

Invitro-Virucidal and Anti-Microbial Activities of Phytochemical Extracts from Garlic (Allium Sativum)

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Abstract: *The findings of the present study had demonstrated that the plant extract possesses anti-virucidal and anti-microbial activities according to standard screening tests and conventional accepted protocols. The results indicated that high yield of the extract was obtained at the end of the ethanol soxhlet extraction with the extract having greenish and smoothly textures. The phytochemical screening of methanolic and ethanolic extracts of Allium Sativum bulbs using soxhlet extraction method revealed the presence of tnnins, saponins and steroids in all the extracts. Flavonoid was present in ethanolic extract while alkaloid was present in methanolic extract. The results further revealed that SPyogenes was the most sensitive to the isolated compound while E. Coli was the least Kpneumonia showed high sensitivity to the isolated compound. The low concentration range for the MIC (2.1-ug/ml-4.0 ug/ml) and the MBC (2.ug/ml-18ug/ml) generally exhibited marked anti-bacterial activities and anti-virucidal potential against the susceptible organisms.*

Keywords: Anti-bacterial activity, bulb extract, AlliumSativum.

INTRODUCTION

At the onset of corona virus pandemic (Covid-19), it was obvious that the disease rendered pharmaceutical ingenuity impotent, ridiculed medical expertise and debased economic foundations. Phytomedically, it becomes necessary to stay safe and healthy by encouraging natural eating habits and stimulating herbal cures from vegetables, herbs, shrubs and medicinal plants from the tropics. Garlic has powerful anti-microbial and anti-virustalic qualities which can interfere with causal microbes behind diseases. It is also a powerful detoxifier which helps in getting rid of waste and toxins out of the body. It also improves immune functions of the body (Block, 1985). Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. It is a well-known fact that plants or herbs produce these

chemicals to protect themselves but recent research demonstrates that they can also protect Humans against diseases.

Resistance to antimicrobial agents has become an increasingly important and a serious global problem. Over the years bacteria in food and viruses in the environment have built resistance to drugs due to the over use or misuse drugs. There is therefore, the need for alternative means which are plant based (herbal cures) in tackling these pathogens. Phytonutrients give colour, peculiar smell and taste to the different parts of the plants such as the fruits, the leaves, the stem, tubers, flowers. With these, the plants are able to destroy or keep attackers at bay. Flavonoid-containing preparations have been used to treat human diseases and this class of natural products is becoming the subject of anti-infective research. Many scientists have isolated and identified the structures of flavonoids possessing anti-fungal, antiviral and antibacterial activity. Flavonoids represent a novel set of classic substances to be used as anti-infectives.

Ajoene, an active sulphurous compound found in the pungent vegetables when combined with antibiotics helps to break down bacterial defenses and viral infections. The natural compound found in garlic, allylsulphide improves the health of non-pathogenic bacteria in the stomach and also improves cognitive health in the elderly (Adoga and Ohaeri, 2006). The chemotherapeutic and potency levels of these phytochemicals present in AlliumSativum can be extracted and tested on widespread micro-organisms grown on different Agar media to establish their anti-microbial activity of garlic with respect to the biodiversity of these bacteria isolates will go a long way in the discovery of novel antibiotics and anti-viral agents to tackle infectious microbes (Abdullahietal, 2010 Anuradhaetal, 2006).

MATERIALS AND METHODS

Collection, preparation, identification of plant materials and phytochemical Analysis.

Procurement of raw material

The plant material, AlliumSativum was procured from a local market in Bokkos. The plant material; was identified by a Botanist in the Faculty of Natural and Applied Sciences, Plateau State University, Bokkos. The seeds were sorted for healthy ones, air-dried and milled using pestle and mortar in the General Laboratory of the department of Biochemistry. The reduced powdered material was stored in air-tight polythene bags protected from direct sunlight until when required for use.

Extraction Technique

A powdered substance from AlliumSativum (2kg) was extracted for 3 days with methanol (4L) by the process of maceration. The solvent was removed in vacuo to afford a brownish product (120gm). 100gm of the extract was suspended in distilled water and filtered. The filtrate was then partitioned with hexane and n-butanol to afford residual aqueous fractions.

Gel filtration

The butanol soluble fraction (2gm) was subjected to gel filtration using the process of sephadex LH-20 eluted with methanol. The level of separation was monitored using thin layer chromatography (TLC) method. Gel filtration process was repeated and a compound was isolated and labelled (SPE) Ethanol Soxhlet extract.

Donsunmate, a strong anti-microbial drug was purchased from a patent medicine store and used as a control.

