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Anitmicrobial, Phytochemical, Ethnobotanical and Proximate analysis of *Allium cepa L.*

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ABSTRACT

Allium cepa is an important plant used in daily food stuff and one of the useful traditional medicinal plant to local people. The plant is said to be native to Asia but is cultivated throughout the world. The fresh, homogenized bulbs were extracted with Ethanol, Dichloromethane and water. The study aim to screen the plant against selected bacterial and fungal strains, qualitative phytochemistry and proximate analysis on the basis of their local uses. Phytochemical analysis gave positive results for steroids, glycosides, alkaloids, phenolic compounds, flavonoids, Resins, oils, carbohydrate and protein. All extracts are active against the selected bacterial strains. *Klebsiella pneumonia* is the most sensitive bacteria while *Pseudomonas aeruginosa* is susceptible but least. The *Fusarium Oxysporum* is more susceptible to ethanolic extract with zone of inhibition 8mm as compared to the *Colletotrichum spp*. in the same extract with 4 mm zone of inhibition. Moisture is 91.05%, Carbohydrate is 80.6%, total Sugar is 3.2%, Protein is 3.90% and Acidity of the extract is 2.1%. So the results justify the use of the plant in various infectious diseases.

Keyword: *Medicinal plants, Ethnobonty, Phytochemicals..*

INTRODUCTION

Medicinal plants are the plant which have medicinal use and contain those secondary conventional drugs such as fox glove and opium poppy, as well as daily use plants, like onion [5]. All drugs obtained from plants have particular therapeutic action. Continued research discovered that dialy food and their individual constituents have similar way of function to modern drugs and sometimes more better without the feared side effects. The use of plants as the principal medications is a general phenomenon. All nations on earth, through transcribed or verbal practice, has relied on the massive diversity of natural chemistry present in curative plants for their therapeutic properties [6].

Following to tomatoes, Onion (Allium cepa L.) is important vegetable throughout the world [1]. It is believed, bulbs of onion have been used as a nutrition source for Ages. Onion comprises of its herbaceous plant part and its edible bulb part. It is perhaps a native to southwestern Asia [7]. There are three main varities- white, red and purple skinned [8].The relative spiciness of onion has both genetic and ecological components. Sulphur complexes in onions have also been reported to be anti-inflammatory both by stopping formation of thromboxanes and by inhibiting the action of platelet-activating factor (PAF). Thiosulfinates condition anti-thrombotic benefits, including antioxidant activity [] 9, 10], low serum cholesterol and enhance in vitro platelet activity [7]. This later effect is important for cardiovascular health by reducing the possibility that platelets aggregate in the blood, a major cause of heart attacks and strokes [12]. Hence, thiosulphinates found in onion have been shown to inhibit in-vitro platelet aggregation [13].

The aim of this research work is to determine the antimicrobial activity of ethanolic, dichloromethane and water extracts, qualitative phytochemical tests and proximate analysis of the locally use medicinal plant onion botanically called *Allium cepa Linn.*

MATERIALS ANT METHODS

Onion Extraction

The Fresh onion were used in this trail. Onions were washed with distilled water and were air dried. Before washing the outer dried peels were removed. Fresh onion bulbs were blended into fine powder and soaked in 100mls of 100mls of 95% ethanol for 48hrs. The juice obtained was poured in a glass beaker. It was filtered using a filter paper after which the extract was air-dried. The dried extract were fractionated with Dichloromethane and we get three fractions of the plant that is Ethanol, Dichloromethane and water extract.

Antimicrobial activity

Bacterial Test strains: *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa and Staphylococcus aureus.*

Fungal test Strains: *Fusarium oxysporum, Colletotrichum spp, Phythium.* Screening Method: Antimicrobial activities of different extracts were evaluated by the agar well diffusion method [14] modified by [15].

Media Preparation: For agar well diffusion method [14, 15] antimicrobial susceptibility was tested on solid (Agar-agar) media in petri plates. For bacterial assay nutrient agar (NA) (40 gm/L) and for fungus PDA (39 gm/L) was used for developing surface colony growth.

Agar well diffusion method

Agar well-diffusion method was followed to determine the antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed with sterile cotton swabs with 24 hour old – broth culture of respective bacteria and fungi. Wells (8mm diameter) were made in each of these plates using sterile cork borer. Stock solution of each extract were prepared in DMSO at a concentration of 2g/100ml. About 100 µl of different solvent extracts were added with micropipette into the wells and allowed to diffuse at room temperature for an hour. Positive and Negative controls were also set and incubated at 37°C for 18-24 h for bacterial pathogens and 28°C for 48 hours fungal pathogens and the diameter of the zone of inhibition in (mm) was calculated.

Qualitative phytochemical analysis

The extract was tested for the presence of bioactive compounds by using the standard methods [16, 17, 18].

