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In Vitro Antibacterial, Antioxidant and Phytochemical Analysis of *Helianthus annuus* Leaves Extract on Some Bacteria Causing Infection

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ABSTRACT

The antibacterial activity of *Helianthus annuus* leaves on some bacterial pathogens (*Escherichia coli*, *Salmonella* spp., *Shigella* spp., and *Staphylococcus aureus)* was evaluated. The extracts were prepared using chloroform, N-hexane and methanol. The antibacterial susceptibility testing of the extracts were carried out using the Agar well diffusion method. The phytochemical analysis was done using standard method while the antioxidant activity was determined using the 2, 2 - diphenyl-1-picrylhydrazyl (DPPH) photometric assay. The antibacterial screening showed that the test organisms were all susceptible to the extract. *S. aureus* showed the highest susceptibility to the extracts while *Shigella* spp. showed least susceptibility. The minimum inhibitory concentration of the methanolic fraction of the extract was determined to be 125 µg/ml on all the test organisms. The phytochemical analysis showed the presence of saponins, tannins, glycoside, alkaloids and flavonoids. The extracts produced a concentration dependent increase in antioxidant activity. The chloroform extract showed greater antioxidant activity while the methanolic extract was least when compared to the ascorbic acid (standard). Therefore this gives a scientific justification for the use of *Helianthus annuus* leave on some bacterial pathogens.

1. INTRODUCTION

The use of herbal medicine in the treatment of disease condition is an age long practice [1]. Several medicinal plants and parts have been used in the traditional management of disease condition and only few of the plants have been scientifically evaluated for the effects claimed thereof [2]. Among the plants used in the traditional medicine is *Helianthus annuus* leaves.

Helianthus annuus L (Asteraceae) is commonly called "sunflower" in English. It is an annual plant native to America and has a cosmopolitan distribution. It usually grows up to 1.5 – 3.5 m in height with large inflorescence, rough hairy stem and broad coarsely toothed leaves [3]; [4]. It is cultivated in some parts of the world for its seed, which serve as an important source of edible oil [4]. The seed oil, shoots and herbs tincture have been used for antipyretic, anti-inflammatory, vermifuge, diabetes mellitus and stomach problem purposes [5]; [6]. The leaves of *H. annuus* are extensively used in South Eastern Nigeria in the traditional treatment of diarrhea, diabetes mellitus, inflammation, bacterial infection and respiratory problems. There is an experimental literature on the antimicrobial activity of the sunflower stem and seed oil [7]; [6]. The antioxidant, antidiabetic [3]; [5] and hepatoprotective [8] activities of *H. annuus* have been reported. There is no available experimental information on the antibacterial properties of *H. annuus* used in South Eastern Nigeria.

The study was aimed to investigate the antibacterial, antioxidant and phytochemical constituents of different extracts of *Helianthus annuus* leaves.

2. MATERIALS AND METHODS

2.1 Source of Plant and Test Organisms used

The leaves of *H. annuus* were collected at university of Nigeria Nsukka premises and identified by Mr. A.O Ozioko, a Taxonomist at Bioresource Development and Conservation Programme (BDCP), Aku Road, Nsukka, Enugu State, Nigeria.

The test bacterial isolates (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica* and *Shigella*) were obtained from the Microbiology Laboratory Federal Medical Centre Umuahia, Abia State, Nigeria. They were purified, sub-cultured and re-identified to ensure purity of the isolates.

2.2 Confirmation of Bacterial Isolates Used

The isolates obtained were confirmed by using some biochemical tests such as catalase test, coagulase test, indole test, citrate test, methyl red – Voges Proskaurer reaction, urease test, hydrogen sulphide production and carbohydrate fermentation tests [9]; [10]; [11].

2.3 Preparation of crude extracts of *H. annuus*

The collected leaves of *H. annuus* were air dried on a laboratory bench at ambient temperature and pulverized into coarse powder with a manual grinder (Corona, China). Fifty (50) gram of the powdered leaves was successively extracted with n-hexane, chloroform and methanol respectively in Soxhlet apparatus maintained at 40^0C. Each of the solvent was run until the eluent became colourless. The extracts were concentrated to dryness in a hot air oven at 40^0C. The extract was stored in a refrigerator at 4^0C throughout the duration of this study.

2.4 Determination of the Antioxidant activity of *H. annuus* extacts

The antioxidant properties of the extracts were carried out. The free radical scavenging property of the extract was analyzed using 2, 2 - diphenyl - 1 -picrylhydrazyl (DPPH) photometric assay [3].

