

Antibacterial Activity of Citrus Sinensis X against Pathogenic Bacterias

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ABSTRACT

Citrus sinensis was tested for antibacterial activity against bacteria strains such as *E.coli*, *pseudomonas aeruginosa*, *klebsiella pneumoniae*, and *staphylococcus aureus*. The extraction was done with water and ethanol. Agar disc diffusion was used to test antibacterial activity in vitro method. On *Escherichia coli*, the antibacterial action of aqueous extract demonstrated a zone of inhibition. On the other organisms, it showed little or no zones of infection, although being seven millimeters in diameter. Inhibition with a diameter of 0-3mm. little inhibitory zones were also visible in the ethanol extract. On test organisms with diagram ranging from 1-3mm the inhibitory concentration that must be met (MIC). Using a two- flow serial dilution method, no zones of contamination were found in the ethanol and aqueous extract. The finding of this of this investigation indicated that the extract have limited promise for treating disorder induced by the organisms.

KEYWORDS: Zone of inhibition, disc diffusion method, test organisms, aqueous extract, ethanol extract

INTRODUCTION

Globally disease are the major causes of death. Antibody resistance is on the rise become a worldwide issue (westh *et al.*, 2004). Many antibiotics clinical of the effectiveness. The emergency is dealing with existence. (Bandow *et al.*, 2003) of a multidrug resistant pathogen. Throughout the course of human history, many infectious disease have been documented in the past. The natural world herbal goods in their purest or us extracts and restricted access to standardized plant extracts. Because of the, there are the more prospects for novel medication leads unrialled.

Accessibility to a wide range of options chemical. As a result a never endelingand exhausting cycle emerges. There is a pressing need to identity novel. Antimicrobials chemical compounds with various chemical structure. For the re-emerging disease as well as new modes of option with the emergence of novel infection disease (Rojs *et al.*, 2003). As a result, are becoming as interested in pathogenic agent have shown these behaviour.

Plants have been used for therapeutic purpose since ancient times a previous civilization (Le Strange, 1997). Medicinal plant or found I throughtout the world despite their small size. The tropics are there you find the majority of them (Calixto, 2000).

Herbal medicine is widely used in Nigeria. As a result of the fact health deteriorating the intensity of the situation is increasing official and other staff expected. To arrive recognize some of the protencies and efficacies of flora native to the area. Some of the diseases that can be found have traditionally been successfully Managed. Malaria, infantile convulsions, and diarrhea are dysentery, gonorrhoea, epilepsy, tonsillilits and fungal infection are all symptoms of dysentery, infections.

Are all things to be concerned about (soforowa, 1996).Biodiversity is undeniably important. The plant kingdom has not evolved, according to scientist. Citrus friuts are grown all throughtout a world including Nigeria, and are members of the plantae family. The Rutaceae family, and the citrus genus 1]

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genus Tangerine (*Citrus Nobilis*) is a citrus fruits. Citrus (*Citrus vitis*), grape fruit (*citrus reticulate*), lime(*Citrus limon*), aurantifolia, lemon (*Citrus limonum*), and sweet orange (*Citrus dulcis*). Citrus *sinensis* L.Osbeck is a small citrus fruits. Evergreen tree with a height range of to 7.5 to 15 meters. The orange fruits is sweet and delicious.

The natural world herbal goods in there purest form or as extracts and unrestricted access to standardized plant extracts. Because of the, there are more prospects for novel medication leads, unrivalled accessibility to a wide range of options chemical. As a result, a never-ending and exhausting cycle emerges. The usage of modern toothbrushes and the use of a chewing stick, also known as Miswak in Arabic, has been recognized for millennia as a tooth cleaning. Human have become accustomed to using interdental cleansers. Ignore the most basic oral hygiene tool, which is a toothbrush. It is a chewing stick. Several research studies have revealed that chewing sticks are made of natural material ascorbic acid, trimethylamine, chloride, and others. Fluoride, silica, resin, and salvadorine are all quality that are antiseptic, astringent, and bactericidal that aids in the reduction of plaque formation and gives anti-infectious properties, removes odours, and increases flavour perception. Several of the Northerners are more likely to utilize chewing stick.