Test of alkaloids:Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's And Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Test of steroid: Crude extract was mixed with 2ml of chloroform and concentrated H2SO4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids.

Test of glycosides:Crude extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H_2SO_4 was added. A colour change from violet to blue to green indicated the presence of glycoside.

Test of saponins: Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for phenols and tannins: Crude extract was mixed with 2ml of 2% solution of FeCl3. A blue-green or black coloration indicated the presence of phenols and tannins.

Test of flavonoids: Crude extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet colour appeared after few minutes which indicated the presence of flavonoids.

Test of carbohydrates: Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

Test for proteins: Crude extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Test of fixed oils: Small quantities of extracts were pressed between two filter papers. An oily stain on filter paper indicates the presence of fixed oil.

Test of resins: Extracts were treated with acetone. Small amount of water was added and shaken. Appearance of turbidity indicates the presence of resins.

Proximate analysis

Proximate analysis of the sample was done by using standerd methods [19, 20].

Determination of Total Carbohydrate:The total percentage carbohydrate content in the onion sample was determined by the difference method. This method involved adding the total values of crude protein, lipid, crude fiber, moisture and ash constituents of the sample and subtracting it from 100. The value obtained is the percentage carbohydrate constituent of the sample. Thus: % carbohydrate = 100 – (% moisture + % crude fiber + % protein + % lipid + % ash)

Determination of Crude Proteins: The powdered onion sample was tested for crude protein content according to the Kjeldahl's method as described in AOAC, which involved protein digestion and distillation.

Protein Digestion: About 2.0 g of the sample was weighed into an ash less filter paper and put into a 250 ml Kjeldahl flask. Then, 1 g of digestion mixture (as catalyst) and 15-20 ml of 98 % conc. Sulfuric acid were added. The whole mixture was subjected to heating in the digestion chamber until transparent residue contents were obtained. Then, it was allowed to cool. After cooling, the digest was transferred into a 100 ml volumetric flask and made up to the mark with distilled water and then distilled using distillation apparatus.

Protein Distillation: Before use, the Markham distillation apparatus was steamed through for 15 min after which a 100 ml conical flask containing 5 ml of 2 % boric acid and 1 or 2 drops of mixed indicator was placed under the condenser such that the condenser tip was under the liquid. About 5.0 ml of the digest was pipetted into the body of the apparatus via a small funnel aperture. The digest was washed down with distilled water followed by addition of 3-4 drops of phenolphthalein and 5 ml of 40 % (W/V) NaOH solution. The digest in the condenser was steamed through until enough ammonium sulfate was collected. The Boric acid plus indicator solution changed color from red to green showing that all the ammonia liberated had been trapped. The solution in the receiving flask was titrated with 0.063 N hydrochloric acid upto a purple end point. Also, a blank was run through along with the sample. After titration, the % nitrogen was calculated using the formula: % Nitrogen = (Vs–VB) × M acid × 0.01401 × 100 W Where, Vs = Volume (ml) of acid required to titrate sample; VB = Volume (ml) of acid required to titrate the blank; M acid= Molarity of acid; W=Weight of sample (g).

Then, percentage crude protein in the sample was calculated from the % Nitrogen as % crude protein = % N x F, where, F (conversion factor), is equivalent to 6.25.

Determination of Total Moisture: The moisture content of powdered onion sample was determined in an oven through drying method (at 105 °C) .The moisture content of the sample was determined by weighing 2 g of sample into a pre-weighed china dish and drying it in an air forced draft oven at a temperature of 105 ± 5 °C till the constant weight of dry matter was obtained. The moisture content in the sample was determined as follows- Moisture (%) = [{(Wt. of original sample – Wt. of dried sample) / Wt. of original sample} × 100]

Determination of Total Sugar: Total sugar is determined by volumetric method (the Lane–Eynon method). The method is used for the quantitative determination of total sugar samples. Results are expressed as percentage of sugar in 100 g of sample.

Reagents: Hydrochloric acid, Sucrose, Copper sulfate solution, alkaline tartrate solution, Fehling's solution, Sodium hydroxide, Sugar standard solution. Method: The method involves the inversion of sugars present in food samples with hydrochloric acid. The sugar present in a specified volume of the hydrolyzed solution was used to reduce copper in the Fehling's solution previously standardized with working standard invert sugar solution. Excess copper was back titrated with the standard sugar solution. The difference in the volume of standard sugar used for the standardization and for back titration is a measure of total sugar content of the sample. Calculation: Total sugar (g per 100 g) = (F−M) × I × 250 × 100 × 100 / (W × A × 50) Where, F = volume of standard sugar solution required to reduce 10 mL mixed Fehling's solution; M = volume of standard sugar solution used in back titration of the sample; I = gram sugar per mL working standard solution; W = weight of sample.