Thin layer chromatography analysis

The TLC analysis was carried out on silica gel percolated glass plate using Butanol as a solvent, Acetic acid and water in the ratio 4:1:5. This solvent system was preferable to enable us view the spots on the plate. The spots on TLC were visualized under ultra violet rays (UV) sprayed with Gibbs reagent.

Phytochemical analysis and determination of phytochemicals

The filtrates were separately evaporated at 40⁰C using soxhlet extractor and used for phytochemical screening.

Test for Tannins

About 0.5g of the extracts were mixed thoroughly with 10ml distilled water and then filtered. 5ml of the filtrate was added to 1ml of 5% ferric chloride solution. The appearance of blue-black greenish precipitate indicated the presence of tannins (Sofowora, 1993).

Test for flavonoids

A few drops of concentrated Hydrochloric acid were added to a small amount of extracts of the plant material. Immediate development of red colour indicated the presence of flavonoids (Sofowora, 1993).

Test for Saponins

About 0.1g of powdered plant material was boiled with 10ml of water for 5 minutes and then filtered. After cooling, 5ml of filtrate was then diluted with water and shaken vigorously. The formation of persistent foam indicated presence of Saponin.

Test for Steroids

About 1ml solution of the plant extracts was added to 1ml sulphuric acid. The appearance of red colour indicated the presence of steroid (Sofowora, 1993).

Test for Alkaloids

About 0.5g of the extracts was stirred with 5ml of 1% Hydrochloric acid on a steam bath and filtered. 1ml of the filtrate was then treated with few drops of Mayer's reagent. A white precipitated was considered as an indication for the presence of alkaloids (Sofowora, 1993).

Anti-microbial Assay

The micro-organisms tested include staphylococcus aureus, streptococcus pyogenes, Bacillus subtilis, corynebacterium ulcerans, pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, salmonella typhi and candida albicans. All the organisms were clinical isolates obtained from the laboratory unit, Plateau state specialist Hospital, Jos, Nigeria. The corona virus isolate was obtained from bats captured in a dilapidated building in Bokkos area of Plateau state, Nigeria. Biochemical tests such as gram staining, Urease, citrate and indole were carried out on each isolated. They were maintained in agar slants in refrigerator (10⁰C) prior to use. Appropriate confirmatory biochemical tests such as gram staining, urease, citrate and indole were carried out on each isolate.

Susceptibility studies

Sensitivity discs of about 6mm in diameter were punched from whatman's no.1 filter paper using a file punch. This was then put onto a Bijou bottle. The sensitivity discs were then sterilized in an autoclave at 121⁰C for 15 minutes and then allowed to cool.

Sensitivity discs were prepared by weighing the appropriate amount of the extracts and dilution in Dimethyl Sulphoxide (DMSO) followed by placing the improvised paper discs in the solution such that each disc absorbs 0.01ml to make the potency of 100ug, 200 ug and 300ug (Cheeshrough, 2004).

Microscopy procedure for the bacterial isolates

The microscopic examination of the organisms was carried out according to the method described by Cheeshrough (2006).

Preparation of smear

Two (2) drops of normal saline was dropped on a clean slide. A loopful of the test organism was then transferred onto the normal saline and spread over a small area and allowed to air-dry. The dried smear was heated fixed by passing the slide through the Bunsen flame three times.

Gram staining

The slide was flooded with crystal violet solution for up to one minute, washed off with distilled water and drained. The slide was then flooded with iodine solution and allowed to act as mordant for one minute. This was washed up with distilled water and rinsed.

The slide was flooded with 95% alcohol for 10 seconds and washed off with distilled water. The slide was flooded with Safranin solution for 30 seconds, washed off and allowed to air-dry. The back of the slide was cleaned with a blotting paper. All slides were viewed under the microscope using oil immersion lens to confirm the isolates (Baher and Silverton, 1993).

Biochemical Tests

Catalase test

Three (3) ml of the hydrogen peroxide solution was poured into a test tube. Using a sterile wooden stick several colonies of the test organisms were removed and was immersed in the hydrogen peroxide solution and the result was recorded.

Urease test

With the use of a dense milky suspension of the test organism in 0.25ml Saline in a small tube, an Urease tablet was added and the tube was closed. The tube was incubated at 37⁰C in a water bath for up 4 hours.

A piece of filter paper was placed in a clean petridish and 3 drops of freshly prepared oxidase reagent was added.

A glass rod was used to transfer a colony of the test organism and smeared on the filter paper. The result was observed and recorded.

Indole test

The test organism was inoculated in a bijou bottle containing 3 ml of sterile peptone water. This was incubated at 37⁰C for 48 hours. 0.5 ml of kovac's reagent was added and shaken gently. The result was carefully observed and recorded.

