In Vitro Antioxidant and Antibacterial Properties of The Leaf Extracts of Cactus Plant (Opuntia Ficus Indica)

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***Abstract*: *Opuntia ficus-indica* has been reported to be used in folk medicine due to its diverse pharmacological effects. Maceration method was employed in the determination of antioxidant activity and antibacterial effects of the plant leaf using Three (3) different solvents (methanol, ethanol and aqueous). The following isolates; *S. aureus, S. pneumoniae, K. pneumonia, E. coli and S. typhi* were tested using agar well diffusion method. Meanwhile, the antioxidant activity of the plant extract was carried out through the determination of total antioxidant capacity assay and reducing power assay. Hence, the leaf extracts showed various levels of activity on the different test organisms; The methanolic leaf extract showed highest antibacterial activity against all tested organisms followed by ethanolic extract and finally aqueuos extract with least antibacterial activity against the tested bacterial isolates as showed in *S. pneumoniae* methanol extracts (17.5±0.66mm), ethanol extract recorded 15.2±0.76mm in *S. aureus*, and aqueuos extract showed 10.7±0.76mm in both *E. coli* and *S. typhi*. On the other hand, the minimum inhibitory concentration in methanolic extract indicated least value in *K. pneumoniae* with 6.2±0.03 mg\mL, followed by ethanolic extract with 12.4±0.32mg\ml in *S. typhi,* and aqueuos extract with 29.5±0.12mg\mL in *E. Coli.* The ability of the methanolic extract to reduce Fe^{3+} to Fe^{2+} and $Cu^{2+}$to Cu^{+} in reducing power assay and total antioxidant capacity respectively using ascorbic acid as a standard reveal it's antioxidant property. The present study suggests that the methanolic leaf extract of *Opuntia ficus-indica* may contain compounds that can form the basis for the development of a chemopreventive agent against free radical damage and development of novel antibacterial chemotherapeutic agent.**

***Keywords and phrases: Opuntia ficus-indica*, Antibacterial activity, Antioxidant activity, Crassulacean acid metabolism, Zone of inhibition, Minimum inhibitory concentration, reductones, free radicals**

I. INTRODUCTION

Opuntia, also known as nopales or paddle cactus, it belong to species: Plantarum, genus: cactus L. division: Magnoliophyta, class: Magnoliopside, order: Caryophyllales, family: *Cactaceae* and sub family of *Cactoideae* [9].

The plant has the fluted stem which helps for better absorption capacity during the rainy season; the stored water helps it to withstand the harsh condition of drought. However, the plant undergoes Crassulacean Acid Metabolism (C.A.M) in which stomata open in the night and close in day hours to prevent water loss through transpiration, and the leaf is in form of spines which protect plant from herbivores, help in photosynthesis, and minimize transpiration [7].

The cactus generally grows in both arid and semi-arid regions of the world, largely distributed in South, North and Central America. However, common species are found in the Madagascar region. The plant grows widely in the desert regions. The plant is considered as an agriculturally important for this arid area. The resident populace generally used this plant as food, fodder, dye, and to some certain extent in phytoremediation [9].

Cactus has been used in traditional folk medicine because of its role in treating a number of diseases and conditions, including diabetes, hypertension, hypercholesterolemia, rheumatic pain, gastric mucosa diseases, asthma, anti inflammatory and exhibit antiviral effects [9]. The plant was also found to have an antibacterial activity, in a way that many bacterial infections caused by some gram negative bacteria such as (*E. coli*, *S. typhi*, and *Entrobacter aerogenes*) and gram positive such as (*S. aureus, Entrococcus faecalis*) could be treated [7].

II. MATERIALS AND METHODS

2.1 Collection of Plant Materials

The leaves of medicinal plants were obtained from Kaura Namoda Local Government Area of Zamfara State. The plant was identified at the herbarium section of the Department of Botany, Bayero University, Kano with the accession number of BUK HAN 0474, and air dried at room temperature. Dried leaves were ground to powder using mortar and pestle then the powder was transferred into closed containers for further use.