Determination of acidity: pH meter was used to calculate the acidity of water extract of the plant.

RESULTS AND DISCUSSION

Local Uses: It is also antiseptic. The bulbs are stimulant. The leaves are diuretic, aphrodisiac and expectorant. Used extensively (both fresh and dry) in cooking as spices and condiment. Its juice is applied to soothe the irritation caused by scorpion and hornet sting. Allium cepa L. Equal amount of extract of Onion bulb and Mint are mixed. One teaspoon of this mixture is taken per hour for a period as needed. This phototherapy is considered to be useful for Cholera. A piece of onion bulb is boiled in mustard oil till it becomes black in colour. 2 or 3 droplets of oil are put in the ear when needed. This is traditionally prescribed for earache. The bulb of the onion is half heated and is used as poultice for the treatment of abscesses. Veterinary uses: for foot and mouth disease, bulbs of onion fed to the animal. [2, 3, 4].

Table.1 Antibacterial activity of *Allium Cepa L.*

Microorganism	Streptomycin	Dichloromethane Extract	Ethanol extract	Water extract
Escherichia coli	±16.2	±13.7	±14.2	±16.2
Klebsiella pneumonia	±16.0	±15.2	±14.5	±10.8
Pseudomonas aeruginosa	±17.2	±10.0	±11.0	±08.5
Staphylococcus aureus	±17.7	±13.0	±12.5	±10.2

The antibacterial activity in table.1 showed that all extracts are active against the selected bacterial strains. From the table it is clear that Klebsiella pneumonia is the most sensitive bacteria while Pseudomonas aeruginosa is susceptible but least. This activity of onion extracts can be acknowledged because of the presence of flavonoids which has been described to have extensive band of antibacterial activity [21]. Herbal preparations have Advantages over the artificial drugs which do not act directly on bacteria but make an opposing atmosphere for them, thus terrifying their survival and they have also been found to discourage the growth of tough strains of microbes [23].

Table.2 Antifungal activity of *Allium Cepa L.*

Microorganism	Dichloromethane Extract	Ethanol extract	Water extract
Fusarium oxysporum	±03	±08	±06
Colletotrichum spp	±02	±04	±03
Phythium spp.	NA	NA	NA

It is indicated from table 2. that the inhibition of fungal microorganisms in which the *Fusarium Oxysporum* is susceptible as compared to the *Colletotrichum spp*. and *Phythium spp*. show no activity against any extract. The inhibitory result of the extracts of plant against selected microorganisms can present the plants as a possible contestant for drug development for the treatment of complaints caused by these microorganisms. The activity of the *Allium cepa* extracts against selected bacterial and fungal components investigated in this study is agreed with other prior works done either singly or with more strains. This study indicated good results matched to the studies stated above. The result proportion may vary from one to another.

Table 3. Phytochemical test of crude ethanolic extract of *Allium Cepa L.*

Phytochemical test	status
Tannin	-
Saponin	-
Cardiac Glycosides	+
Alkaloids	+
Flavonoids	+
phenols	-
resins	+
Oils	+
Steroids	-
Carbohydrates	+
Proteins	+

The phytochemical test (table.3) of the onion ethanolic extract showed the presence of Cardiac Glycosides, Alkaloids, Flavonoids, and resins, Oils, Carbohydrates and Proteins while Tannin, Saponin, phenols and Steroids were not showed by the tests applied. . Alkaloids are involved in relaxation of muscles and relieve nasal congestion, also components of quinine and aspirin. Cardiac glycosides are antidotes for heart failure, irregular heartbeats. Terpenes are psychoactive chemicals found in cannabis. [24] has reported that flavonoids possess both bacteriostatic and bacteriocidal effects on some strains of bacteria; further, they inhibit the activity of reverse transcriptase and proteases. Both vegetables contain them;

hence their consumption in moderation is beneficial to the body.

Table 4. Nutritional composition of *Allium Cepa L.* in percentage

Moisture	Carbo hydrate	total Sugar	Protein	Acidity
91.05	80.6	3.2	3.90	2.1

It is clear from the Table 4. that the Nutritional composition of onion in percentage, where carbohydrate are highest in percentage while total sugar percentage is 3.2 and acidity of the extract is 2.1 measuring with pH meter.

CONCLUSION:

In conclusion, *Allium cepa L.* extract possess a wide-range of activity against a panel of bacteria responsible for the most common bacterial diseases. The antimicrobial activity of *Allium cepa L.* could be attributed to various phytochemical constituents (flavonoid, phenolic and alkaloid compounds) present in the respective crude extracts. Additional work on the types of phytoconstituents and refinement of individual groups of bioactive mechanisms may expose the exact potential of the plant to inhibit several pathogenic microbes and inspire the expansion of a novel broad spectrum herbal antimicrobial formulation in the future.

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