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# PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL EFFECT OF *CUCURBITAPEPO* FRUITS (SQUASH) ON *STAPHYLOCOCCUSAUREUS.*

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## ABTRACT

The aqueous and organic extract of the fruits of Cucurbita pepo traditionally used for the treatment of infectious disease were screened for the presence of phytochemical and its antimicrobial activity against Staphylococcus aureus. Theethanolic extract (i.e. organic extracts) show the highest activity while the aqueous extract shows the least activity against the test organism. Preliminary phytochemical screening indicated the presence of Alkaloid, Tannin, Saponins, Anthraquinones, Glycoside and Flavonoids on both the extracts. The result also revealed that, the fruit extract of Cucurbitapepo had a bacteriostatic and bactericidal effect on Staphylococcus aureus, this will provide a scientific support for the local medicinal use of the fruit of Cucurbitapepo.

**Keywords:** *Phytochemical Screening, Antibacterial activity, Cucurbitapepo, Staphylococcus aureus and Antimicrobial activity*

## INTRODUCTION

Squash is the edible immature fruits of *Cucurbita pepo* L. It is a herbaceous, monoecious, annual plant belongs to family Cucurbitaceae and cultivated for human consumption (cooked flesh). Previous phytochemical studies on *Cucurbita* species led to the isolation of cucurbitacins, phenolic acids, phenolic glycosides, ascorbic acid conjugates, sterols, fatty acids and flavonol. (Mohamed et al., 2009)

In nature many plants and plants seed provided source of medicine at the earlier times. Plants have proven to be the most useful in curing diseases and provide an important source of pharmacy and medicine. Plants have great significance to

the health of individuals. The medicinal importance of these plants lies in some chemical substances that produce a distinct physiological action on the body of human. The major importances of these bioactive constituents of plants are Steroid, Terpenoids, Tannins, Carotenoids, Flavonoids, Alkaloids and Glycosides. Plants in all aspect of life have served as important material for drug development. Antibiotic and Antimicrobial components like Saponins, Glycosides, Flavonoids and alkaloids are found in plants. Medicinal plants are the foundation of many important drugs of the modern world. (Chonoko and Rufai, 2011). Plants are now playing an important role in many medicines like allopathic medicine, herbal medicine, homoeopathy and aromatherapy. Many of these local medicinal plants are used as spices and food items. Many plants are cheaper and more simply to get to most people especially in the developing countries and these plants have lower incidence of side effect after use (Spencer, 2008).

Phytochemical are non nutritive chemical compounds which occur naturally in plants, or the chemical which is derived from plants. The word Phytochemical came from Greek word *Phyto*– plant and chemicals. The term phytochemical is generally used to those chemicals that may have biological importance but are not established as important nutrients. In a narrower sense the terms phytochemical describe the number of secondary metabolic compounds found in plants. Scientists estimate that about 10,000 different phytochemicals having the capability to have an effect on diseases like cancer and metabolic syndrome (Schinor, 2007).

Antibacterial activity is method to destroying or suppressing the growth or reproduction of bacteria. The term antibacterial terms derives from Greek word —anti that means against. The compound which destroys or suppresses the growth or reproduction of bacteria, and that type of compound or agent having such properties is called antibacterial agent or antibacterial compounds. These are the either drugs or any plants material that destroy or inhibit the growth of bacteria, chemotherapeutic agents also having ability to prevent or treat bacterial infections (Tshibangu, 2002).

*Staphylococcus aureus* is the most common cause of *Staphylococcus* infection. It is

spherical bacterium, frequently living on the skin and in the nose of a person. *Staphylococcus aureus* can cause a range of illness from minor skin infections such as pimples, impetigo, boils, scalded skin syndrome and abscesses to life threatening diseases, such as pneumonia and toxic shock syndrome (TSS). Its incidence is from skin, soft tissue, respiratory bone joint, endovascular wound infections (Klein, 2002).

*Staphylococcus* food poisoning is the major types of food intoxication caused by improperly stored or cooked food, particularly food such as processed meat, chicken salad and ice-cream in which *Staphylococcus aureus* grow (Klein, 2002).