Citrus sinensis is the botanical name for sweet orange, which belong as to the Rutaceae family. *Citrus sinensis* is a well-known citrus, with global output estimated to be in the billions of dollars. 120 million metric tones. In tropical and subtropical areas, orange trees are frequently grown. It is grown in a variety of climes for its sweet juice and therapeutic properties. Orange peel extract has immunity boosting, stomachic, digestive system tonic, and immune boosting effects. Mechanism that has been show to be effective against colic, stomach distress, and cancer. Its primary purpose is to treat and prevent vitamin deficiency. The pithy axis is made up of a number of tightly paced segments. Each segments grows from a single carpel and is encircled by a thin, translucent endocarp or “ray” from which multicellular hairs fill the segment. Each of these hairs’ massive call pulp vesicles fills with juice and forms the edible part of the fruit for which the crops are farmed. The seed is located on the ripe fruit, the axle placenta is close to the central axis and contains around 40-45 percent petunia when combined, acids, proteins, and minerals from essential oils (**Aschoff et al.,2015**).

The fruit typically contains 80-90 percent sugar and acids, with the proportions variable. Orange peel has

also been shown to have antibacterial properties in the literature. Discovered that extract has significant antibacterial action (against intestinal infections). It can be derived from orange peels Orange peel extract was also discovered to be beneficial. Akdemir is effective against *klebsiella pneumonia*. Naturally goods have always been and will continue to be vital sources of nutrition. *Citrus sinensis* is the botanical name for a strum that belongs to the Rutaceae plant family. Sweet oranges are thought to have originated thousands of years ago in Southern China. They are the most popular now widespread and well-known. Sweet oranges (*Citrus sinensis*) can be grown in most parts of the trophics where they are more than five years of sunshine. It can be produced from seeds, however buying budded is preferable. *Citrus sinensis* is a widely distributed citrus fruit.

The pithy axis is made up of a number of tightly packed segments. The pulp and seed, which are 90 percent water, 5-10 percent sugar, and 1-2 percent petunia when combined. With its application in science and technology, nanotechnology is developing as a rapidly rising area. Gold, silver, and plantinum nanoparticle a are widely used in a variety of applications, used in medicine there is an ever-increasing demand to create an no hazardous ingredients are used in this ecologically friendly nanoparticle production technique.

Nanoparticle are small that can be used in a variety of applications are made using a number of chemical and physical techniques, which are unfriendly to a environment various biological system, such as yeast, are now used green chemistry techniques. For the creations of nanoparticles, fungus.

Because the isolated oil contains aromatic chemicals which are responsible for the oil’s scent. It is possible that the volatile oil has antibacterial properties. As a result, our primary goal is to assess antibiotic efficacy. Citrus oil’s action. Diarrhea is defied as the passing of three or more loose or liquid stools per day, or more frequently than the individual’s typical bowel movements. It’s usually a sign of a gastrointestinal illness, which can be caused by a wide range of bacteria, viruses, and parasites. Severe diarrhea causes dehydration and can be fatal, especially in young infants and those who are malnourished or have weakened immune systems. Every year, this is the second largest causes death in children. Many medicines are used to treat diarrhoea; however, two or more are frequently required for therapy; consequently, their continued use leads to bacterial resistance and a loss of antibacterial activity. Natural goods can be used to supplements your diet.

It also contains limonoids, synephrine, hesperidin, falconoid, polyphenols, pectin, and enough amounts of folacin, calcium, potassium, thimine niacin, and magnesium. These biologically active compounds help to prevent arteriosclerosis, cancer, kidney stones, and stomach ulcers, as well as lower cholesterol and blood pressure, improving human health. They also have anti-inflammatory, antibacterial, larvicidal, and antifungal properties. Although reports imply that leaves, flowers, and fruits have a high concentration of bioactive compounds, few studies have looked into the pharmacological effects of albedo and flavedo. The goal of this study was to confirm that the albedo and flavedo of two varieties of *C. sinensis* cultivated in Yucatan have a dual spasmolytic and bactericide effect.