2.2 Preparation and Processing of Plant Extracts

Leaves of *Opuntia ficus-indica* were collected in bulk for preparation of extracts. The leaves were also collected and air dried at room temperature in the Department of Biochemistry, Bayero University, Kano Nigeria for Seven (7) days for the determination of antibacterial and antioxidant properties. Maceration method was used during extraction. The samples were ground into coarse powder using motor and pestle and stored in an air tight bottle prior to analysis. After weighing 40g of the ground sample of the leaves, it was dissolved in 200 ml of aqueous solution in an air tight bottle.

However a 40g of the sample was also weighed and dissolved in 400 mL ethanol solution in an air tight bottle and as well 40g of the sample was weighed and dissolved in 400 mL methanol solution in an air tight bottle. All were shaken and kept for Forty Eight (48) Hours (h). The extract was filtered using a chess cloth to obtain filtrates of the respective solvents of aqueous, methanol and ethanol. The ethanol and methanol filtrate was allowed to evaporate for Seven (7) days and then concentrated to dryness and weighed. The aqueous filtrate was also kept in a water bath and allowed to evaporate for Seven (7) days and then concentrated to dryness and weighed.

2.3 Determination of the Potency of the Herbal Preparation

The agar diffusion method was used to investigate the antibacterial activity of the crude extracts according to Shakeri *et al.,* [3]. Within Fifteen (15) minutes after adjusting the turbidity of the inoculums suspension, a sterilized swab was aseptically dipped into the suspension. The dried surface of a Mueller- Hinton agar plates were inoculated by streaking the swab over the entire sterile agar surface with bacteria. A sterilized cock borer of an internal diameter of about 6 mm was used to punch holes in the medium and plant extracts were dispensed into the respective labelled holes. Amoxicillin was used as control. Triplicates of each plate were made and the procedure was repeated for each microorganisms. The plates were kept in the refrigerator for about Four (4) hours for complete diffusion of the extract and incubated at 37ºC for 24 hours. After the incubation period, the diameter of each zone of inhibition was measured in millimetres (mm) with zone measuring scale.

2.4 Determination of Minimum Inhibitory Concentration (MIC) of the Crude Extracts

MIC for each test organism was determined by following the modified agar well diffusion method of Shahidi *et al.,* [12]. A two-fold serial dilution of each extract was prepared by first reconstituting the dried extract (100 mg/ml) in 20% DMSO followed by dilution in sterile distilled water (1:1) to achieve a decreasing concentration range of 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, and 3.125mg/ml and thesame procedure applied to control antibiotic. A 100µl volume of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with 100µl of standardized inoculum of microbial strain (mc farland standard). All test plates were incubated aerobically at 37^0C for 24 hours and observed for the inhibition zones. MIC, taken as the lowest concentration of the test extract that completely inhibited the growth of the microbe, showed by a clear zone of inhibition (>6mm), was recorded for each test organism.

2.5 Total Antioxidant Capacity Assay

The total antioxidant capacity (T A C) of leaf extract of *Opuntia ficus-indica* was determined by phosphomolybdate method according to Jayaprakasha *et al.,* [8]. An aliquot (30ml) of different concentrations (0.2 to 1.0 mg/ml) of the test extracts were mixed with 3ml of the reagent solution (0.6M sulphuric acid, 28mM sodium phosphate, 4mM ammonium molybdate) taken in test tubes. The tubes were capped with aluminium foil and incubated in a boiling water bath at 90°c for 90 minute. The reaction mixture was allowed to cool at room temprature and the absorbance of solution was measured at 695nm against a blank containing 3ml of reagent solution and the appropriate volume of the dissolving solvents. The blank was incubated under the same conditions as the test sample. Ascorbic acid was used as standard reference compound to compare the activities of the extracts.

