UJPICR Volume 2 Number 1 :June, 2022
UMYU Journal of Pure and Industrial Chemical Research
Volume 2 No. 2 December, 2022.
ISSN:**2814 – 1628/2814 - 1636**
DOI: https://doi.org/10.55688/ujpicr22.001

Phytochemical Screening and Antibacterial Effect of Onion against Oral Pathogenic Bacteria

Usman Salifu Oma*[1], Onoja Alexander David[1], Salihu Ali Audu[1], Momoh T.B.[2], Buluku T. Gabriel[3], Okpanachi A.A[1]

[1]Department of Chemistry, Prince Abubakar Audu University Anyigba, Kogi State, Nigeria

[2]Department of Plant and Environmental Biology, Prince Abubakar Audu University Anyigba, Kogi State Nigeria

[3]Department of Chemistry, Benue State University, Markudi Benue State

*Corresponding Author: usman2great@gmail.com, +2347062184747

ABSTRACT

Dental caries remains a major health problem. This problem along with the antibiotic resistance has created a renewed interest to search for new other antimicrobial substances from various sources including medicinal plants. This study was carried out to evaluate the antimicrobial properties of onion and its effect on oral pathogenic bacteria responsible for tooth decay and mouth odour. Two were used in the study (shallot and red Creole), and their activities against the oral pathogenic bacteria were compared with fluoride toothpaste. The method of extraction employed was soxhlet extraction using methanol as the solvent. The extract at different concentrations was then applied to the cultured bacteria (*streptoccous mutans*). The results shows that Red Creole (*allium cepa* .varcepa) at 200mg/ml, 100mg/ml and 50mg/ml shows 6mm, 4mm and 3mm zones of inhibition, respectively, while the Shallot (*allium cepa* var. Aggregatum) species at the same concentrations respective concentrations shows inhibitions of 3mm, 1mm and 0. The results of chemical test carried out revealed that tannin, saponin, alkaloid, anthraquinones and flavonoid were present in the extract.

Keywords: antibiotic, antimicrobial, dental caries, inhibition, oral pathogenic bacteria

INTRODUCTION

Natural products are believed to possess biochemical and pharmacological characteristics that can promote many health-beneficial effects in treating diseases and hence, there is an increasing demand to seek for therapeutic drugs from these sources [1]. Due to the unmatched availability of chemical diversity and biodiversity, some of these natural products

involve medicinal plants, either as pure compounds or standardized crude extracts. To date, studies involving natural products especially plants have increased tremendously throughout the world particularly in edible ones and a number of collected evidences prove the ability of plants to exert therapeutic effects [2]. Their contribution towards different branches of study such as chemistry, medicine, and pharmacology and drug discovery is undeniable. The use of plants in Nigeria traditional practice as either extracts or infusion is a widespread practice in the treatment of common infections such as malaria, epilepsy, infantile convulsion, diarrhoea, dysentery, flatulence, tonsillitis, bacterial and fungal infections, mental illness and worm infections [3, 4].These antimicrobial attributes of medicinal plants are products of chemical constituents that may include alkaloids, tannins, glycosides, flavonoid and volatile oil. [5]. Approximately 50,000 people are killed annually due to various infections [6]. Thus, the development of antibiotics and other therapies are of absolute necessity and it is not too far-fetched to say that there are effective treatments or remedies that are readily available to treat different kinds of infections [7]. This is where plant-based natural products comes into picture, whereby studies which focus on the potentials of active compounds found in plants to act as antimicrobial agents are being conducted [8].To achieve this, modern scientific and technology are presently engage in active research to investigate the potency and activities of these plants. The aim of these studies is to determine the phytochemical constituents and investigate the antibacterial activities of two species of onion: Red Creole (*allium cepa .varcepa*) and Shallot (allium cepa var. Aggregatum) against oral bacterial.

Figure 1: Shallot onion (allium cepa var. Aggregatum)and Red Creole onion (allium cepa .varcep) (adapted from Seror, 2000)

Methods and Materials

The solvent used for plant extraction was methanol. The reagents used for phytochemical screening includes: 10% Hydrochloric acid (HCl) sigma Aldrich, 0.1M Hydrochloric acid, concentrated tetraoxosulphate (VI) acid (H_2SO_4) BDH of England, 2% tetraoxosulphate (VI) acid, 10% Ammonia (NH_3), 0.1M Sodium

hydroxide (NaOH) BDH of England, Dragendoff's reagent, Chloroform ($CHCl_3$) CDH Analytical Chemicals, Distilled water LOBA CHEM. The apparatus used in this study were weighing balance, measuring cylinder, test-tubes, test-tube racks, beakers, conical flasks, needles and syringes, funnel, filter paper, water bath, sample bottles, mortar and pestle, spatula, stirring rod and soxhlet extractor.

