Effects of *Helianthus annuus* and *Anthocleista djalonensis* on *Staphylococcus aureus* and *Escherichia coli*

ABSTRACT

Plant based medicines have been a part of traditional healthcare in most parts of the world for thousands of years. The medicinal plants are of great interest to human health. It has been proven that antimicrobials of plant origin work more efficiently with fewer side effects. The study aimed to identify the phytochemicals in the extracts of *Helianthus annus* and *Anthocleista djalonensis* and examine the effects of extracts of these plants on the growth of clinical bacterial isolates. Leaves of *Helianthus annus* and *Anthocleista djalonensis* were extracted in methanol, distilled water and hot water. The extract solution (100%) was further diluted to various concentrations (75%, 50%, and 25%). The data obtained were analyzed by Analysis of Variance (ANOVA) to determine significant ($P< 0.05$) effects. Significant differences between means were determined using Duncan's Multiple Range Test (DMRT). The phytochemical screening indicated the presence of alkaloids, phenols, glycosides, flavonoids, terpenoids, saponins and quinone. The extracts of the plants inhibited the growth of the bacteria tested with varied effectiveness. The maximum antibacterial activities were observed in the aqueous extracts. Thus the extracts of *Helianthus annus* and *Anthocleista sapo* can be used in the development of new pharmaceuticals that address unmet therapeutic use.

Key words: phytochemicals, plant extracts, *Helianthus annus* and *Anthocleista djalonensis*

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INTRODUCTION

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Plants are abundant source of phytochemicals which are antimicrobial molecules (Hemaiswarya *et al*., 2008). It had been reported that plants contain antimicrobial substances (Gullo *et al.*, 2006). Many studies have shown that medicinal plants contain coumarins, flavonoids, phenolics, alkaloids, terpenoids, tannins, essential oils, lectin, polypeptides, and polyacetylenes (Valli *et al*., 2012; Parvin *et al*., 2014) . These bioactive compounds are used as a starting point for antibiotics synthesis in order to treat infectious diseases (Rahman and Anwar 2007). Natural medicines have been used to boost health since time

immemorial, and the success of modern medical science largely depends on drugs originally obtained from natural sources (Kebede, 2021).

Sunflowers are botanically classified as *Helianthus annuus*. They are a large plant and are grown throughout the world because of their relatively short growing season (Adetunji *et al*., 2014). Among the plants used in the traditional medicine is *Helianthus annuus* leaves.

The Anthocleista species are trees and shrub-like plants presently in the Gentianaceae family, and formerly of the Loganiaceae family, in the major group Angiosperms. The genus Anthocleista contains about 14 species distributed in tropical Africa, in Madagascar and on the Comoros (Anyanwu *et al*., 2015). The traditional medicinal uses of Anthocleista is in the treatment of stomach ache, fever, constipation, inflammatory diseases, diabetes, wounds, etc. (Ateufack *et al*., 2014

Escherichia coli is a pathogenic bacteria transmitted by infected food. Many diseases caused by *E. coli* have been reported worldwide (Paproski *et al*., 2015).

Staphylococcus aureus is a gram-positive bacterium colonized on the skin, and present in the nose of 25% to 30% of healthy people (Grundmann, *et al*., 2006). It causes a range of skin infections, pneumonia, cardiovascular infections and other infections globally (Rasigade *et al*., 2014, Tong *et al*., 2015).

Therefore, the objectives of the study was to determine the phytochemicals present in different extracts of *Helianthus annuus* and *Anthocleista sapo* and examine the antibacterial activities of these plants.

Materials and Methods

Plant Collection and Identification.

Plants were collected from Anchor University campus environment.

Test organisms

Staphylococcus aureus and *Escherichia coli* clinical isolates bacteria collected from Nigerian Institute of Medical Research NIMR and incubated in the incubator at 37.0 ^{0}C.

Preparation of extracts

Preparation of Aqueous Extracts of *H. annuus* and *A. djalonensis*

Plants of *Helianthus annuus* and *Anthocleista sapo* were harvested and separated into shoots and roots. 100 g of the shoots were finely ground with blender so as to release the phytochemicals in the plant. The ground plant material was soaked in 500 ml of distilled water for three days. The solution was filtered through cheese cloth to remove debris and then centrifuged after which it was filtered through Whatman No 1 filter paper. This extract solution (100%) was diluted appropriately with water to give 75%, 50%, and 25% concentrations of the aqueous extracts while distilled water served as control. Hot water extract was prepared by boiling the distilled water extract to obtain hot water extract of the different concentrations.