Coagulase test

Each test organism was inoculated in a test tube containing 0.2ml of plasma and 1.8ml of normal Salina. It was then incubated at 37⁰C for 48 hours. Four (4) drops of 3% hydrogen peroxide was added. The solution was carefully observed and result recorded.

Oxidase test

A dense "milky" suspension of the test organism in 0.25ml Saline was collected in a small tube. An Urease tablet was added and the tube was closed and incubated at 37⁰C for 4 hour using a water bath. A piece of filter paper was placed in a clean Petri dish and 3 drops of freshly prepared oxidase reagent was added. A glass rod was used to transfer a colony of the test organism and smeared on the filter paper. The result was observed and recorded.

Rapid Antigen test

Strains of corona virus isolate was confirm using the rapid antigen test where anti bodies were detected to confirm the presence of the virus isolated from captured bats.

Preparation of turbidity standard

Barium sulphate suspension at 1.0% (W/V) was prepared as follows:

One percent (1% V/V) solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid in 99cm³ of water. One percent (1% W/V) solution of Bariumchloride was also prepared by dissolving 0.5g of dehydrated BarliumChloride in 50cm³ distilled water.

Barium Chloride solution (0.6cm^3) was added to 99.4cm^3 of sulphuric acid solution to yield 1.0% (W/V) Barium suspension. The turbid solution formed was transferred into a test tube as the standard for comparison (Osadebe and Ukwueze, 2004).

Standardization of inoculums

A loopful of each of the test isolates was picked using sterile wire loop and emulsified into 3, 4 ml of sterile physiological Saline. The turbidity of the suspension was then matched with that of 0.5 McFarlands standard

Sensitivity testing

Using sterile Swab stick, standardized inoculums of each isolate was swabbed onto the surface of Saboraud dextrose agar in separate petri dishes. Disc of the extracts was placed on to the surface of the inoculated media. Then, the plates were inverted and allowed to stand for 30 minutes for extracts to diffuse into the agar. After which, the plates were incubated aseptically at 35°C for 18 hours.

Zone of inhibition formed around each of the extracts was measured using a meter rule (National committee for Clinical Laboratory Standards, NCCLS, 1999).

Minimum inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MIC) of the extracts were determined using the tube dilution method. Dilution of the plant extracts were incorporated in nutrient broth in 1:1 ratio. Initial rough estimates of the MIC values of the plants extracts against the test organisms were estimated to determine the range of MIC values.

The following concentrations were prepared for each extract, using the dilution formula: 400, 200, 100, 50, 25, 12.5, 6.25 mg/ml. In addition, 0.1 ml of standard suspension of the test organisms was added to each tube. The tubes were incubated at 37°C for 24 hours. A tube containing extract and growth medium without inoculums was included to serve as control. The presence of growth (turbidity) or the absence of growth (clear solution) at the end of incubation period was recorded. The MIC was defined as the lowest concentration of the extract inhibiting the visible growth of each test organism (Vollekobial et al, 2001).

Minimum Bactericidal concentration (MBC)

The contents of the MIC tubes and the preceding tubes in the serial dilution were sub-cultured into appropriately labeled nutrient agar plates by dipping a sterile wire loop into each test tube and streaking the surface of the labeled nutrient agar plates. The plates were then incubated at 37°C for 24 hours after which they were observed for colony growth.

The lowest concentration of the Sub culture with no growth was considered as minimum bactericidal concentration (Vollekevia et al, 2001).

RESULTSTable 1: physical properties of *Allium Sativum* extracts.

Physical parameter	Ethanol	Methanol
Weight extracted (g)	24	24
Weight of extract (g)	6	7
Percentage yield (%)	4	3.4
Colour	Green	Green
Texture	Smooth	Smooth

Legend:

High yield (4%) of the extracts was obtained at the end of the ethanol soxhlet extraction with the extract having greenish and smoothly textured.

Table 2: results of phytochemical screening of *A. Sativum*

Phytochemical	Method	Ethanol
Tannins	+	+
Saponins	+	+
Flavonoids	-	+
Alkaloids	+	-
Steroids	+	+

+ = Presence of metabolite. - = Absence of metabolite the phytochemical screening of methanolic and ethanolic extracts of *AlliumSativum*bulbs using soxhlet extraction method revealed the presence of tannins, saponins and steroids in all the extracts. Flavonoid was present in ethanolic extract while alkaloid was present in methanolic extract.

Table 3: Results of susceptibility of SPE to various pathogenic test organism.

