripening stages containing various qualitative and quantitative number of phytochemicals (Al-Diahan, 2012).

In recent decades an increasing tendency towards the use of natural substances as food additive instead of the synthetic ones has been observed. Natural phytochemicals, such as phenolic compounds, which extracted from many types of plants, are gaining importance that adds to foods, due to their benefits for human health, showed best antioxidant activity. In addition to antioxidant activity, several studies demonstrated the antibacterial activity of phenols and/or phenolic extracts. Besides nutritional values, dates are rich in phenolic and phytochemical components which changes during ripening stage and may possessing antimicrobial activity. Several studies have been done on the antioxidant activity of dates but the literature reviews are poor about studies on the antibacterial activity of dates. Thus, the purpose of the present investigation was to investigate the antibacterial activity of dates ether, ethanol, and water extracts in different ripening stages against some Gram-positive and Gram-

negative bacteria in vitro and in situ (in food) in order to establish their biological activity for value (Sohaimy, 2013).

Nutritional Value of Date Fruits

Date fruits have been reported to contain 6.5%–11.5% total dietary fibers (up to 90% of which is insoluble and 10% of soluble dietary fiber), approximately 1% fat, 2% proteins, and 2% ash. It is also a rich source of phenolic antioxidants. Similarly, the soft date fruit is mostly composed of invert sugars (fructose and glucose) with little or no sucrose, whereas the dry ones have high proportion of sucrose. Accordingly, the fruits are classified based on their sugar type into (i) invert sugar types containing mainly glucose and fructose (e.g., Barhi and Saidy), (ii) mixed sugar types (e.g., Khadrawy, Halawy, Zahidi, and Sayer), and (iii) cane sugar types containing mainly sucrose (e.g., Deglet Nour and Deglet Beidha). Date fruits contain a wide variety of essential nutrients, making them very nutritious. The ripe fruits mostly contain sugar (80%), with smaller amounts of protein, fiber, and trace elements including boron, cobalt, copper, fluorine, magnesium, manganese, selenium, and zinc. In view of the nutritious and antioxidant-rich contents of date fruits, there have been attempts to develop functional foods from it (Abdul and Alliath 2008).

Table 1: Nutritional value of date fruits (Deglet Nour)

Nutrient	**Content (per 100 g)**
Energy	1178 kJ (282 kcal)
Carbohydrates	75.03 g
Sugars	63.35 g
Dietary fiber	8 g
Fat	0.39 g
Protein	2.45 g
Beta-carotene	6 µg
Vitamin A	10 IU
Thiamine (vitamin B_1)	0.052 mg
Riboflavin (vitamin B_2)	0.066 mg
Niacin (vitamin B_3)	1.274 mg
Pantothenic acid (vitamin B_5)	0.589 mg
Vitamin B_6	0.165 mg
Folate (vitamin B_9)	19 µg
Vitamin C	0.4 mg
Vitamin E	0.05 mg
Vitamin K	2.7 µg
Minerals	
Calcium	39 mg
Iron	1.02 mg
Magnesium	43 mg
Manganese	0.262 mg
Phosphorus	62 mg
Potassium	656 mg
Sodium	2 mg
Zinc	0.29 mg
Water	20.53 g

Source: USDA Nutrient Database

Antioxidant and Antimicrobial Properties of Date Fruits

Date fruit is used as traditional medicine in some cultures for the treatment of ailments such as intestinal disorders, fever, bronchitis, and wound healing. This is because of their wide variety of essential nutrients. Recent studies have provided more evidence for the use of date fruits for medicinal purposes. Accordingly, preclinical studies have shown that date fruit has antibacterial and anti-inflammatory activity. More studies, especially human trials, are however still needed to evaluate the clinical validity of these findings. Similarly, the mechanism of action of the compounds in date fruits warrants investigation (Tengberg, 2012).

Materials and Methods

Materials

Beaker, cotoon wool, conical flak, funnel, weight balance, Bunsen burner, wire loop, aluminium foil, cork borer, masking tape, petri dish, autoclave machine, water bath, methanol, distill water, filter paper, swab stick, date fruits, test tube, ruler e.t.c.

Collection of Sample

Date fruits were collected and brought to the laboratory in sterile containers.

