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Antimicrobial Activity of Ethanolic Extract of *Helianthus annuus* Stem

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Abstract
In vitro antimicrobial and phytochemical properties of the Stem extract of *Helianthus annuus* was prepared using ethanol as solvent, the extract was evaluated using agar diffusion and broth dilution method against *Staphylococcus aureus ,Eschericha coli ,Aspergillus niger and Candida albicans.* The ethanolic extract of *Helianthus annuus* showed an antimicrobial activities against *Staphylococcus aureus, Aspergillus niger and Candida albicans* while *Escherichia coli* was resistant to the extract. The antimicrobial activities varies from 2.00 mg/ml to 24 mg/ml. The ethanolic extract was found to have a pH of 4.8. The minimum inhibitory concentration of the extract ranged from 50-70 mg/ml. The ethanolic stem extracts of *Helianthus annuus* on *Candida albicaus* and *Escherichia coli* were found to be bactericidal .Ethanolic stem extract of *Helianthus annuus* was bactericidal on *Staphylococcus aureus*, and fungicidal on *Aspergillus niger* and *Candida albicans.*
Key words: Antimicrobial activity ,Minimum Inhibitory Concentration, Phytochemical , *Helianthus annuus.*

INTRODUCTION

Modern medicine has evolved from folk medicine and traditional system only after thorough

chemical and pharmaceutical screening. The use of synthetic compounds led to a decline in the use of plants in modern medicine. However, synthetic medicine can cause side effects and as a result people are more favorable to use natural compounds obtained from plants. Thus, plants remain a major source of medicinal compounds. About 20,000 plant species are used for medicinal purposes (Penos, 1983). Seventy four percent of 119 plant-derived drugs were discovered as a result of chemical studies to isolate the active substances responsible for their traditional use (Farnsworth and Soejarto, 1991). So plants, especially the higher plants contain a variety of substances, which are useful as food additives, perfumes, and in treatment of various diseases as medicine due to their versatile therapeutic potential (Mukherjee and Wahile, 2006).

Plants are considered world's best chemists. Their unparalleled biosynthetic capacity has been exploited through their use in medical and commercial applications. Development of multiple drug resistance has necessitated the search for alternative sources of antimicrobials. Antimicrobial activity is due to the active substances synthesized during secondary metabolism of the plants (Kiranmayee Rao *et al*., 2010).

Several investigators had reported that plants contain antibacterial or antimicrobial substances (Adetunji *et al* .,2011; Zaria *et al.,* 1995; Ibekwe *et al*., 2001; Akujobi *et al*., 2004).

Thus, the aim of this study was to evaluate the antimicrobial properties of a common herbaceous species *Helianthus annuus*, against different groups of microorganisms. Moreover, the plant extract was subjected to preliminary phytochemical screening to analyze the possible antimicrobial compounds it contain. The study provides scientific evidence on the use of this plant which is being utilized traditionally as herbal medicines.

Materials and Methods

Sources of materials and collection of plants materials

Fresh stem samples of *Helianthus annuus* was collected at the environment of Nigerian stored product research institutes, Ilorin, Kwara State, Nigeria. The plants was identified at the Herbarium unit of the Department of Plant Biology, University of Ilorin. The plants were dried in the sun until the moisture content was reduced to a level of 10%. The plant was then pounded in a mortar, and further ground into a fine powder of about 80 mesh using a clean electric blender and stored at $37^{O}C$ in polythene bags until use.

Collection and Maintenance of the Test Organisms

The microorganisms used were obtained from the clinical isolates from the Department of microbiology and parasitology laboratory of the university of Ilorin Teaching Hospital, Ilorin, Nigeria. The bacteria were maintained on Nutrient agar slant at $4^{0}c$ and fungi are maintained on potato dextrose agar slant at $4^{O}C$. The isolates were subcultured unto fresh media at regular interval before use.

Preparation of plant extracts

The fresh plant materials were air-dried for a period of three weeks and four days and they were grounded into powder using mortar and pestle. The grinded stem were sieved to get fine powder that was used for the extraction.