Natural items, such as plants have long been a feature of traditional medicinal systems (such as Chinese, Ayurvedic, and Egyptian). A medicinal plant is one that includes compound that can be utilized for therapeutic reasons or are precursors for chemopharmaceutical semi-synthesis one or more of its organs (stem, root, leaves, rhizomes, fruits, flowers, and seeds). Such a plant (medicinal plant) will be sections that are used in the treatment or control of a disease state, and hence has biochemical components known as phytochemicals that are important in medicine. Plant-derived bioactive compounds are known as phytochemicals.

They are classified as secondary metabolites since the plant that produced them does not require them. Phytochemicals are produced naturally in all parts of the plant. Including the bark, leaves, stem, root, fruits, seeds, and so on. The majority of the medications used in the conventional medicine were initially derived from plants. Many recent studies have demonstrated that herbs strengthen the immune system by boosting the formation of disease-fighting white blood cells.

Citrus sinensis (Sweet orange) may be grown in most parts of the tropics if the growing season is longer than five months and rainfall is evenly distributed throughout the year. The trees can be produced from seeds, however buying them budded is preferable. *Citrus sinensis* is a widely distributed citrus fruit. Evergreen, sometime prickly trees that grow up to 12 metres tall and have ovate elliptic leaves. The leaves are usually 7-10cm long, dark green, and channeled at the base. The leaves have a distinct aroma, and the flowers are fragrant as well. White blossoms with a pleasant scent are smaller. In humid climates, the colour changes from deep yellow to orange. When ripe, the fruit becomes green. The sweet orange (*Citrus sinensis*) is a tropical and annual crop.

Bailey, 2002. The exocarp and mesocarp of a classic sweet orange are leathery and preserve the fruit.

The fruit's epidermis has a thick cuticle and a variable number of stomata, while the exocarp, or flavedo, is a layer of irregular photosynthetically active parenchyma cells that is green in immature fruit and turns orange as it matures, gap between cells. The albedo is the name given to the mesocarp. It's chock-full of vitamin C and sugars. Pectin and cellulose are both found in pectin. The flesh of the fruit is made up of the mesocarp and exocarp. The growth of carpels of the ovary, which are disposed of in the centre of the fruit, occupies the centre of the fruit. Several densely packed segments create a ring around the pithy axis. Each section grows out of the previous one. A single carpel with a thin, translucent endocarp or "ray" around it from which multicellular hairs sprout to fill each section each massive cells.

Higher plant's potential as a source of novel pharmaceuticals is yet mostly untapped. Only a small percentage of the estimated 250,000-500,000 plant species has been studied. Phytochemically, as well as the percentage subjected to biological or chemical tests. The number of people who go through pharmacological screening is significantly fewer, as a result. Only a phytochemical investigation of a particular plant will reveal. It has a fairly limited range of ingredients. Random as a method for discovering new biologically active compounds, screening.

As a method for discovering new biologically active compound screening molecules has been very fruitful in the field of antibiotics. The huge potential of plants as an antibacterial source. Antibacterial medicines are pharmaceuticals that have antibacterial activity. Currently I'm working on *Citrus sinensis* also known as sweet orange, is a member of the Rutaceae family. It is the most widely cultivated tree fruit on the planet. The sweet orange is a delicious fruit. Evergreen flowering tree that can reach a height of 9-10 metres height. Its fruit is a tonic, cardiogenic, laxative and fortifying fruit. Anti-helminthic and anti-fatigue. It has antibacterial, anti-inflammatory, and antioxidant properties. The leaves are lustrous and leathery, and they are alternately oriented. Oranges are thought to help with digestion and cholesterol reduction. Oranges have high concentration of vitamin C. Specially in the peel and the white layer directly beneath it. Citral, an aldehyde found in the peel, is anti-inflammatory. Vitamin A's action as a result, anyone who consumes large amounts of orange peel should be used to produce. Plant have been used for therapeutic purposes since the Vedic era period.

However, until a few decades ago, herbal medicine was widely used.