Collection and preparation of onion

The Bulbs of Red Creole and shallot onions were collected from Anyigba Market of Dekina Local Government Area, Kogi State. The bulbs were properly rinsed in water, air dried for 14 days, and then size-reduced into a coarse powder to increase surface area using mortar and pestle.

Preparation of methanol extract of the bulb

400ml of methanol each was added into two round bottom flasks that were attached to the soxhlet extractor and a condenser. The soxhlet extractor is placed on an electronic heating mantle (isomantle), 40g of the coarse powder of Red Creole (*allium cepa .varcepaand*) and Shallot (allium cepa var. Aggregatum) onions were loaded into the thimble of the soxhlet extractor. The isomantle is switched on and supplies heat to the soxhlet extractor for the extraction with methanol. The isomantle is switched off after the colour is fully discharge into the round bottom flask. The extract is then allowed to evaporate of the solvent giving a powdered extract.

Phytochemical Screening:

Chemical tests were carried out on the methanol extract of the bulbs using standard procedures to identify the constituents as described by Trease and Evans (1989)

Test for saponins (Frothing test)

To a small portion of the extract in test tube, 10 ml of distilled water was added and then shaken continuously for 30 seconds. The solution was allowed to stand for 5minutes; the formation of a persistent froth indicates the presence of saponins.

Test for flavonoids -Sodium hydroxide test

Two drops of 10% Sodium hydroxide was added to the solution of the extract, yellow coloration indicates the presence of flavonoids

Test for tannins–

Lead acetate test

To a small portion of the extract, 4 drops of lead sub acetate solution was added, the

formation of a cream coloured precipitate indicates presence of tannins.

Test for alkaloids–

Dragendoff's Test

The extract (0.2g) was dissolved in 2 ml of 1% aqueous hydrochloric acid with continuous stirring in a water bath. The mixture was filtered and few drops of Dragendoff's reagent was added, rose red precipitate indicates the presence of alkaloids.

Test for Anthraquinones- Bontrager's Test

A small portion of the extract was dissolved in 5ml chloroform,shaken and filtered. To the filtrare, an equal volume of 10% ammonia solution was added with continuous shaking, bright pink colour in the aqueous upper layer indicates the presence of anthraquinone.

Antibacterial Screening:

The materials used for this work: weighing balance, Petri dishes, disinfectants, pipette, measuring cylinder, glass rod, glass slides, autoclave, water bath, beakers, sterile cotton swab, pipette, microscope, aluminum foil, spatula, distilled water, methanol, hot air oven, spirit lamp, masking tape, Chocolate nutrient agar (CNA), nutrient broth and antibiotic.

Sterilization of glass-wares and equipment:

All glass wares were thoroughly washed with detergent and rinsed with distilled water. They were dried in hot air oven and then sterilized at 160^{o}C for 2 hours. Media were prepared according to manufacturer's specification and sterilized by autoclaving at 121^{o}C for 15 minutes. The working bench was disinfected before carrying out any experiment to avoid contamination and to ensure aseptic working conditions.

Preparation and Dilution of Extracts:

The dried crude extracts were collected in clean 30 ml beakers and left open for five days for complete evaporation of residual solvents. To get an initial stock concentration of 200 mg/ml, 2 g of dried extract was suspended in a clean test tube containing 10ml of sterile distilled water. A two-fold serial dilution was subsequently performed from the first dilution to get two (2) more concentrations: 100mg /ml and 50mg /ml.

Test on organisms:

Stock cultures of the test organisms were gotten from the laboratory of Good shepherd

hospital, Anyigba, Kogi State, Nigeria. Bacteria used to test the antibacterial activity of the extract were oral pathogenic bacteria which includes *Streptococcus mutans.*

Media preparation:

The media used included nutrient broth (Tulip Diagnostics Limited, India), nutrient agar (Titan Biotech Limited, India). All media were prepared according to the manufacturer's instructions.

Determination of antibacterial activities of the extract.

Antibacterial activity

The antibacterial activity of the extract was evaluated by a modified method of agar well diffusion assay [9]. About 25ml of prepared nutrient agar was poured into sterile Petri dishes at 50–60 °C and allowed to solidify under aseptic conditions for 3 hours. Subsequently, a sterile cotton swab was dipped into 24 hour bacterial suspensions of the test organisms (which had been standardized to turbidity of 0.5 McFarland). Each agar plate was inoculated appropriately by evenly streaking over the solidified agar media. Inoculated plates were left to stand for about 1 hour to allow the surface of the agar plates dry. Thereafter, six wells of 6 mm in diameter were made in each inoculated plate using a sterile cork borer. About 100µl of the test samples were dispensed into the wells using sterile pipettes. The plates were incubated at 37 °C for 24. All tests were replicated to minimize errors. The diameters of growth inhibition zones around the wells were measured using a vernier caliper, and the values of the zones of inhibitions were recorded in millimeters (mm).