Test organism	Mean zone of inhibition (MM)	
	SPE (20mg/ml)	Donsunmate (5.ug/nil)
Staphylococcus aureus	20	19
Streptococcus pyogenes	21	23
Bacillus subtilis	0	25
Escherichia coli	16	13
Klebsiella pneumonia	20	12
Salmonella typhi	19	25
Pseudomonas aeruginosa	19	16
Candida albicans	0	0
Corona virus isolates	2	1

0 = NO activity.

Streptococcus pyogenes was the most while Escherichia coli was the least. However, Klebsiella pneumonia equally showed high sensitivity to the isolated compound.

Table 4: Minimum inhibitory concentrations and minimum Bactericidal Concentrations of SPE against the test organisms.

Test organism	Concentration (ug/ml)	
	MIC	MBC
Staphylococcus aureus	2.1	8
Streptococcus pyogenes	2.2	9
Bacillus subtilis	R.I	12
Corynebacteriumulcerans	R	R
Escherichia coli	4.0	18
Klebsiella pneumonia	2.1	4
Salmonella typhi	2.2	8
Pseudomonas aeruginosa	2.2	18
Candida albicans	R	R
Corona virus isolate	1.1	2

R = resistant and shows no activity against organisms the low concentration range for the MIC (2.1 ug/ml – 4.0 ug/ml) and the MBC (2 ug/ml – 18 ug/ml) suggests that the compound has a very good antimicrobial activity against the susceptible organisms compounds or substances with MICs of less than 100ug/ml are regarded as having strong antimicrobial activity as well as anti-virucidal potential.

DISCUSSION

Results indicate high yield (4%) of the extract obtained at the end of the ethanol soxhlet extraction. The extract had greenish and smooth texture. The high yield of the extract was as a result of high solubility of the plant material in the solvent. The higher yield of methanol extracts was in line with the findings of IWU and Okafor in 2005. Previous works had indicated that phytonutrients rich sources can be identified by the colour which is conferred on them by pigments. Phytonutrients and green colour of leaves and bulbs come from the chlorophyll (Ohaeri, 2003).

The phytochemical screening of methanolic and ethanolic extracts of Alliumsativum bulbs using soxhlet extraction method revealed the presence of tannins saponins and steroids in all the extracts. Flavonoid was present in ethanolic extract while alkaloid was present in methanolic extract. This agrees with the work of Oheari and Adoga, (2006) which showed that Tannins and Saponins when present in the plant, they exhibit antibacterial activities. This is in line with the findings of look and Okafor in 2005 when they reported the presence of carotenoids, flavonoids, phenols and phytoestrogens in bulbs and other plants. Again, several authors had reported that loss can be treated with extracts with of Alliumsativum due to high content of phytonutrients (I will, 2019; Iwu and Okafor, 2005).

Zero indicated no activity on the mean zone of inhibition S. pyogenes was the most sensitive to the isolated compound. While E.Coli was the least. However, K.pneumonia compound equally showed high sensitivity to the isolated. The observed anti-bacterial effect of the isolate

may be due to the presence Tannins, flavonoid and saponins which have been shown to possess anti-bacterial properties. This is in agreement with Luttrodtetal (1999) who carried out similar work on plant extracts of EucalyptusCamaldulensis against Saureus and EColi and attributed the ingredients. These phytonutrients have been shown scientifically to benefit human beings in disease management. They function either as anti-oxidants, anti-inflammatory agent or immune system booster: They also act as detoxifier of foreign substances especially in the liver where they also support a healthy liver (Ohaeri and Adoga, 2006).

The low concentration range for the minimum inhibitory concentration MIC (2.1µg/ml-4.0µg/ml) and the minimum bacterial concentration (MBC) (2µg/ml-18µg/ml) suggest that the compound has a very good antimicrobial activity against the susceptances with MICs of less than 100µg/ml are regarded as having strong antimicrobial activity as well as anti-virucidal potential (Luttrodtetal, 1999; Tang et al, 2003).

This result is in line with that of Williams et al (2009) who reported that the leaf extract of GuieraSenegalensis inhibited the growth of various micro-organisms at different concentrations (Williams et al, 2009). High minimum bacterial concentration value is an indication of low activity while low minimum bacterial concentration value indicates high activity as reported by Baker and Silverton (1993); Wu et al, 2007; and Yamada, (1991).

CONCLUSION

The research work supports the tradition use of Alliumsativum bulb extract for the treatment of various infections and demonstrated its role as an anti-bacterial agent for the treatment and management of infections caused by bacteria and viral particles. The study also provides that the plant extract has the potential for the production of potent, safe and cost-effective antimicrobial agents and anti-virucidal drugs.

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