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Antimicrobial and antihelminthic activities of *beetroot plant*

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Abstract

The antimicrobial and antihelminthic activities of beetroot plant was analyzed in this work against some bacterial isolates of clinical significance, the isolates were obtained from the University of Port Harcourt teaching hospital and tested against the beetroot extract. The disc diffusion and well in agar technique was used for the antimicrobial analyzes and the bacteria isolates used include *Escherichia coli*, *Bacillus* sp., *Klebsiella* sp. *Salmonella* sp., *Staphylococcus auerus* and *Candida albicans*. The juice extract of the beetroot ample proved to be more effective on the bacterial isolates than the ethanol extract; the extract was effective against Staphylococcus sp., *Bacillus* sp. and *Escherichia coli* but wasn't effective on *Salmonella* sp. and *Klebsiella* sp. the beetroot samples was less effective on the worm. The phytochemical compounds present in beetroot samples include saponin, tannins, alkaloids, flavonoids, cardiac glycosides. From the result obtained in this study, it shows that beetroot plant is very nutritional and can serve as a source of a variety of nutrients and natural occurring chemical compounds.

Keywords: Antimicrobial; Antihelmintic; Beetroot and plant

1. Introduction

The exploration of new medicinal properties of various plant specifics has induced the attention of the scientists towards biologically active compounds for every illness known to man; nature has provided an answer in the form of one herb or a combination of roots and other plant part. According to the Oxford Advanced Learners Dictionary, a herb (root) is a plant with a soft stem that dies down to the ground after flowering and whose seeds and leaves are used in medicine. Since the last couple of decades, the reason behind this is that bioactive compound, posse's potential pharmacological activities (qualities). Early man considered most illness to be the effects of the super natural because plants were so central to medicine throughout history to having a culture effect on most of these illness. The plant root system constitutes the major part of plant body.

It is the view of herbalist that all plants were specifically created to cure certain disease (Krutch, *et al*., 1976) this suggest that the number of herbs known to man may exceed that the modern drugs since practically all plants have been used by men in ancient times as a cure of illness. Modern scientists have disproved this view from their studies. We can see that some plants exist which are of known medicinal value or which may even be harmful to life.

The beetroot is a plant that has natural antianamic, antibacterial, antcarcinogenic, antipyretic, antiiodiant, antisclerotic, detoxicant and diuretic properties. This medicinal plant has been used as a medicine to 2000 years.

Different parts of plants (roots, leaves, flowers, fruit, stem, bark, and have been successfully used to treat numerous diseases. Owing to their antioxidant activity, they can influence a number of physiological processes. Thus protecting

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the organs from the damaging effect of free radical and inhibiting the development of unwanted microorganism (Ames, 1995) However, synthetic antioxicts, such as butyrate hydroxyltoluene (BHT) reaction of lipid peroxidation, have been proven to be carcinogenic and to cause liver damage, the use of plants in the food industry in place of synthetic preservatives antioxidants or other food additives have significantly increased over the last few years due to their ability to produce biological active substances.

Objectives

- To examine the antimicrobial effect of beetroot plant on some clinical isolates.
- To examine the antihelmintic effect of beetroot.
- For recommendation and nutritional health and benefit of beetroot.

From the middle age, beetroot was used for treating varieties of condition especially illness relating to digestion and blood. Beets are believed red to originate along the costs of Mediterranean (sea beets) and were first cultivated for their edible leaves. These beets were white and black and became known as Roma-beets. By the end of the 15th century, cultivated forms of beets were found throughout Europe and used not only or their leaves but also for the roots.

The sugar beets also originally a long pointed white roots, was developed in the 1700s by the Prussians for its sugar contents, originally sugar beets contain 6% sugar content and today the sugar beet has been cultivated to contain up to 20% sugar contact.

Beet root has been regarded as a laxative, a cure for bad breath, coughs and headaches and even as aphrodisiac. It is grown widely in Germany and France and in laser amounts in other European countries, Africa Asia, and South America.