2.5 Determination of the phytochemical composition of *H. annuus* fractions

The fractions were tested for the presence of alkaloids, flavonoids, tannins, glycosides, saponins, terpenes/sterols, carbohydrates, and starch using the standard procedures [12].

2.6 Antimicrobial sensitivity Test

Antimicrobial activity of each extract was evaluated using agar well diffusion technique [13]. Mueller Hinton agar was prepared and poured into four culture plates and then inoculated with the test bacteria (*E. coli*, *Staphylococcus aureus*, *Salmonella enterica* and *Shigella* spp.) in each plate from the pure stock culture and was incubated at 37^0C for 24 hrs. After which two to four colonies each of the test organism were aseptically transferred into 5 ml of sterile normal saline in a test tube and mixed thoroughly for uniform distribution. Sterile swabs were then used to spread the bacterial cultures uniformly on the dried surfaces of the prepared Mueller Hinton agar plates.

Adequately spaced holes were made in the plate using an improvised cork-borer (radio pole 5 mm). 0.2 ml of each fraction at 2000 µg/ml concentration were put in each hole aseptically and were kept for two to three hours to allow the extracts to diffuse into the agar medium and incubated at 37^0C for 24 hrs. Gentamycin (280 µg/ml) was used as positive control and the zones of inhibition diameter were measured and recorded after 24 hrs.

2.7 Determination of Minimum Inhibitory Concentrations (MIC) of extract

The lowest concentration of the extracts that inhibit the growth of test organisms is the minimum inhibitory concentration (MIC) [1]. The initial concentration of the plant extract (2000 µg/ml) was diluted using double fold serial dilution. The different concentrations were 125, 250, 500, 1000 and 2000 µg/ml. Two milliliters of sterile nutrient broth was poured into sterile test tubes and then inoculated with 0.1 ml of prepared inocula, after which 0.1 ml of each concentration of the different extracts were added to the tubes and incubated for 24 hrs at 37^0C to determine the Minimum Inhibitory Concentration. One of the tubes served as the negative control as it contained only the nutrient broth and the inocula.

2.8 Determination of Minimum Bactericidal Concentration (MBC) of the Crude Extract

The minimum bactericidal concentration is the lowest concentration of antimicrobials that will kill microorganisms after 24 hrs incubation. The MBC is determined by sub-culturing the tubes with no visible growth on a fresh nutrient broth.

3. RESULTS

3.1 Phytochemical analysis of the extracts of *Helianthus annuus*

The result of the phytochemical analysis of the fraction of *H. annuus* is presented in the Table 1. The hexane fraction contains glycosides and flavonoids, chloroform fraction contains saponins, tannins and glycosides while methanol fraction contains saponins, tannins, glycosides, and alkaloids.

Table 1: Phytochemical Analysis of *Helianthus annuus* Leaves

Test for	Methanol	Chloroform	n-hexane
Saponins	+	+	–
Tannins	+	+	–
Glycoside	+	+	+
Alkaloids	+	–	–
Starch	–	–	–
Flavonoids	+	–	+

3.2 The Antioxidant Activity of the extracts of *Helianthus annuus* leaves

The result of the *in vitro* antioxidant effect of the extracts of *Helianthus annuus* on DPPH photometric assay is presented in Table 2. The extracts of *H. annuus* produced a concentration dependent increase in antioxidant activity. They produced their optimum antioxidant activity at 400 µg/ml concentration. The antioxidant activities of the extracts are in the following order: chloroform > hexane > methanol.

Table 2: The Antioxidant Activity of *Helianthus annuus* Leaves

Concentration (µg/ml)	Hexane fraction	Chloroform fraction	Methanol fraction	Ascorbic acid
25 µg/ml	29.24 ± 5.56	26.91 ± 2.17	9.46 ± 3.23	95.51 ± 0.19
50 µg/ml	7.80 ± 5.68	30.24 ± 1.22	5.88 ± 0.92	95.67 ± 0.03
100 µg/ml	14.01 ± 6.40	40.62 ± 3.22	12.30 ± 0.61	95.75 ± 0.18
200 µg/ml	62.30 ± 5.02	61.19 ± 1.52	17.89 ± 2.33	95.17 ± 0.16
400 µg/ml	99.11 ± 0.16	85.01 ± 1.39	38.18 ± 0.70	94.97 ± 0.14