2.6 Reducing Power Assay

Antioxidant activity of the leaf extract of cactus plant were determined to assess their ferric ion (Fe^{3+}) reducing ability according to Anandjiwala *et al.,* [1]. Different concentrations (0.2 to 1.0 mg/ml) of each extract were prepared and 1ml of each concentration was mixed 2.5ml of phosphate buffer (0.2M, pH 6.8) and 2.5ml of potassium ferricyanide. The mixture was incubated in a water bath at 50^0C for 20min. To this mixture, 2.5mL of 10% trichloacetic acid was added and then centrifuged at 3000rpm for 10 min. The upper layer of the solution (2.5ml) was mixed with 2.5ml distilled water and 0.5mL of 0.1% ferric chloride was added. Absorbance of the pert Prussian blue solution formed was measured at 700nm. Ascorbic acid were used as standard reference compound for comparison and prepared in same concentrations as the extracts.

Data Analysis

Values were expressed as mean ± standard deviation. Data were analyzed by one-way ANOVA using the SPSS Version 20. Values of $P<0.05$ were considered statistically significant.

III. RESULTS

TABLE 1: zone of inhibition (mm) using different solvent extract (methanol, ethanol and aqueous)

S/N	ISOLATES	AMMOXICILLIN	METHANOLIC EXTRACT	ETHANOLIC EXTRACT	AQUEOUS EXTRACT
1	S. aureus	24.03±0.34[a]	16.50±0.50[b]	15.17±0.76[c]	9.17±0.76[d]
2	S.pneumoniae	24.50±0.55[a]	17.50±0.66[b]	15.00±0.76[c]	6.77±0.76[d]
3	K. pnoumoniae	23.30±0.47[a]	14.00±0.50[b]	12.83±0.50[c]	9.50±0.50[d]
4	E coli	18.50±0.06[a]	14.50±0.50[b]	12.00±0.50[c]	10.17±0.76[d]
5	S. typhi	27.00±0.75[a]	16.83±0.71[b]	12.50±0.50[c]	10.67±0.76[d]

Table 1: zone of inhibition (mm) measured using different solvent extract (methanol, ethanol and aqueous) in each isolate, Amoxicillin was used as a positive control. Data were expressed as mean ± SD; values with different superscript along the rows were considered significantly different (P<0.05)

TABLE 2: Minimum Inhibitory Concentration (Mg/Ml) Using Different Solvent Extract (Methanol, Ethanol and Aqueous)

S/N	ISOLATES	AMMOXICILLIN	METHANOLIC EXTRACT	ETHANOLIC EXTRACT	AQUEOUS EXTRACT
1	S. aureus	4.20±0.22[a]	6.30±0.09[b]	18.30±0.58[c]	35.5±0.46[d]
2	S. pneumoniae	4.90±0.94[a]	8.33±0.14[b]	24.0±0.06[c]	38.0±0.35[d]
3	K. pnoumoniae	4.20±0.74[a]	6.23±0.03[b]	19.5±0.31[c]	36.5±0.23[d]
4	E coli	3.50±0.16[a]	6.30±0.05[b]	16.5±0.32[c]	29.5±0.12[d]
5	S. typhi	6.60±0.05[a]	6.50±0.43[b]	12.43±0.32[c]	39.0±0.35[d]

Table 2: Minimum inhibitory concentration (mg/ml) measured using different solvent extract (methanol, ethanol and aqueous) in each isolate, Amoxicillin was used as a positive control. Data were expressed as mean ± SD; values with different superscript along the rows were considered significantly different (P< 0.05)

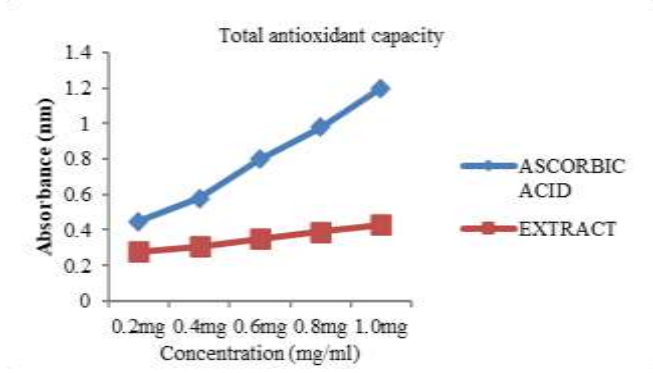

Figure 1: Total antioxidant capacity of *Opuntia ficus indica* Methanolic extract on line graph using ascorbic acid as a standard.

