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Phytochemical and Antimicrobial Potentials of Aqueous Leaves Extracts of *Helianthus annuus* (Sunflower): A Natural Preservative in Food Industry

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ABSTRACT

Helianthus annuus (sunflower) is an important plant that belongs to the family Asteraceae and usually used as oil in the food industry. This study investigates the phytochemical and antimicrobial potentials of aqueous leaf extracts from *Helianthus annuus* (AqHa). The *H. annuus* leaves were collected from botanical section of Ajayi Crowther University, Oyo and were taken to the laboratory and processed into aqueous extract using standard method. The Aqueous leaf extracts from *Helianthus annuus* (AqHa) were assessed for phytochemical attributes, Fourier transform infrared Spectroscopy (FTIR), antioxidant activity and antimicrobial potential against food pathogens using standard procedures. Phytochemical analysis of the assessed extracts revealed the presence of some plant metabolites including flavonoids, steroid, tannins and saponins while FTIR showed the presence of some key functional groups such as O-H stretch, H-bonded, O-H stretch, H-bonded, Methylene C-H stretch, Methyne C-H stretch and C=C Conjugated bonds.

The peak at 2355.00 cm^1 and 1735.60 cm^1 were related to stretch vibration of Methylene C-H and C=C, respectively both denoting the presence of fat while the highest peak (3759.57 cm^{-1}) was related to hydroxyl group. AqHa had the highest zone of inhibition (13 mm) against *Staphylococcus aureus* while the least (7 mm) inhibition was on *Staphylococcus* spp. The highest antioxidant activity was evaluated as Hydrogen peroxide scavenging assay (56 and 87%) and reducing power scavenging assay (49% and 83%). Aqueous leaf extracts from *Helianthus annuus* (AqHa) contains phytochemical compounds, had antioxidant activity and antimicrobial potentials on food pathogens, and could be used as an alternative in the development of plant based antimicrobials or as preservatives in food industries.

INTRODUCTION

Sunflower (*Helianthus annuus*), an annual plant belonging to the family Asteraceae is more commonly known as a decorative plant with its bright yellow petals, brown center and plays a very important role in meeting the food demands and fulfilling nutritional benefits. However, for many centuries, it has been used as a medicinal plant due to its numerous therapeutic benefits. The use of sunflowers as a medicinal plant can be traced back to American culture where it was used for a wide variety of health purposes (Han *et al*., 2017; Kumar *et al.,* 2020; Cocciardi *et al.,* 2021). Sunflower plants are easily adaptable, can grow in a range of climatic condition that requires full sun exposure and well-drained soils. In traditional medicine, different parts of the sunflower plant have been used for the treatment of various ailments. For example, sunflower leaves have been used for the treatment of respiratory infections, fever, and arthritis, while the seeds and its oil have been used to alleviate pain caused by arthritis, anti-inflammatory and analgesic properties (Cocciardi *et al.,* 2021). The sunflower plant contains phytochemicals such as flavonoids, phenolic acids, and tannin which have been reported to possess various pharmacological activities such as antimicrobial, antioxidant, and anti-inflammatory properties (Kumar *et al.,* 2020; Karunakaran *et al.,* 2020).

Sunflower oil is rich in important fatty acids such as linoleic acid (55–70%) and oleic acid (20–25%). Oleic acid has mono-saturated omega-9 fatty acids, which help to reduce the risk of heart attack by converting the low-density lipoproteins to high-density lipoproteins (Perezvich et al., 2021). It also helps in oil stability, reduces oxidative stress and works as a preservative. Higher contents of poly-saturated fatty acids (31%) has also been identified in sunflower compare to sesame (25.5%), flax (22.4%), cottonseed (18.1%), peanut (13.1%) and soybean (3.5%) (Ikram *et al.,* 2021). Sunflower seeds are rich in nutrients, including vitamins E, magnesium, and selenium, which are essential for maintaining healthy body and preventing various diseases. The consumption of sunflower seeds can also help to regulate blood sugar levels and cholesterol, and could also lower the risk of heart disease. Its seeds are used for the treatment of different diseases such as chest infection, cold, heart diseases, chronic pulmonary infection and pertussis (Grunvald et al., 2014). In addition, the plant contains oleic acid which suppresses Her-2/neu gene that is responsible for formation of breast cancer (Nounou, 2015).

Another significant health benefit of sunflower is its antioxidant properties and this protect the body from oxidative stress that contribute to various diseases such as cancer. The antioxidants in sunflower also help prevent cellular damage, reduce inflammation, and slow down the aging process.

Furthermore, sunflower have been found to have antibacterial and antifungal properties, which makes them useful in treating various skin conditions. Sunflower extracts have been used for the treatment of acne, eczema and psoriasis. The flower is more than just decorative plants but a natural remedy for a wide range of health problems. Due to the presence of phytochemicals such as phenolic compounds, antioxidants, poly-saturated fatty acids, it is regarded as important medicinal plant with nutraceutical value.

However, scientific evidence on the efficacy and safety of sunflower leaf extract for antimicrobial purposes is limited and inconsistent. *Helianthus annuus* for therapeutic applications is quick, inexpensive, environmentally friendly, and safe. The aim of this study was to determine the phytochemical and antimicrobial potentials of aqueous leaf extract of *H. Annuus* (sunflower).