Preparation of Methanol Extracts of Shoots of *H. annuus* and *A. djalonensis*

The same procedure described above for the preparation of water extract was carried out except that 20 g ground plant part was extracted in methanol. The solution was filtered through cheese cloth to remove debris and then centrifuged after which it was filtered through Whatman No 1 filter paper. The filtrate was dried using the petri plates and dissolved in 100ml of water to serve as the stock methanol extract solution. This extract solution (100%) was diluted appropriately with water to give 75%, 50%, and 25% concentrations of the methanol extracts while distilled water served as control.

Phytochemical Screening of the *H. annuus* and *A. djalonensis* water and methanol extracts.

Phytochemical screening for alkaloids, phenols, flavonoids, saponins, terpenoids and glycosides and were carried out according to the methods of Sofowora (1982) and Ghani (1998).

Antimicrobial activity of the extracts

The antimicrobial activity was carried out according to the agar well diffusion assay by (Irobi *et al*. (1994)) against *S. aureus* and *E. coli* clinical isolates bacteria. The agar diffusion seeded plate method was employed to assess the antimicrobial activity of *H. annuus* extracts and *A. djalonensis* . Tryptone Soy Broth was prepared according to the manufacturer's instruction and it was then used to grow the organism while Mueller-Hinton agar was used for the sub-culturing of the bacteria organisms. Sterile Petri dishes were prepared and labelled for each extract and organism. Agar bottles containing 20 ml freshly prepared Muller Hinton agar were cooled to 45 ºC and inoculated aseptically with 0.1 ml of the test organisms. The agar bottles were rotated and swirled to allow for mixing. The bottle contents were poured into the corresponding labelled petri dishes and allowed to set.

A sterile cork borer was used to bore 6 mm holes (wells) into the solidified seeded agar in the plates. A micropipette was used to introduce 0.1 ml of different concentration of extracts into the wells. The plates were left for 30 min to allow for diffusion of the extract into the agar and incubated at 37 ºC for 24 h. The diameters of zone of inhibition produced by the extracts were measured and their mean values reported

Statistical Analysis

The data obtained were analysed by Analysis of Variance (ANOVA) to determine significant ($P< 0.05$) effects. The significant differences between means were determined using Duncan's Multiple Range Test DMRT. The result of the study is presented as Mean ± standard error of the trials.

RESULTS AND DISCUSSION

The screening of the water and methanolic extracts of the test plants indicated the presence of glycosides, tannins, phenols, alkaloids, flavonoids and saponins (Tables 1 and 2) The inhibitory effects of different concentrations of aqueous and methanol extracts of *H. annuus* and *A. djalonensis* are shown in Tables 3 and 4. Both aqueous and methanol extracts had activities against two of the test microorganisms used. The highest zone of inhibition for *E. coli* when treated with *A. sapo* extract was recorded in water extract with a zone diameter of 27.0 ± 0.6 mm at 100%, while the lowest zone of inhibition was recorded in ethanolic extract (Table 3). The highest zone of inhibition for *S. aureus* was recorded in water extract with a zone diameter of 21.3 ±1.9 mm followed by hot water extract with zone of inhibition of 20.0 ± 0.6 mm, while the least zone of inhibition was recorded in methanolic extract. *H. annuus* 100 % hot water extract recorded the highest zone of inhibition with diameter of 17.0 ± 0.1 on *E. coli*, while the highest zone of inhibition on *S. aureus* was 16.5 ± 0.1 mm in 100 % aqueous extracts (table 4)

Increasing drug resistance of pathogens and negative consequences of antibiotic usage has led to the search for alternative medicines. A wide range of plants express complex mixtures of secondary metabolites within each species (Arguedas and Coley, 2005). Phenols, terpenoids, flavonoids, glycosides, tannins, alkaloids, steroids, saponins and resins are some important phytochemicals found in plants (Tiwari *et al*., 2011). In this study, the screening of the water and methanolic extracts of *H. annuus* and *A.* glycosides, tannins, phenols, alkaloids, terpenoids, flavonoids and saponins indicated the presence of glycosides, tannins, phenols, alkaloids, terpenoids, flavonoids and saponins. These chemicals like phenolics, terpenoids and alkaloids and their derivatives are potential inhibitors of bacteria.