Because of this, natural medications have been supplanted by synthetic drug. Their immediate impact Allopathic drugs have side effects. Green medicines is reversing the global trend. Plant have long been thought to have therapeutic properties. Poultices and infusions of hundreds, if not thousands, of herbs have been used by people on all continents. Plants that have been around since the beginning of time. According to the World Health Organization (WHO). Plants would be the best source of a wide range

MATERIAL AND METHODS

PLANT MATERIAL COLLECTION AND IDENTIFICATION

The plant material used in this project Citrus sinensis leaves were collected from kodaikanal, Tamilnadu, India. Sample were bought to laboratory in polythene bags and cleaned thoroughly with fresh water to remove adhering debris and associated biota.

THE PLANT EXTRACT'S PREPARATION

Water was used to rinse the newly acquired plants and were dried in the oven using a moisture extraction system. The drying took place in a 65C over for four hours 36 hours. The dried leaves were ground into powder. Thomas Wiley mill type E.D.5 was used to grid the powder.

PREPARATION OF AQUEOUS EXTRACT

In 100mls of distilled water, ten grammes of crushed leaves were weighed and macerated. The ingredients were whirled vigorously. Following that, after a 24-hour period of interval stirring, the whatman No.1 filter was used to filter the mixture into a clean beaker (Azoro,2002), and the filtrate was concentrated until it was completely dry, evaporation in a steam bath bset at 100C. After filtering, the filtrate had the following colour: The ethanol extract was a dark brown colour. The Aqueous Extract exhibited a dark green colour. The extract were obtained after evaporation. As well as weighing 1.6g aqueous extract 1.6g ethanol extract. The extracts were kept in the refrigerator at a temperature of 32 F. For use, set the temperature to 0C. The yield was recovered in the form of the aqueous extract.

PLANT EXTRACT CONCENTRATION PREPARATION

Weighing 0.2g of each, the aqueous and ethanol crude extracts were reconstituted. 2mL distilled water, 2mL distilled water, 2mL distilled water, 2m 100mg/ml dimethyl sulphoxide, 50 percent assay for Disc Diffusion. Baker and his colleagues described a disc diffusion approach. Pallister (1998) was selected as a candidate for the presidency of the United States of

Amercian. Determining the antibacterial properties of extracts. Whatman Filter paper No.1 was utilized. Using a circular disc cutter, the filter paper was cut into round discs. A perforator with a 6mm diameter. The compact disc boiling for 30 minutes was used to treat it. Denature and entirely ruin the entire chemical that was employed in its preservation as well as to prevent the extract from becoming inactive include into the discs when the water has reached a boil, remove it from the heat. Included into the discs when the water has reached a boil, remove it from the heat. The disc was placed in a glass.

DETERMINATIONS BASED ON A CLOSER LOOK

MOISTURE CONTENT

Moisture content each sample's moisture content was measured. The gravimetric approach was used to determine the results. A fresh samples (five) were weighed and their weights were calculated was put into a previously weighed container. Wetness can be dried for three hours using an electric dryer 105 C in the oven, chilled in a desiccators, and reweighed. This procedure was carried out once at regular intervals until a consistent weight was achieved. The amount of moisture lost was calculated and expressed as a proportion of the total weight of the sample.

CONTENT OF ASH

The furnace determined the ash content. Gravimetric method of incineration a calculated. Each sample was weighed(5g) and placed on a Porcelain crucible wasd previously weighed and at 550C, it was placed in a muffle furnace. The sample was left to burn until it turned grey.

CONTENT OF FATS

Using a continuous solvent extraction approach, the fat content was assessed. Extractor for Soxhlet. Each has a gramme (5g) of each a weighted porous wrapped was used to wrap the sample sheet of paper (Whatman filter paper No 40). In a Soxhlet column, a wrapped sample was deposited. Mounting a flask on top of a weighted oil extraction flask comprising around 300mL petroleum ether (40-45%) (Boiling point: 60C). The sample that had been package was the fat content was determined by defatting twice. The weight difference between each sample is calculated and expressed. As a proportion of the total weight of each sample.