Results and Discussion

The results of the phytochemical screening according to Table 1 of the extract indicated the presence of medicinally active constituents; the presence of tannins, saponins and alkaloids in these extracts explains the wide activities of the plant against the diseases caused by the test organisms in this study since they have been identified as highly antiviral and antibacterial agents [10]. Also Sherifat*et al.* [11] reported similar finding in the phytochemical component of Allium cepa (Onion).

As shown in Table 2 above, Red Creole (allium cepa .varcepa) extract showed maximum activity against the microorganisms tested, with 200mg/ml the

inhibition zone was 6mm, at 100mg/ml it was 4mm and at 50mg/ml it was 3mm It was also observed that there was no much difference in the effectiveness of the extract at the lower concentrations of 100mg/ml and 50mg/ml against the sensitive organisms. For the Shallot (allium cepa var. aggregatum) extract showed minimal activity against the organisms tested, with 200mg/ml it show inhibition zone at 3mm, 100mg/ml shows 1mm and none at 50mg/mmIt was also observed that there was little/no effectiveness of the extract at the lower concentrations of 100mg/ml and 50mg/ml against the sensitive organisms. Although the effect of onion extract showing better inhibitory on bacterial has been reported by Corzo-Martínez et al. [12] and Škerget et al. [13]. Also Kim [14] showed the effectiveness of onions extracts on various strain of bacterial (S.mutans JC-2, S. sobrinus OMZ176, P. gingivalis ATCC 33277 and P. intermedia ATCC 25611), and the effects were bactericidal against cultured and resting bacterial cells.

However the fluoride toothpaste (close up toothpaste) which serves as control specimen showed maximum activity against the organisms tested, at concentration of 200mg/ml shows 11mm 100mg/ml it give inhibition zone of 7mm and at 50mg/ml gives 6mm . It was also observed that there was much difference in the effectiveness of the extract at the lower concentrations of 100mg/ml and 50mg/ml against the organism.

The result showed that the extract demonstrated a concentration dependent antibacterial activities as degree of activities varies with change in concentration (higher concentration had higher effect). The methanolic extracts of Red Creole exhibited very strong activity against the oral pathogenic bacteria the shallot however on the other hand exhibited minimal activity.

Table 1: Phytochemical Constituents of Crude Methanol Onion Extract (CME) of Red Creole (*allium cepa .varcepa*) and Shallot (allium cepa var. Aggregatum)

Constituents	Test	Observation	Inference
Anthraquinone	Bontrager	No color change	-
Alkaloids	Dragendoff	Presence of a red precipitate	+
Saponins	Frothing	Formation of persistent froth	+
Flavonoids	Sodium Hydroxide	Presence of a yellow colored precipitate	+
Tannins	Lead acetate	Presence of a cream colored precipitate	+

+ = present, - = absent

Table 2: Antibacterial activity of methanolic extract of Red Creole (*allium cepa .varcepa*), Shallot (allium cepa var. Aggregatum) and Fluoride toothpaste on the tested microorganisms showing zones of inhibition in mm with various concentrations.

Species of onions used Plus control	**Organisms used**	**Concentration (µg/ml)**	**Zone Inhibition (mm)**
Red Creole (allium cepa .varcepaand)	*Streptococcus mutans*	200 100 50	6 4 3
Shallot (allium cepa var. aggregatum)	*Streptococcus mutans*	200 100 50	3 1 0
Fluoride toothpaste (Close up toothpaste**)** **Control**	*Streptococcus mutans*	200 100 50	11 7 6

Conclusion

The finding of this study provides an insight into the usage of this bulb in traditional medicine for the treatment of common bacterial infections and oral pathogenic bacteria responsible for mouth odour and tooth decay. It is concluded that Red Creole extract has a more significant antibacterial activity against *Streptococcus mutans* which is the major causative bacteria for tooth rot and mouth odour than Shallot. Therefore,

Onion extract can be considered as an antibacterial agent to prevent human dental caries.Fluoride toxicity is a condition in which there are elevated levels of the fluoride ion in the body. Although fluoride is safe for dental health at low concentrations, sustained consumption of large amounts of soluble fluoride salts is dangerous it can cause the following maladies; dental fluorosis, skeletal fluorosis, thyroid problems, acne and other skin related problems. Although the zone of inhibition of fluoride toothpaste is great; at elevated levels however it is harmful to the body than that of Herbal toothpaste in which onion is an active ingredient. I will suggest that the concentration of onion and other active ingredients present in herbal toothpaste be increased so as to match that of fluoride and as consequences herbal toothpaste should be a suitable replacement for fluoride toothpaste if well formulated.

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