3.3 Antimicrobial activity of extracts of *Helianthus annuus*

The result of antimicrobial activity of different extracts of *H. annuus* is presented in the Table 3. The various extracts of *H. annuus* (hexane, chloroform, and methanol extracts) produced various decrease of inhibition against the test organism (*E. coli, Staphylococcus aureus, Salmonella enterica and Shigella* spp.). The methanol extracts at 1000 µg/ml concentration produced 10, 13, 5 and 3 mm diameter zone of inhibition against *E. coli, S. aureus, S. enterica* and *Shigella* spp. respectively. The chloroform extracts produced 6, 8, 3 and 1 mm diameter

zone of inhibition against *E. coli, S. aureus, S. enterica* and *Shigella spp*. respectively while the hexane extracts produced at 1000 µg/ml concentration produced 1, 3, 1 and 1 mm diameter zone of inhibition against *E. coli, S. aureus, S. enterica* and *Shigella spp*. respectively. The antimicrobial activities of chloroform and hexane extracts were significantly ($P < 0.05$) lower when compared to gentamycin. The antimicrobial activity of methanol extracts against *E. coli* and *S. aureus* were comparable to the antimicrobial activity of gentamycin.

Table 3: Antimicrobial activity of extracts of *H. annuus* (Zone of Inhibition diameter (mm))

Bacteria	Methanol extract	Chloroform extract	Hexane extract	Gentamycin
Escherichia coli	10	6	1	13
Staphylococcus aureus	13	8	3	15
Salmonella enterica	5	3	1	15
Shigella species	3	1	1	12

3.4 Minimum Inhibitory Concentration (MIC) of *Helianthus annuus* of Different Extracts

The result of the minimum inhibitory concentration (MIC) of the extracts of *H. annuus* is in the Table below. The MIC of methanol extract against *E. coli, S. aureus,* and *S. enterica* was 125 µg/ml concentration while the MIC against *Shigella spp.* is 250 µg/ml concentration. The MIC of chloroform extract against *S. aureus* and *S. enterica, E. coli* and *Shigella* spp. were 125, 250 and 500 µg/ml concentration respectively. The MIC of Hexane extract against *S. aureus, S. enterica* was 250 µg/ml concentration while the MIC against *E. coli* and *Shigella spp.* was 500 µg/ml concentration. The MIC of the methanol extract was 125 and 250 µg/ml concentrations were lower than the MIC of gentamycin (280 µg/ml).

Table 4: Minimum Inhibitory Concentration (MIC) of *Helianthus annuus* of different extracts

Extract	*E. coli*	*S. aureus*	*Salmonella enterica*	*Shigella* spp.
Hexane	500µg/ml	250µg/ml	250µg/ml	500µg/ml
Chloroform	250µg/ml	125µg/ml	125µg/ml	500µg/ml
Methanol	125µg/ml	125µg/ml	125µg/ml	250µg/ml
Gentamycin (control)	280µg/ml	280µg/ml	280µg/ml	280µg/ml

4. DISCUSSION

The leaves of *H. annuus* were successively extracted in three different solvents (n-hexane, chloroform and methanol) in the order of increasing polarity to yield three fractions; n-hexane, chloroform and methanol fractions. The phytochemical analysis, antioxidant and antibacterial activities of the fractions were investigated using standard methods [1]; [3]; [14].

The fractions produced varied degree of antibacterial activities against the bacterial isolate used in the study. The antibacterial activities of the extracts may be mediated by some of the phytochemical constituents of the extracts [15]; [7]. The phytoconstituents particularly tannins and flavonoids are known to induce antibacterial activity due to their possession of ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins etc. [15]; [7]. The methanol fraction produced the highest antibacterial activity while the n-hexane fraction produced the least antibacterial activity when compared to other fractions. The difference in the degrees of antibacterial activity may be attributed to the variation in the nature and concentration of the phytochemical constituents of the fractions [14]. The study is in agreement with the work of [7] on the seed of *H. annuus* and the only variant from the report is the MIC which they reported to be in the range of 10 – 50 µg/ml while the MIC of the present study was in the range of 125 – 500 µg/ml. The difference in the MIC of the two studies may be due to difference in the vegetative part of the plant used (Evans 2009) and the antibiotic resistant pattern of the bacterial used in the study [16]; [17].

The extracts demonstrated a concentration dependent increase in antioxidant activities. The chloroform fraction had the highest antioxidant activity while the methanol fraction had the least antioxidant activity when compared to other fractions. The antioxidant activities of the

fractions are in the following decreasing order; chloroform fraction > n-hexane fraction > methanol fraction. The difference in the antioxidant activity may be due to variation in the nature and concentration of the phytochemical constituents of the fractions [14]. The demonstrated antioxidant activity of the fraction may be of advantage in the management of oxidative stress that may accompany bacterial infection [18].

5. CONCLUSION

The study has demonstrated that *Helianthus annuus* leaves can be used in the treatment of *E. coli*, *Staphylococcus aureus*, *Salmonella enterica* and *Shigella* related infections and justify its use in the traditional medicine for this purpose.

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