Several researchers have reported that plants contain bioactive substances (Kilani, 2006; Babu *et al*., 2007; Maswada and Elzaawely, 2013). Some researchers have suggested that antimicrobial components of the plant extracts (terpenoid, alkaloid and phenolic compounds) interact with enzymes and proteins of the microbial cell membrane causing its disruption to disperse a flux of protons towards cell exterior which induces cell death or may inhibit enzymes necessary for aminoacids biosynthesis (Gill and Holley, 2006). Other researchers attributed the inhibitory effect of these plant extracts to hydrophobicity characters of these plants extracts which enable them to react with protein of microbial cell membrane and mitochondria disturbing their structures and changing their permeability (Tiwari *et al.*, 2009).

Extensive works have been carried out on the antibacterial activities of plant extracts. The antistaphylococcal activity in this study is in accordance with a previous study. For example, it has been reported that the ethanolic extract of *Berberis hispanica* roots was active against *S. aureus* (Aribi *et al*., 2017). The sensitivity of *E.coli* confirmed the activities obtained in previous screening against the *E.coli* (Pauw and Eloff, 2014). Also, Makhafola and Eloff (2012) in their preliminary investigation of the antibacterial activity of crude acetone extracts of *Ochna* spp. reported that *E.coli* was the most sensitive bacterial species amongst the tested bacteria. The results of the present study corroborate the reports of previous workers (Singh, 2013) and (Adetunji, 2011).

Table 1. Phytochemical profile of *H. annuus* plant extract

Phytochemicals	Distilled water extract	Hot water extract	Methanol extract
Glycosides	+	+	–
Flavonoids	+	+	+
Saponins	+	+	–
Phenols	+	+	+
Terpenoids	+	+	+
Alkaloids	+	+	+
Quinone	+	–	–
Anthraquinone	+	–	–

+ indicates the presence of the phytochemical compound

- indicates the absence of the phytochemical compound

Table 2. Phytochemical profile of *A. djalonensis* plant extract

Phytochemicals	Distilled water extract	Hot water extract	Methanol extract
Alkaloids	-	-	-
Glycoside	+	+	+
Flavonoids	+	+	+
Saponins	-	-	-
Phenols	+	+	+
Terpenoids	+	+	+
Quinones	+	+	+
Anthraquinones	-	-	-

Table 3: Antimicrobial activity of *H. annuus* extracts

		E. coli	*S. aureus*
H. annuus extract	Extract concentration	Zone of inhibition (mm)	Zone of inhibition (mm)
Hot Water Extract	100%	17.0 ± 0.1	16.5 ± 0.1
	75%	15.5 ± 0.1	15.0 ± 0.1
	50%	15.0 ± 0.0	14.0 ± 0.1
	25%	13.0 ± 0. 1	07.0 ± 0.0
Water Extract	100%	13.5 ± 0.1	16.5 ± 0.1
	75%	12.5 ± 0.1	15.0 ± 0.1
	50%	11.5 ± 0.2	14.0 ± 0.1
	25%	10.5 ± 0.3	7.0 ± 0.1
Methanol Extract	100%	-	-
	75%	-	-
	50%	-	-
	25%	-	-

Table 4 Antimicrobial activity of *A. djalonensis*

		E. coli	*S. aureus*
	Extract concentration	Zone of inhibition (mm)	Zone of inhibition (mm)
Hot Water Extract	100%	25.3 ± 0.9	20.0 ± 0.6
	75%	21.3 ± 0.9	17.3 ± 0.3
	50%	17.7 ± 0.9	16.0 ± 1.5
	25%	-	2.0 ± 2.0
Water Extract	100%	27.0 ± 0.6	21.3 ±1.9
	75%	24.0 ± 0.6	17.7 ± 0.9
	50%	19.6667 ± 0.3	11.0 ± 0.6
	25%	-	-
Methanol Extract	100%	9.0 ± 0.6	18.0 ± 1.2
	75%	7.3 ± 0.9	10.3 ± 5.2
	50%	-	4.0 ± 4.0
	25%	-	-

CONCLUSION

The development of multiple antibiotic resistant organisms has constituted a global problem as far as treatment of some infectious diseases is concerned. There is the need for the application of herbal products for the bio-control of diseases.This study showed that these plants are effective antibacterial agents against *S. aureus* and *E. coli* and have the potential to be utilized for the treatment of bacterial infections. Phytochemical research is required to investigate new natural active compounds derived from plants that possess antimicrobial effects.

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