Method of Extraction

Date fruits were opened, and the seeds were removed. The seedless fruits were washed with distilled water and kept under a room temperature for 4 weeks to dry. The Date fruits were then grinded with mortal into powder form. 50g of the grinded date fruits was weighed and added to methanol in a conical flask and left in the shaker incubator 150 rpm (rotating/min) for 48hours, the mixture was filtered and poured into a conical flask and kept in a water bath to dry for 1 week, respectively.

The extract was covered with aluminum foil (to avoid oxidation) and stored at the room temperature for the future analysis.

Source of Organism

The test organism was collected from FMC (Federal Medical Centre) in Zungeru, Microbiology Department.

Preparation of Media

Molarhiton agar was weighed at 38g and dissolved in 1000ml, for four plate (one petri dish contains 20ml of media.

After the calculation 1.52g of molarhiting agar was dissolved in 40ml of water.
The media was autoclave for 15min in 121^0C
It was allowed to cool at the range of 40^0C.
The media was dispensed and allowed to solidify.

Inoculation of the organism and incubation

A sterile swab strick was used to isolate (E.coli and S.aureus) on the surface, and was then allow it to diffuse.

The Bunsen burner was placed around the media so as to disinfectant the table and unseen organism.
The cork borer was sterilized, and make a well hole was made at the culture plate.
The extract and Dsno was mixed and put into the well, and the antibiotic that serves as control has (Amoxillin).
Then incubate at 37 for 24hours.

Minimum Inhibitory Concentration (MIC)
The MIC of an antimicrobial agent is the lowest (i.e., minimal) concentration of the antimicrobial agent that inhibits a bacterial isolate from multiplying and producing visible growth. Extracts were prepared in different concentration 50,100,200 mg/ml, and added into a sterile tube containing 1 ml of nutrient broth and bacterial cultures were inoculated as 105 cells/ml and incubated at 37°C for 24 hours. Controls were incubated like nutrient broth with only extract and another tube with only bacterial cultures. After incubation, results were examined for turbidity in the tubes with control and also to confirm the results, loop of culture was inoculated in agar plates by streaking

Result and Discussion
Antibacterial activity of methanolic extract of Date fruit (*Phoenix Dactylifera L.*) in test organisms.

Table 2: Average zone of inhibition (mm) of methanl extract of Date fruit

Test organism	**200mg/ml**	**100mg/ml**	**50mg/ml**	**Amoxillin**
Staphlococcus aeureus	17	11	Nil	21
Escherichia coli	15	Nil	Nil	12

Key = Amoxillin, AMX

The result above shows antibiotic susceptibility of the isolates. from the table 2 it indicates that the conventional antibiotic is effective on the isolates. but E.coli tend to be less sensitive to amoxillin. The methanolic extract 200mg/ml was more effective against staph. Aureus. And 50mg/ml show no zone of inhibition against Staph. Aureus. And E.coli.

Table 3: Antibacterial activity of water extract of date fruit (*Phoenix Dactylifera L.*) on test organism

Test organism	200mg/ml	100mg/ml	50mg/ml	Amoxillin
Staphlococcus aeureus	24	11	Nil	12
Escherichia coli	9	Nil	Nil	12

Key = Amoxillin, AMX

Table 3 above show antibiotic susceptibility of the isolates. It indicates that the conventional antibiotic are effective on the isolates but *E.coli* tend to be less sensitive to amoxillin. The water extract 200mg/ml was more effective against staph. Aureus. And 50mg/ml shows no zone of inhibition against *Staph aureus.* and *E.coli* indicating that the effectiveness of the extract depends on its level of concentration.

Table 4.

Phytochemical	Fruits (mg/100g)
1. Alkaloids	1591.0 ± 15.39
2. Flavonoids	3360 ± 0.00
3. Anithraquinine	169.2 ± 15.21
4. Saponin	1.37 ± 0.10
5. Terpenoids	1.97.0 ± 0.12
6. Tannin	685.0 ± 0.00

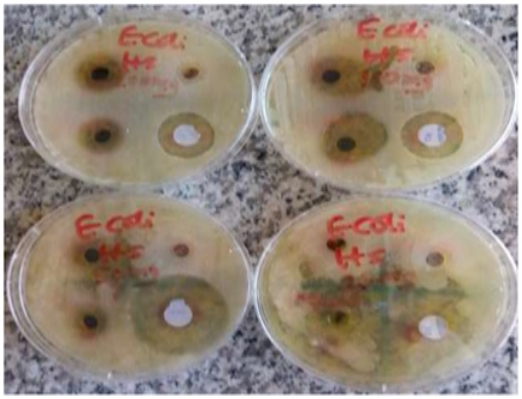

Figure 1: Antibacterial activity of Date fruit extract on E.coli.