The beetroot plant serves as indispensable constitute of human diet supplying the human body with mineral, vitamins and certain hormone precursors in addition to protein and energy (Oyenuga *et al*., 1975). Seeds have nutritive and calorific values which make them necessary in diets (Odemelam, 2005). It is also rich in betaine which is prescribed to lower toxic levels of homolyste which contributes to the development of heart disease, stroke and peripheral, vascular disease and healthy liver function (Vali *et al*., 2007) and contains a significant amount of vitamins A, C, and also calcium, 1ron, phosphorus, potassium, protein, and carbohydrates, (Chinoyedua *et al*., 2009). The derivation of essential minerals like iron would much like others serve as a broad treatment for cancer (Hunell, 2003). The beetroot greens are high in vitamin A. Like many modern vegetable beetroots was first cultivated by Romans by the 19th century it held great commercial value when it was discovered that beetroot be converted into sugar which serves as a source of carbohydrate.

Plants are primary source of medicine, fiber, food, shelter and other items in everyday used by humans with roots, stems, leaves and flowers, fruits and seeds providing food for humans (Hemingway, 2004). A wide variety of plants have been used in past and present times in the treatment of various diseases. The juice is also good for blister and cleansing digestive quality of open obstructions of the Liver and spleen-good for headaches and all affection of the brain. The juice is also good for blisters and blains of skin and as decoration water. (William Booth, 1810).

2. Material and methods

The beetroot samples was purchased at fruit garden market in Port Harcourt and packed in a sterile container

2.1. Sample collection

The beetroot samples was purchased at fruit garden market in Port Harcourt and packed in a sterile container

2.2. Materials

Petri dishes, conical flasks, test-tubes, measuring cylinders, Pipette, glass slides, 1.8mm sieve plate, blender, wire loop, inoculating needle, disk, forceps, masking tape, swab stick, cock borer.

2.3. Sterilization

Glass wires such as conical flasks, test tubes, bijou bottles, Petri dishes were sterilized by autoclaving at 121℃ at 15 psi for 15 minutes. Other materials such as inoculating wire loop was sterilized by heating to red hot over Bunsen burner flame and the cork borer was dipped into ethanol sterilized before sterilization.

2.4. Media preparation

The appropriate quantities of each media used were measured based on the manufacturer's standard and poured into conical flask, then the required quantities of distilled water were added and then shake very well to dispensed and particles and sterilized by autoclaving. After sterilization about 20 ml of each media was ascetically poured into the Petri dishes and 5ml into the test tube for slant and stab cultures. The media used were nutrient agar (NA) and saboraud dextrose agar (SDA).

2.5. Nutrient agar (NA)

This is a general purpose medium used for the cultivation of microorganisms. The medium was used for the enumeration of total viable bacteria count (TVBC) and was prepared by dissolving 28 g of powder in 1000 ml of distilled water, swirled to homogenize and sterilized at 121℃ at 15 psi for 15 min allowed to cool before dispensing into Petri dishes.

2.6. Seaboard dextrose agar (SDA)

This medium is recommended for isolation and identification of fungi 39 g of powder was suspended in 1000 ml distilled water and sterilized at 121℃ for 15 psi at 15 mins, after which it was allowed to cool and 1ml of lactic acid was added before dispensing into Petri dishes.

2.7. Preparation of beetroots samples

The beetroot samples were washed and grinded using a greater after which the water content was extracted.

2.8. Collection of clinical isolates

Pure clinical isolates of *Staphylococcus* sp., *Bacillus* sp., *Klebsiella* sp., *Escherichia coli*. and *Salmonella* sp. was obtained from patients at the University of Port Harcourt Teaching Hospital and was cultured by swabbing on the surface of the prepared nutrient agar and incubated at 37℃ for 24 hours. Further test were done to identify and characterize the isolates obtained from the hospital.

2.9. Characterization of the isolates

The isolation and characterization of bacterial isolates were based on cultural, morphological and biochemical test. The cultural characteristics of isolates which include the colony shape, size, surface texture, elevation, pigmentation were determined by direct observation of the colonies on the cultured plates and Gram staining was also done to differentiate between Gram positive and negative organisms.

2.10. Gram staining

Gram staining was carried out to differentiate between Gram positive and negative organisms. A smear was made on the glass slide using a sterile wire loop to pick a drop of distilled water and placed on the slide, the wire loop was sterilized again by flaming and an inoculums was picked and placed on the slide and heat fixed by passing the slide over the Bunsen burner flame. The slide was flooded with crystal violet (primary stain) for 60 seconds, rinsed with water to wash unbound dye. Gram's iodine (mordant) was added for another 60 seconds and rinsed with water after which the decolorizing solvent (95% alcohol) was added for 30 seconds to remove unbound dye and immediately rinsed with water followed by the addition of safranin for counterstaining (secondary stain) for another 30 seconds and rinsed with water. Excess stain was wiped off from the slide using cotton wool. The slide was air dried and examined under the oil immersion objective of the microscope (Chessbrought 1991).