Fifty grams of the finely ground powder was introduce into different conical flask and 200ml of

absolute ethanol and cold aqueous was added to each of the different conical flask containing grounded *Helianthus annuus* respectively. Each conical flask are then covered with aluminum foil and placed on mechanical shaker. The suspensions was shaked for 48hours at 190rev.per.min. After 48hrs, the extract was decanted and passed through a muslin cloth and later filtered with a Whatman No.1 filter paper (110mm). The filtrate obtained was evaporated to dryness at 45^{o}C, and the residue obtained were reconstituted in 95% ethanol as stock concentration of 250mg/ml.

Preparation and standardization of bacterial inocula

Preparation and standardization of bacterial inocula was done using the method described by Adetunji *et al.* (2011). Five colonies of each of the test organism growing as a pure culture on nutrient agar were transferred into 5ml of sterile saline solution. Each culture was incubated for 4 to 5 hours to produce a growth of the same turbidity visually as 0.5ml of 1% Barium chloride to 99.5ml of 1% sulphuric acid.

.**Antimicrobial assay of the plant extracts**

Prepared sterile potato dextrose agar and Mueller Hinton agar plates were inoculated with standardized organisms of 0.1ml of a day old culture. Glass spreader was used in spreading the inocula evenly on the surface of the agar and excess are drained off. A sterile cork borer of 5mm diameters was used to make five (5) ditches on the plates. The bacteria were inoculated into Mueller hinton agar while *Candida albicans* and *A. niger* were inoculated into potato dextrose agar.

Varying concentrations of the extracts 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml were prepared from the stock concentration of the extracts. 0.5ml of each concentration of the extracts were dispensed into each of the ditches in the plates that are appropriately labeled. The fifth ditch in the plates were picked as control by adding 0.5ml of appropriate solvent use for the different extraction. The plates were done in duplicates and left on the bench for few minutes for the extract to diffuse into the agar and later incubation at 37^{O}C for 24hours. After incubation the zone of clearance around each ditch was measured using a metric ruler by taking measurement of the zone of clearance around the ditch. The diameter of the cork borer was removed from the diameter of the zone of clearance and this made or represented the antibacterial activity measured or diameter of the zone of inhibition.

Phytochemical Screening of the Leaves Extracts

Phytochemical screening was done in order to detect the presence of plant constituents such as alkaloids, tannins, saponins, phenolics, phlobatannis, flavonoids and glycosides using the methods described by Adetunji *et al*., 2011.

a. **Test for saponins**

Two milliliter of the aqeous and ethanolic extracts in a test tube was shaken for two minutes. Fronting which persisted on shaking was taken as evidence for the presence of saponins.

b. **Test for Alkaloids**

Three milliliter of the ethanolic and aqueous extracts was stirred with 5ml of 1%HCl on a steam bath for twenty minutes. The solution obtained was cooled and filtered and the filtrate was added to few drops of Mayer's reagent/picric acid. A cream precipitate indicated the presence of alkaloid.

c. **Test for Phenolics**

Two drops of 5% ferric chloride were added to 5 ml of the ethanolic and aqueous extracts in a test tube. A greenish precipitate was taken as an indication of phenolics.

d. **Test for Tannins**

A volume of 1ml of freshly prepared 10% KOH was added to a volume of 1ml of the ethanolic extracts and aqueous extracts. The presence of a dirty white precipitate was taken as indication of tannins.

e. **Test for Steriods**

To a volume of 1ml of the extracts, five drops of concentrated $H_2SO_4$was added. Red colouration indicated the presence of steroids .

f. **Test for Phlobatanins**

To a volume of 1ml of the ethanolic and aqueous extracts, 1% HCl acid was added. A red precipitate was taken as the presence of phlobatannis .

g. **Test for flavonoids**:

To a volume of 3ml of the ethanolic and aqueous extract, 1ml of 10% sodium hydroxide was added. A yellow colouration indicated the presence of flavonoids.

h**. Test for Glycosides:**

To a volume of 3ml of the ethanolic and aqueous extract, 2ml of chloroform was added. H_2SO_4acid was careful added to form a lower layer.
A reddish brown colour at interface indicated the presence of a steroidal ring.