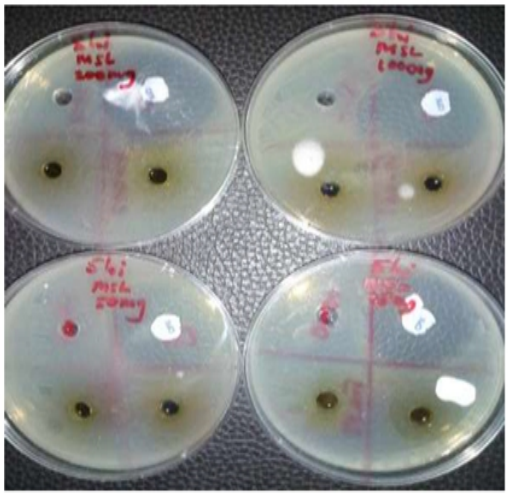

Figure 2: Antibacterial activity of Date fruit extract on Staphlococcus. Aureus

Discussion

Date fruits are considered as staple fruit in (Arabian peninsula) and they are widely cultivated in semi-arid regions. The extraction of Date fruit was obtained using methanol as solvent and the photochemical analysis of the date extract revealed the presence of alkaloid, anthraquinone, flavonoid, glycoside, phenol, saponin, steroid and tannins which agrees with

the finding of Ladipo et al. (2010) that shows that *Date* contains all the phytochemicals mentioned earlier.

The antibacterial analysis of the methanolic extract of *Date* revealed that the extract has antibacterial activity against *E. coli*. This could be as a result of the presence of one of the phytochemicals or the interaction of two or more of the bioactive compounds against the test organism which agreed with the work of Abdullahi (2012) and also supported the traditional uses of the plant in the treatment of various bacterial enteric diseases such as diarrhea, dysentery and other gastrointestinal infections (Nwinyi et al., 2009). From the result obtained, there was decreased in antibacterial activity with decreased in concentration of the extract as shown the methanolic extract 200mg/ml was move effective against staphlococcus Aureus. And 50mg/ml show no zone of inhibition against Staphlococcus Aureus. And E.coli.

However, there was no antibacterial effect of the extract on E.coli (Figure 4.1) and *S.aureus*(Figure 4.2). This resistance may be due to the lipid content on the membranes of these bacteria that prevented the permeability of the active phytochemicals into the cell or the low quantity of alkaloid (1591.0 ± 15.39) extracted with methanol (Abdullahi, 2012). However, this partially agreed with the work of (Ladipo *et al.* 2010), who reported that the methanol extract of Date fruit has antibacterial activity against E .coli and *Staphlococcus. Aureus*.

But (Justina and Solomon 2017) suggested that difference in phytochemical constituent of the fruit of Date be as a result of the planting location, seasonal and environmental variations. This could also have effect on the antibacterial activity of the date fruit extraction E .coli and *Staphlococcus Aureus*. Therefore, the fruit extract of Date could serve as natural antibacterial agent and herbal drug against gastroenteritis.

Conclusion

In conclusion, Date fruits are a good source of vitamins minerals simple carbohydrate, dietary fibres and is used for various medical purposes. The results obtained revealed that Date fruits possesses antimicrobial activities, therefore, could be a good source of medicine for the treatment of infectious disease caused by *E.coli* and *Staph. Aureus*.

Recommendations

Considering the significant findings of this research, it is therefore necessary to draw with the following recommendations.

1. There is need to investigate the antimicrobial potency of the fruit against wider range of clinical isolates of pathogenic organisms in order to obtain a more accurate evaluation of the plants therapeutic potential.
2. It will be necessary to elucidate the mechanism of action and as well as their levels of toxicity to assess their clinical applicability.

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