## Methods

*Cucurbita pepo (*Squash) fruit was bought from Damaturucentral market in February, 2016. Authentication of the plant species was done by comparing it with the voucher specimens available at the botanical Laboratory of the Department of Science Laboratory Technology. The squash was properly washed and sliced. The back peel and seeds were air dried, ground to powder as described by Mukhtar and Tukur (1999).

## Extraction

The aqueous extract of the powdered fruit of the *Cucurbita pepo* (60g) was added to 100cm$^3$ distilled water in a 500cm$^3$ beaker. The content was boiled for 20minute, and allowed to cool, filtered through a cotton wool and re-filtered using a filter paper.(Dobell, 1993).This gave aqueous extract. The *Cucurbita* (100g) was steeped in ethanol for 23 hours in a conical flask. The content of the flask was stirred using magnetic stirrer, the extract was filtered. This gave ethanol extract.

Soxhlet Extraction method was then used with ethanol as the solvent. In each case the solvents were evaporated to obtain the extract using rotary evaporator (Fatope et al., 1993).

## Phytochemical screening

### Test for Saponins

The plant extract (1cm$^2$) was transferred into a test tube. Distilled water (1cm$^2$) was added to the test tube and shaken vigorously. Persistent froth that last for about 15 minutes would indicate the presence of saponins (Sofowora, 1993).

### Test for Tannins

Two ml of each of the extracts, 2cm$^2$wasdissolved in distilled water in separate test tubes and 2-3 drops of 5% ferric chloride (Fe

$Cl_3$) was added. A green –black or blue colouration would indicated the presence of tannin (Ciulci, 1994)

**Test for Flavonoids**

To Each of the extracts ($2cm^2$) was transferred into a test-tube. 10% sodium hydroxide($1cm^3$) was added followed by the addition of 3 drops of dilute hydrochloric acid (HCl). A change in colour from yellow to colorless indicate positive test (Trease and Evans, 2002).

**Test for Alkaloids**

Each of the extracts ($1cm^3$) was stirred with 1% aqueous HCl ($3cm^3$) on a hot water bath and then filtered. Each of the obtained filtrate was treated with Meyer's reagent. A precipitate indicates presence alkaloids (Trease and Evans, 2002).

**Test for Steroids**

Salkowski test was adopted in which each of the extracts ($2cm^3$) was transferred into a test tube containing chloroform ($2cm^3$), concentrated ($H_2SO_4$) was subsequently added to form a lower layer. A reddish brown ring at the interface of the two liquids and a violet colour in the supernatant layer indicated the presence of steroids (Sofowora, 1993).

**Test for Anthraqinones**

Each of the extracts ($1cm^3$) was shaken with $5cm^3$ of benzene, followed by $1cm^3$ of 10% Ammonia and shaken. The appearance of pink, red or violet colour in Ammonia (lower) phase indicate the presence of Anthraqinones (Shelland, 1994).

**Test for Glycosides**

50% $H_2SO_4$($10cm^3$)was added to $10cm^3$ of each of the extract in a test tube, the mixture was heated in boiling water for 15minute and $10cm^3$ of Fehling's solution was added and the mixture was boiled. A brick-red precipitate observed on both extracts indicate the presence of Glcosides (Trease and Evans, 2002).

**Determination of Antimicrobial Effects**

The determination of antimicrobial effectiveness against specific pathogens is essential to proper therapy. Testing can show which agent are most effective against a pathogen and estimate of the therapeutic dose (Klein, 2002).*Staphylococcus aureus* was isolated from clinical laboratory, Ben Medical Laboratory Damaturu, Yobe State and used for the tests. Isolate test for coagulate activity. A positive Congolese test was observed in

each of the extract, which showed immediate clumping and indicated the presence of Staphylococcus aureus (Bakers, Silverton and Pilaster, 1998). For the coagulase activity, the Slide test was carry out.

## DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION:

The minimum inhibitory concentration (MIC) of the extracts was estimated for the test organism in triplicates. To 0.5ml of varying concentration of the extracts (20.0, 18.0, 15.0, 10.0, 5.0,1.0 and 0.5mg/ml), 2ml of nutrient broth was added and then a loopful of the test organism was introduced to the tubes. The procedure was repeated on the test organism using nutrient broth only to serve as control. All broth cultures were then incubated at $37^0$ C for 24 hours. After incubation, the tubes were then examined for microbial growth by observing turbidity. The lowest concentration of the extracts which result in no growth after 24 hours of incubation is the minimum inhibitory concentration (MIC) (Bakers, Silverton and Pilaster, 1998).

## RESULTS

## PYTOCHEMICAL SCREENING

The extracts was obtained using three (3) different methods for maximum precaution and tested separately. The methods which include; the aqueous extract, organic extract (maceration and soxhlet extractions) all produce similar results. The result of the phytochemical screening in all the methods reveals the presence of alkaloids, saponins, tannins, anthraquinones, steroid, Flavonoids and glycosides.

**TABLE 1; PHYTOCONSTITUENT**

| Extract | Alkaloid | Tannis | Saponnin | Glycoside | Anthraquinone | steroid | Flavonoid |
|---|---|---|---|---|---|---|---|
| Ethanol (Macaretion) | + | + | + | + | + | + | + |
| Ethanol (Soxhlet extraction) | + | + | + | + | + | + | + |
| Aqueous( using distilled water) | + | + | + | + | + | + | + |

KEY; + = Present, - = Absent.

**TABLE 2: ANTIMICROBIAL ACTIVITIES OF THE FRUIT OF *CUCURBITA PEPO***

| Dilution mg/m | Zone of Inhibition (mm) | | |
|---|---|---|---|
| | Zone of Ethanol | Water | Control |
| 100 | 10 | 6 | - |
| 80 | 8 | 5 | - |
| 60 | 7 | 5 | - |
| 40 | 5 | 3 | - |

**TABLE 3: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Fruits of *Cucurbita pepo;***

| MIC/Mg/M | MBC/Mg/M |
|---|---|
| 7.5 | 10.0 |

## DISCUSSION

The result of this experimental work of preliminary phytochemical screening conducted on the extract of squash indicated the presence of phytoconstituent. The extraction was conducted in three (3) different ways to ensure maximum precaution. The first method is the use of soxhlet extractor with Ethanol as the solvent, the second method also ethanol was used as a solvent, but with manual agitation using magnetic stirrer. Lastly the extract were obtained in aqueous medium using distilled water. However, the result of all the three (3) different extract obtained indicate the presence of alkaloids, saponins, tannis, anthraquine, flavonoid, steroids and glycocide.

The aqueous and ethanolic extracts were screened for their antimicrobial effect on *Staphylococcus aureus*, and the entire results demonstrated a higher activity of (10mm zone of inhibition) at 100mg/m). The minimum bactericidal concentration of the extract (7.5mg/m) showed that it had a side effect on the test organism at different concentration. This is an indication that the plant fruit extract when purified will prove to be potent in the treatment of *Staphylococcus aureus* and other related infection.

The result obtained indicate the existence of antimicrobial compound in the extracts of the fruit of *Cucurbita pepo* and this shows a good concentration between the reported use of the fruit in the traditional medicine against infectious diseases.

## CONCLUSION

The squash was extracted and tested for phytoconstituent and

screened for antimicrobial effect on staphylococcus, the phytoconstituent obtained are alkaloids, saponins, tannis, anthraquine, flavonoid, steroids and glycocide. However, the extract was found to have a bactericidal effect on *Staphylococcus aureus,* hence justifies its local use as medicine for most infectious diseases.

## RECOMMENDATIONS

From the result of the research work obtained, the following recommendations are made;

1. It is strongly recommended that the squash should be used in the treatment of staphylococcus infection due to its antimicrobial effects against staphylococcus aureus.
2. Due to the literature and the phytoconstituent obtained in this work, the squash are appetizer, laxative, diuretic, and hence of medicinal value. It is therefore recommended that farmers should be encouraged to produce more squashes in abundance.
3. More research work should be conducted to ascertain the antimicrobial and phytochemical effect of *Cucurbita pepo* fruit.

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