2.11. Biochemical tests

The identities of the isolates were confirmed using biochemical tests (Cowan and Steel, 1994). Tests carried out include as indole, catalase, motility, oxidase, methyl-red production, voges-proskauer reaction, citrate and, triple sugar iron agar test (TSIA) and sugar fermentation tests for all isolates.

2.12. Indole production

The test was used to determine the ability of certain microorganisms to break down the amino acid tryptophan in the medium into indole in the presence of the enzyme tryptophanase. The test organism was inoculated into test tubes

containing 10ml of sterile tryptone broth and incubated for at least 48 h at 35-37℃. After which 0.5ml of Kovac's reagent was added to the media and shoked gently and examined for a red colour in the surface layer which indicates a positive result and no colour change indicates negative result. (Chessbrought 1991).

2.13. Catalase test

This test was done to differentiate between bacteria that produce the enzyme from non-catalase producing bacteria.

A drop of distilled water was placed on a clean glass slide after and a colony was picked using a sterile inoculating loop and emulsified with water and a drop of hydrogen peroxide. The production of bubbles is an indication that oxygen was given off which indicates a positive result (Chessbrought 1991).

2.14. Citrate utilization

This test was used to determine if an organism can utilize citrate as its sole source of carbon and energy. The citrate test uses a medium in which sodium citrate is the source of carbon and energy. In the simmon citrate agar, the pH indicator is bromothymol blue, which is green neutral pH and becomes blue when the medium become alkaline.

Slopes slant of Simon's citrate agar was prepared in bijou bottles and the test organisms was inoculated by streaking the surface and stabbing the butt with a sterilized inoculating needle and incubated at 35℃ for 48 h and was observed for a bright blue colour in the medium which indicates a positive result (Chessbrought 1991).

2.15. Methyl red and voges proskauer tests

This test is made up to two tests; methyl red and voges proskauer test. The methyl red indicates the production of sufficient acidic products from the fermentation of glucose while the voges proskauer test indicates the production of acetoin from the fermentation of glucose. The MR-VP broth (10ml) was inoculated with the test organisms and incubated for 48 h. After incubation, 5ml of the test culture was transferred aseptically to a clean test tube for the VP test. 3-4 drops of methyl red was added to the first test tube. A positive reaction is indicated by a distinct red colour showing the presence of acid. A yellow colour indicates a negative result.

For the voges- psokauer test, 0.6ml of alpha-napthol and 0.2ml of 40% potassium hydroxide was added to the second test tube. The broth was left to stand for 15 min for colour development after thorough agitation. If acetoin was produced, there will be a red colour change. A yellow to brown colour indicates a negative result (Chessbrought 1991).

2.16. Oxidase test

The test was used to determine the presence of cytochrome oxidase. Kovac's oxidase reagent is turned purple by organisms containing cytochrome C as part of their respiratory chain. Few drops of kovac's oxidase reagent was put on strips of filter paper and allowed to dry, sterile wire loop will be used to pick a loopful of the isolates from the culture media and streaked across the filter paper and will be observed for a colour change (Chessbrought 1991).

2.17. Motility test

The motility test was done to determine if the organism is motile or not motile by moving away from the line of inoculation. A sterile wire loop was used to pick an isolate, stabbed directly into the Centre of the test tubes containing the motility agar and incubated for 18-24 h at 37℃. A diffused growth away from the line of inoculation indicates a positive result and no diffused growth indicates a negative result.

2.18. Starch hydrolysis test

This test was carried out to ascertain if the organism is capable of utilizing glucose or starch as its sole source of carbon on a solid media after incubation. The Petri plate containing the test organism was flooded with gram's iodine and the presence or absence of a zone of clearance was observed (Chessbrought 1991).

2.19. Sugar fermentation test

This test was done to check the ability of the organism to ferment carbohydrate as its sole source of carbon, the triple iron agar contains three sugar; glucose, sucrose, lactose in the ratio 1:10:10. If the bacteria ferment sucrose and / or lactose, it turns yellow but if only glucose was fermented, the agar turns yellow from the acid produced.

2.20. Triple sugar iron agar test

The triple sugar iron agar was weighed and dissolved in distilled water 10 ml was dispensed into test tubes, autoclaved at 15 psi, for 15 min and 121 ℃. After which it was slanted and allowed to cool. The isolates was inoculated into sterile test tubes using inoculating needle and streaked across the top of the slant and incubated for 24 h at 35-37 ℃. After which colour change was observed indicating a positive result and gas production was observed.