AMOUNT OF PROTEIN

The sample's protein content was the Kjeldahl method was used to determine the value. 0.5grammes of 10cc of water added to each sample. In a Kjeldahl, concentrated sulphuric acid (H₂SO₄) flask for digestion A selenium catalyst pill was used. Added to it, and the result was a concoction that was digested

by heating under a fume cupboard until a clear. A solution was found. Each of the digest was carefully transferred into a 100 mls volumetric flask and made up to the mark with distilled water.

CONTENT OF CARBOHYDRATE

Arithmetic difference was used to estimate the carbohydrate content of the test samples. The carbohydrate content of the food was calculated represented as NFE (nitrogen free extract) as follow % CHO (nitrogen-free extract)=100-percent where a denotes protein fat, c denotes ash, and d denote fibre.

BIOCHEMICAL TEST

TEST FOR INDOLE

Take sterilized test tubes containing 4ml of tryptophan broth. Inoculate the test tubes aseptically by taking the growth from 18 to 24 hrs culture. Incubate the tube at 37C for 24-28 hours. Add 0.5 ml of Kovac's reagent to the broth culture. Observe for the presence or absence of ring.

CATALASE TEST

Transferred pure culture of organisms from the agar to a clean glass slide with a plastic loop. Immediately add 3 drops of H₂O₂ into slide. Observe the release of the bubbles it show the positive result. Take about 3 to 4 ml of H₂O₂ into a test tube. A small amount of bacterial culture was introduced into the test tube with the help of inoculation loop. Observe the release of air bubbles and find out the result

TEST FOR TRIPLE SUGAR IRON (TSI)

Triple sugar iron slant are prepared is sterile condition. Prepare triple sugar iron slant are taken inoculated in the TSI tubes by streak method. Inoculation tubes were incubated at 25C to 37C for 24 hours. After the incubation the test tube were taken and the result were notes.

TEST FOR METHYL RED (MR) AND VOGES-PROSKAUER

Take a sterile test tube and prepare MR-VP broth. Using sterile inoculation loop the test organisms are inoculated to the MR-VP Broth. The test tube are incubated at 37C for 24-48 hrs. Then add 5 drops of methyl red indicator into the test tubes and observe the result. To prepare the MR-VP medium. After incubation add VP- reagent I and II to the set of test tubes as well as maintained the control tube. Shake the tube gently for 30 seconds will the caps of the two expose the media to O₂.

UREASE TEST

The given culture was inocubated with Christensen urea agar tube. The inoculated tube was incubated at 37 C for 24-48 hrs. After incubation period the result were observed.

CITRATE TEST

Inoculate the test organisms in the simmon's citrate agar medium. Incubate at the 37C for 24 hours. Observe the colour change green to blue colour.

BACTERIAL STRAIN

Bacterial strains that have been tested *staphylococcus aureus* and *Escherichia coli* were the germs utilized in thr antibacterial test. Clinical isolates from E. coli

ANTIBACTERIAL ACTIVITY TEST

Muller Hinton agar was prepared.15-20ml of Muller Hinton agar was poured into petriplates and allowed to solidify. Agar surface of each plate was streaked by a sterile cotton swab with the reference bacterial strain. Agar plate was punched with a sterile cork borer of 4 mm size marked as A,B,C,D.100 µl of aqueous extract, ethanol extract, gentamycin and streptomycin poured with micropipette in the well. The plates were allowed to stand by for 30 minutes. The plates were incubated at 37 ° C for 24 hours. After incubation observed the result.

RESULT AND DISCUSSION

PERCENTAGE EFFECTS PLANT EXTRACTS YEILD

Plant extract yields as a percentage of total yield calculated as a percentage of the starting amount seen in a pulverized sample of plant material. Citrus sinensis ethanol extract while giving a yield (16g) equals 16 percent. The aqueos produced a yield (1.5g) that corresponded to 15% of the total Plant Extracts Have Antibacterial Activity.

PHYTOICHEMICAL SCREENING RESULT

The test's preliminary phytochemical screening citrus sinensis plants revealed the resence of alkaloid, saponin, flavonoid, steroid, tannin, alkaloid, saponin, flavonoid, steroid. The absence of cyanide and the presence of triperthenes glucoside.