Results

The screening for antimicrobial activity of the stem of the plants used in this study revealed that the plant extracts had varying effects on the growth of the clinical isolates. *Candida albicans* was found to be the most susceptible organism to all the extract while *Escherichia coli* was resistance to all the extract. The antimicrobial activities of the leaf extract of *Helianthus annuus* on the test organisms are shown in Table1.
The ethanolic stem extract of *Helianthus annuus* antimicrobial effect on C*andida albicans, Staphylococcus aureus* and *Aspergillus niger* when tested in vitro. Table 1 shown the antimicrobial effect of ethanolic stem extract of *Helianthus annuus* on test organism. *Escherichia coli* was resistant to the extract of the plant part because No zone inhibition was recorded with all the concentration of the plant extract used.

The MIC and MBC/MFC values obtained for the extract on *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger* and *Candida albicans* varied. For instance, the MIC values of 70mg/ml was obtained for ethanolic extract of *Helianthus annuus* on *Staphylococcus aureus.*

Discussion

The result of this study have shown that the ethanolic stem extract of *Helianthus annuus* possess antimicrobial effect on *Staphylococcus aureus*. This antimicrobial activity exhibitor could be due to complimentary nature of the active principle Candida *albicans,* and *Aspergillus niger*. This may probably due to the insolubility or partial solubility of the active ingredients of the plants or it may be due to the presence of low concentration of diffusible water soluble active constituents. Oil are generally soluble in methanol and ethanol, it will make all soluble active components to dissolve in the solvent (methanol and ethanol) (Cowan, 1999). The solubility of some of the active ingredients in methanol and ethanol enhances their inhibitory nature on the test isolates. The result shown that the higher the concentration of the plant extract, the higher the zone of inhibition and the lower the concentration of the plant extract the lower the zone of inhibition.

Candida albicans was the most susceptible organism among all the test isolate with zone of inhibition of 24 mm from ethanolic stem extract of *Helianthus annuus* (Table 1). This may suggest that the active ingredient in all the extracts penetrated the fungi cells to appreciable degree and caused reduction in fungal growth. The antimicrobial effect of medicinal plants on microorganisms may depend on the type of medium used to culture to microorganisms (Giesse, 1994). The antimicrobial agent may be incapable of diffusing through the cell wall or membrane of the microorganism as a result of the complexity in the organism's cell structure. It was recorded and shown that *Escherichia coli* was the most resistant organisms among the tested microorganisms because there was no zone of inhibition observed on the plate tested with , ethanol stem extract of *Helianthus annuus* . In addition, the absence of activity may also be due to number factors such as age of plant, time of collection of plant materials and climate which might in turn affect the amount of active ingredients in the plant material (Oshodi *et al.,* 2004)

Generally, antimicrobial activity of plants is affected by the nature of biologically active component present in the plant, the method of extraction of the plant as well as the extractant used. Excessive heating which often affect biologically active substances such as flavonoids essential oils and other heterogenous phytoconstituent present in the extracts may also influence their activity (Oshodi *et al*., 2004 and Akinyemi *et al*., 2005)

The MIC of ethanolic stem extract of *Helianthus annuus* was high on the test microorganisms . This means that antimicrobial substances in the extracts were bactericidal and fungicidal at higher concentrations of the extracts. The result of the MBC and MFC of this research work is in agreement with the observation reported by Olorundare *et al*., (1992).

The physical properties of the stem extract of the plants used were shown in Table 4. The pH of the ethanolic plant extract was discovered to be acidic. This variation in pH may account for difference in the antimicrobial nature of the extracts because acid generally tend to inhibit the growth of microorganisms. It will cause osmotic imbalance for the cell or bacteria and fungi, which may eventually leads to death of the microorganisms.