2.21. Phytochemical screening

The beetroot samples was screened for their qualitative phytochemical screening using qualitative analysis showed the presence of many constituents including alkaloids, tannins, phlobatannins, cardiac glycosides, flavonoids, saponin etc.

2.21.1. Alkaloids

0.5 g of the plant materials and 10 ml of 5% dilute hydrochloric acid was heated for 5 min, filtered and 2 ml of Dranfend off, Hager's, Meyers was added to the filtrate. Any yellow or brown precipitate is taken as positive result.

2.21.2. Tannins

0.5 g of the plant materials and distilled water is heated on a steam bath for 5 min, allowed to cool and filtered; 2 ml of ferric chloride (5%) was added and observed for a blue- black precipitate which is a positive result.

2.21.3. Phlobatannins

0.5 g was shaken or stirred with a magnetic stirrer for 15 min, filtered and 1% hydrochloric acid is added and het for 30 min, observed for colour change.

2.21.4. Anthraquinones, free and combined

0.5 g of the samples is extracted with 100 ml of chloroform and stirred and allowed to dissolve for 20 min, the filtrate was added 10% Ammonia solution. A red or violet colour at the ammonia lower indicate the presence of free anthraquinones (Bonthragers free hydroxy anthraquinnones).

2.21.5. Combined Anthraquinones

Dilute sulphuric acid 10% is used to extract the plant the plant on heat, benzene is added and swirled and the benzene layer is collected and 2 ml of ammonia solution was added. A red or violet colour in the ammonia lower layer indicates presence of combined Anthraquinones.

2.21.6. Flavonoids

0.5 g of the sample was boiled with 10 ml of distilled water for 5 min and filtered. A piece of magnesium metal was added to the filtrate in the test tube, 2 ml of concentrated sulphuric acid is added to react with the metals and to form a layer between the acid and plant filtrate with a ring at the inter phase, the presence of flavones (flavonoids).

2.22. Carbohydrate, test for reducing and presence of sugar

2.22.1. Reducing sugar

0.5 g of the plant is also boiled with water and treated with Fehling solution A+B and heated and colour change was observed during heating.

2.22.2. Saponin

The plant sample was shaken vigorously with 20 ml of distilled and allowed for 15-20 min. if the forming persist for duration of 20 min, it indicates the presence of saponin.

2.22.3. Emulsion test from the frothing test

2 ml of olive oil is mixed with the filtrate and shaken to form a cloudy or slippy formation by the side of the test tube. This indicates the presence of saponin.

2.23. Cardiac glycosides

Liebermann test, the samples was extracted with 10 ml of methanol filter, 2 ml of 3.5% dinitrobenzoic acid and 1 ml of NaOH was added, if an immediate violet colour is observed which indicates a positive result.

2.23.1. Salrvoski test

10% chloroform is used to extract 0.5 g of the plant and 2 ml concentrated H_2so_4 is used to form a brown interphase which gradually changed to blue- green indicating the presence of steroids nucleus.

2.23.2. Keller killiani test for de-oxy sugar

0.5 g of the extract is exhausted in acetic acid containing 5 drops of 5% ferric chloride solution with 1 ml of sulphuric acid a brown ring at the interphase which indicate the presence of de-oxy sugar for cardiac glycoside.

3. Results and discussion

Result obtained in table 1 and 2 show that the phytochemical screening of beetroot plant (Beta vulgaris) include tannins, alkaloids, carbohydrate, saponin, cardiac glycosides but anthraquinones was absent for the qualitative screening and saponnins gave the highest frequency yield.

Table 1 Qualitative analysis of the phytochemical screening of beetroot samples

Phytochemical tested	Result
Alkaloids	
• Drangendorffs test	+
• Hager's test	+
• Meyers test	+
Tannins	
• Ferric chloride test	+
Phlobatannins test	
• Hydrochloric acid test	+
Anthraquinones test	
• Free anthraquinnonnes	-
• Combined Anthraquinnones	-
Flavonoids	
• Shinota test for presence of flavones	+
Carbohydrate	
• Molisch test for simple sugar	+
• Fehling test for sugar reduction	+
Saponin	
• Frothing test	+
• Emulsion test	+
Cardiac glycosides	
• Liebermann test	+
• Salwoski test	+
• Kller Killiani test	+

+= Present, -= Absent

These extracted chemicals have also been reported in similar studies (Adamu *et al*., 2007; Okullu *et al*., 2010). The extraction of these phytochemical from the beetroot could be attributed to the antimicrobial, anti-inflammatory and humectants properties of beetroot plant as reported by other researchers (Manosroi *et al*., 2010). The hemolytic, immune stimulating efficacy of saponin have been documented (Ray *et al*., 2014).