PLANT EXTRACTS HAVE ANTIBACTERIAL ACTIVITY

Agar disc were used to test the antibacterial activity of a *citrus sinensis* extract in vitro. Diffusion was tested against four different bacterial species. The highlights the suppression of microbial growth ethanol and aqueous extracts of the plant species that have been tested. The aqueous extract is a mixture of aqueous extract and a citrus sinensis had a prominent zone of inhibition of *Escherichia coli* with a diameter of 7.00mm.

MINIMUM INHIBITORY CONCENTRATION

The test is used to determine how effective a drug is. The MIC was calculated using the ethanolic method. *Citrus sinensis* orange aqueous extracts the organism were subjected to a two-fold serial dilution test.

Utilising the disc diffusion method. As a previously stated, none of the dilutions indicated any zone of they were resistant to inhibition. That's how the MIC works was a terrible waste of time.

PHYTOCHEMICAL SCREENING RESULTS

The test preliminary phytochemical screening had little or on inhibition zone, as well as. the impact of ethanol extracts on all of the tests creatures whose inhibition zones were not more than or equal to 3.00mm. Gentamycin (2mg/ml) has the opposite effect. (positive control) revealed large areas of all of the test organisms are inhibited, which is in comparison to the concentration of 100mg/ml.

The inhibitory activities of ethanol extracts were found to be somewhat higher than those of aqueous

extracts, except on all of the test organism. The aqueous extract was tested on *Escherichia coli*. There was no inhibiting effect. Despite this, there is a minor difference. As a result, it can be assumed that the possible that the active ingredients in the plant herb are more potent, ethanol-soluble, as used in ethnomedicine. Regardless of the ethanol extract's presence, aqueous extract has higher inhibitory properties. Antibacterial action was minimal. Results the size of the beads varied from 1-3mm in diameter. Consequently the inhibitory concentration at its lowest level (MIC). There was no zone of inhibitiob detected. The MIC result can be linked to the fact that its initial concentration was decreased by twofold repeated dilution. From a concentration of 100mg/ml to a concentration of 10mg/ml.

ANTIBACTERIAL ACTIVITY OF THE EXTRACTS

BACTERIAL SPECIES	AQUEOUS	ETHANOL
<i>Escherichia coli</i>	+	+_
<i>Pseudomonas aeruginosa</i>	-	+_
<i>Klebsiella pneumioniae</i>	+_	+_
<i>Staphylococcus aureus</i>	+_	+_

Key +=Inhibition (>3.00)

+_ =Trace

- =Resistant

PERCENTAGE YEILD OF THE CRUDE EXTRACTS OF *CITRUS SINENSIS*

PLANT SPECIES	EXTRACT TYPE	WEIGHT OF PULVERIZED SAMPLE USED	WEIGHT OF EXTRACT PERCENTAGE OF YEILD OF EXTRACT
Citrus sinensis	Aqueous	10.0g	1.5g 15%
	Ethanol	10.0g	1.6g 16%

BIOCHEMICAL CHARACTERITATON ION OF BACTERIAL ISOLATED FROM PATIENT DENTAL CARIES

S. No	Gram	Shape	Citrate	Indole	Mr	Catalase	Urease	Gas	Organism
1	-	Rod	-	-	+	+	+	+	<i>K.pneumoniae</i>
2	+	Rod	+	-	-	+	+	+	S.aureus
3	-	Rod	+	-	+	+	+	+	<i>Proteus Mirabilis</i>

CONCLUSION

The findings of the plant extract continue the various searches for new information. Plant based medications that are less the are effective. Accessible to those from poor socioeconomic backgrounds in the treatment of infectious disorders bacteria. Antimicrobials have the potential to be developed from as a result, higher plants appear to be rewarding. To the developed of a phytomedicine that can be used as a treatment against microorganisms antimicrobials derived from plants. As they have immense therapeutic potential can accomplish the goal with fewer adverse effect these are frequently linked to synthetic antimicrobial. More research is required antimicrobials originating from plants are being

investigation of plants, as well as their pytochemical method of action should be investigated further

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