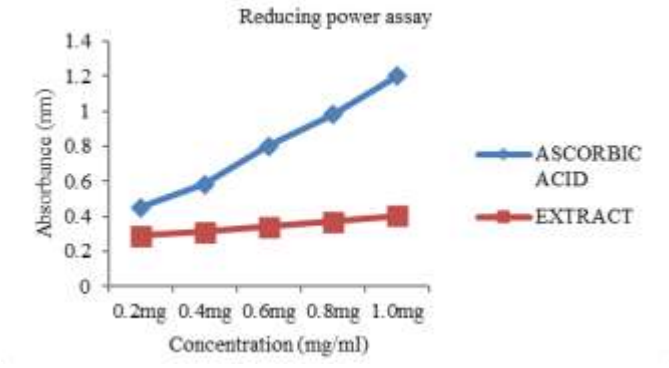

Figure 2 Reducing power assay of Opuntia ficus indica Methanolic extract on line graph using ascorbic acid as a standard.

V. DISCUSSION

The result for antibacterial activity of *Opuntia ficus indica* which is presented in table 1 and 2 that determined the zone of inhibition (mm) and minimum inhibitory concentration (mg/ml) clearly indicated that all the extracts have antibacterial activity against tested organisms. The antibacterial activity of all solvent extracts and their potency was assessed using stock solution of 100mg/ml. The leaves extract of *Opuntia ficus indica* (methanol, ethanol, and aqueous extracts) were evaluated for its activity against five bacterial isolates; *Staphylococcus aureous*, *Streptococcus pneumonae*, *Klebsiella pneumoniae*, *Eschirecia coli* and *Salmonella typhi*. However Amoxicillin was used as control throughout the experiment.

In this research, amoxicillin was found to have highest zone of inhibition followed by methanolic extract, then ethanolic and finally aqeuos extracts in each test organisms. This is in agreement with antibacterial study carried out by Sanchez and García, [10], using *Opuntia ficus indica* extracts on *Vibrio cholerae* and the research carried out by Gebrekidan and Aragaw [5] using skin fruits extracts of the plant.

In the determination of minimum inhibitory concentration using different solvent extract. There was reciprocal relationship with zone of inhibition. The least minimum inhibitory concentration was found in control used

(amoxicillin) followed by methanolic extract, then ethanolic extracts, and finally the water.

This may be as a result of the ability of methanol to extract phytochemicals responsible for antibacterial activity of plants [11]. These antibiotic principles were the defensive mechanism of plants against different pathogens [6]. And hence the antibacterial activity of *Opuntia ficus indica* may form the basis of further investigation to isolate active components, elucidate the structure and evaluate it against wide range of bacteria with subject to find new therapeutic principles [13].

Figure 1 and 2 was the result of antioxidant activity of *Opuntia ficus indica*, in the determination of total antioxidant capacity, *Opuntia fucus indica* extract has shown a great antioxidant potential through its effects on reduction of Cu^{2+} ion to Cu^{+}ion using ascorbic acid as a standard.

However, In the determination of reducing power, *Opuntia fucus indica* has shown a great antioxidant potential through it's effects on reducing Fe^{3+}ion to Fe^{2+} using ascorbic acid as a standard.

The reducing power of the extract was generally associated with the presence of reductones which have been known to exert antioxidant action by quenching the free radicals chain in donating a hydrogen atom [4], similarly, in total antioxidant capacity the extract shows electron donating capacity and thus they may act as radical terminators, transferring reactive free radicals species into more stable non-reactive product [2].

VI. CONCLUSION

The research revealed that the plant has both antioxidant and antibacterial activity, antioxidant activity of plant may be as a result of compounds that inhibit free radicals and acting as reducing agents, therefore the plant extract could be used in prevention of free radical damage that result in lipid peroxidation. However, methanolic extract showed the highest inhibition zone in all the bacterial strains tested compared to remaining extracts thus, *Opuntia ficus-indica* can form the basis for the development of novel broad spectrum antibacterial chemotherapy.

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