Study of the phytochemical screening of ethanolic extracts of the stem of *Helianthus annuus* used in this study, showed that the extract contain secondary metabolites (Table 3). The presence of these biologically active substances may have been responsible for the anti-bacterial and antifungal activities reported in this particular work. The results of the study have shown that stem *Helianthus annuus* possess pharmacologically active component capable of inhibiting or stopping the growth of pathogenic microorganisms used. The result showed that the extracts from stem of *Helianthus annuus* can be better purified to manufacture drugs for use in the treatment of skin infection, stomach upset, candidaisis and other diseases causes by the tested isolates.

The ability of ethanolic stem extract of *Helianthus annuus* to inhibit some organisms may be due to the active component it contain such as saponin, Tannins, steroids, Alkaloids, phlobatannins and flavonoid and phenolics . Flavonoid have ability to complex with bacterial cell wall which often lead to inactivation of the protein and loss of function (Tsuchija *et al*., 1996) phenolics inhibit enzymes by reaction with sulfhydryl groups or through non-specific interaction with protein thus toxic to microorganisms (Mason and Wasserman, 1987).

Alkaloids have been reported to interrelate with DNA of microorganisms; Tannins inactivate microbial adhesins, enzymes, cell envelope transport protein (Cowan, 1999). Scalbert 1991 reviewed the antimicrobial properties tannins. He listed 33 studies which had documented the inhibitory activities of tannins up to that point. According to these studies, tannins can be toxic to filamentous fungi, yeast and bacteria. (Jones *et al.,* 1994). The primary benefits of using plant-derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are of then associated with synthetic antimicrobials. (Iwu, 1993).

This finding is significant because most bacteria and fungi have been reported to be resistant to the action of most antimicrobial agent available. Therefore, the active components identified in the extracts should be purified. Secondary screening should be carryout on the purified active components and in vivo test should be carry out. The chemical structure of the active component of the plant extract should be determined for possible industrial synthesis.

From the above studies on the ethanolic stem extract of *Helianthus annuus*, it is concluded that the traditional plants may represent new sources of anti-microbials with stable, biologically active components that can establish a scientific base for the use of plants in modern medicine. These local ethnomedical preparations and prescriptions of plant sources should be scientifically evaluated and then disseminated properly and the knowledge about the botanical preparation of traditional sources of medicinal plants can be extended for future investigation into the field of pharmacology, phytochemistry, ethnobotany and other biological actions for drug discovery.

Table 1: Antimicrobial effect of ethanolic stem extract of *Helianthus annuus* on test organisms.

CONCENTRATION	**ORGANISM/ZONE OF INHIBITION (mm)**			
(mg/ml)	***Staphylococcs Aureus***	***Escherichia Coli***	***Aspergillus niger***	***Candida albicans***
200	11	NIL	12	24
100	6	NIL	6	13
50	2	NIL	4	7
25	NIL	NIL	NIL	4
Control	NIL	NIL	NIL	NIL

(NIL: No inhibition)

Table 2: Minimum inhibitory concentration and minimum bactericidal/fungicidal concentration of ethanolic stem extracts on the tested organisms

Test organisms	***Helianthus annuus***	
	MIC	**MBC/MFC (mg/ml)**
Staphylococus aureus	70	90
Aspergillus niger	80	80
Candida albicans	50	70
E. coli	-	-

Table 3: Phytochemical analysis of ethanolic *Helianthus annuus* stem extract.

		Helianthus annuus
S/N	**Active Component**	**Ethanol**
1	Saponin	+
2.	Tannins	+
3.	Flavonoid	+
4.	Terpenoid	-
5.	Phenolics	+
6.	Steroids	+
7.	Alkaloids	+
8.	Phlobatannins	-
9.	Glycoside	-

KEY + = Present

\- = Absent

Table 4: Physical properties of the stem extract of the tested plant

Extract	***Helianthus annuus***	
	Colour	**PH**
Ethanolic Extract	Dark Green	4.8

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Dr. Adetunji received scientific awards like TWAS, CSIR etc. Presently he is involved in the screening of medicinal plant bioproducts and their antimicrobial effects. He has formulated various edible coatings containing antimicrobials compounds that can extend the shelf life of fruits and vegetables.