Flavonoids have been reported to have anti-oxidant free radical scavenging properties as they prevent oxidative cell damages and anti casinogenic (Pietta, 2000). Its presence in the intestinal tract also helps in prevention of heart diseases. (Okwu *et al*., 2004). Cardiac glycosides have been reported by (Terease *et al*., 2002) to be used in the correction of hear disorders and its presence in beetroot could be use in the treatment of diseases associated with the heart (Anyasor *et al*., 2011). Anthraquinnones were absent in the beetroot plant. The bacterial isolates used for the antimicrobial sensitivity were tested based on their implication in human diseases such as skin diseases, typhoid, urinary tract and respiratory problems. The bacteria isolates used include Bacillus sp., *Salmonella* sp., *Staphylococcus* sp., *Klebsiella* sp and *Escherichia coli*. And the fungal isolate used was *Candida albicans*.

Table 2 Percentage and quantitative result for the beetroot sample analyzed

S/no	Phytochemical analysed	Percentage (%)
1	Alkaloids	24.01
2	Tannins	13.15
3	Phlobatanins	2.15
4	Anthraquinones	1.15
5	Flavonoids	61.40
6	Carbohydrate	30.14
7	Saponin	61.42
8	Cardiac glycoside	15.05

In table 3, *Klebsiella* sp. and *Salmonella* sp. and *Candida albicans* were not susceptible to the ethanol beetroot extract but *Escherichia coli*, *Bacillus* sp. and *Staphylococcus* sp. were susceptible to the ethanol extract with 10 mm, 6 mm and 14 mm zones of inhibition respectively for the disc diffusion test and 8 mm, 8 mm, 4 mm for the well in agar during the antimicrobial testing.

Table 3 Antimicrobial activities of ethanol extract of beetroot plant on bacteria isolates

S/no	Bacteria	Disc method (zone of diameter inhibition)	Well in agar method (zone of diameter inhibition)
1	*Escherichia coli*	10 mm	8 mm
2	*Staphylococcus* sp.	14 mm	8 mm
3	*Klebsiella* sp.	0 mm	0 mm
4	*Salmonella* sp.	0 mm	0 mm
5	*Bacillus* sp.	6 mm	4 mm
6	*Candida albicans*	0 mm	0 mm

For the beetroot juice extract, *Klebsiella* sp. and *Salmonella* sp. and *Candida albicans* were not susceptible to the beetroot juice extract but *Escherichia coli*, *Bacillus* sp. and *Staphylococcus* sp. were susceptible to the ethanol extract with 10 mm, 5 mm and 16 mm zones of inhibition respectively for the disc diffusion test and 7 mm, 5 mm, 11 mm for the well in agar in the antimicrobial test in table 4. The anti-helminthic result showed that the beetroot plant extract was not completely effective on the worm after 24 hours of exposure to the extract.

Table 4 Antimicrobial activities of juice extract of beetroot plant on bacteria isolates

S/no	Bacteria	Disc method (zone of diameter inhibition)	Well in agar method (zone of diameter inhibition)
1	*Escherichia coli*	10 mm	7 mm
2	*Staphylococcus* sp.	16 mm	11 mm
3	*Klebsiella* sp.	0 mm	0 mm
4	*Salmonella* sp.	0 mm	0 mm
5	*Bacillus* sp.	5 mm	5 mm
6	*Candida albicans*	0 mm	0 mm

4. Conclusion

From the results obtained from this research work, the importance of beetroot (Beta vulgaris) cannot be over emphasized as it contains natural bioactive compounds of different importance that can serve as natural remedies for some infection and beetroot plant have little anti-helminthic effect which could be further investigated.

Generally the presence of the phytochemical in the beetroot plants suggest the importance of eating beetroot as it has medicinal properties and can be used traditionally and industrially. It can therefore be concluded that Beta vulgaris can contribute significantly to the health of both men and animals and should be used as a source of nutrients.

Compliance with ethical standards

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Disclosure of conflict of interest

None

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