MATERIALS AND METHODS

Sample collection and Preparation of the plant leaves extract

H. annuus leaves were collected from Ajayi Crowther University campus and transported to the laboratory for further analysis. Fresh and healthy *H. annuus* leaves were thoroughly washed with clean water, re-washed with sterile distilled water and air-dried at room temperature in a shade for 7 days. The air-dried leaves were mashed using mini mortar and pestle, and blended into powder form using an electric blender. The powdered samples were kept in air tight plastic containers.

Preparation of aqueous extracts from the leaves of *H. annuus*

Fifty grams (50 g) of the dried powder were weighed and soaked in two different flask containing 250 mL of distilled water, and stirred for five minutes. The flask was covered with foil, and then allowed to stand for 48 hours. The suspensions were shaken vigorously and filtered using Whatman filter paper and the filtrate was evaporated under vacuum using a rotary evaporator at 40 ºC. The viscous semi solid extract *H. annuus* leaves was subjected to phytochemicals analysis.

Phytochemical screening of the aqueous leaves extracts

Test for alkaloids

Three (3) mL of the aqueous extract was added to a test tube containing 1 mL of 1% HCL , and allow to heat for twenty minutes in a water bath . After cooling 0.5 mL of Mayer's reagent was added. The appearance of creamy white color indicates the presence of alkaloid.

Test for phenols

One (1) mL of the extract was introduced into a test tube containing 1 mL of distilled water. 2 drops of 5% ferric chloride solution was added to the extract. The formation of orange like color signified the presence of phenols.

Test for glycosides

About 3 mL of the extract was introduced into a test tube containing 2 mL of distilled water and a few drops of 5% aqueous NaOH were added. The formation of yellow colour indicates the presence of glycosides.

Test for tannins

About 10% of lead acetate was prepared. 2 mL of the extract was then mixed with 1mL of 10 % lead acetate. The development of white precipitate showed the presence of tannins.

Test for steroids

During the detection of steroids, 2 mL of chloroform was added to 2 mL of the extract and 2 mL of concentrated H2SO4 was added to the mixture. The presence of red colour at the bottom of the test tube indicates the production of steroids.

Test for flavonoids

About 3 mL of the extract was added into a test tube containing 10 mL of distilled water, and 1 mL of 10% NaOH was also added. The presence of intense yellow colour indicates a positive test for the confirmation of flavonoids.

Test for saponins

About 2 mL of the extract was introduced into a test tube containing 1 mL of distilled water. The solution was mixed vigorously in a graduated cylinder for 30 seconds. The development of a foamy layer in the solution indicates a positive test for saponins.

Test for antraquinones

About 10 mL of the leaf extract was dispensed into a cylinder and filtered. The filtrate was added into a test tube containing 5 mL of 10% ammonia solution and was agitated for few seconds. The upper aqueous layer was observed for bright pink coloration as an indicative for the presence of antraquinones.

Test for Terpenoids

Five (5) mL of the extract was mixed with 2 mL of chloroform and the mixture was evaporated to dryness. About 3 mL of concentrated sulphuric acid was carefully added to the dried residue form a layer. A reddish brown coloration of the interface was formed which indicates a positive result for the presence of terpenoids.

Fourier Transform infrared Spectroscopy (FTIR) Assay

For FTIR assay, 5 mg dried extract powder was encapsulated in 100 mg of potassium bromide (KBr) pellet and translucent sample discs of 16 mm diameter were prepared. FTIR analyses of the samples were performed in THERMO NICOLET IS10 FTIR Spectrometer (THERMOSCIENTIFIC). The samples were run at infrared region between 400-4000 cm-1 and standard DLATGS detector was used at 2.8 mm/sec mirror speed. Fourier transform infrared spectroscopy (FTIR) was used to identify the functional groups or compounds in the leaf extract.

Antimicrobial potential of the aqueous leaf extracts against food borne pathogens.

The antibacterial potential of the aqueous leaf extract against food pathogens was done according to the method of Owoseni and Ajayi (2010). Five food borne pathogenic microorganisms were used in this study consisting of three Gram positive bacteria (*Staphylococcus* spp, *Staphylococcus aureus* and *Escherichia coli)* and two Gram negative bacteria namely *Klebsiella* spp. and *Pseudomonas* spp. All the bacteria were isolated from food samples and analysis was done in the Microbiology laboratory unit of Ajayi Crowther University, Oyo.

Antioxidant Assay

Hydrogen peroxide scavenging

Hydrogen peroxide scavenging assay of *Helianthus annuus* was done using hydrogen peroxide solution (Gulcin *et al.,* 2004). Two different concentration (200 and 400 uL) of the plant extract were dispensed in the test tube containing 0.4 mL of phosphate buffered saline (PBS) and 0.6 mL of hydrogen peroxide. The mixture was allowed to stand for 10 minutes and incubated at 30^oC. After incubation, the value of the hydrogen peroxide scavenging was measured using UV spectrophotometer at 230 nm absorbance. The value of leaf extract without hydrogen peroxide was also recorded.

Determination of reducing power

The reducing power was evaluated using the method of Virtanen *et al.,* (2007). Two different concentrations (200-400 uL) of the aqueous leaf extract were added into a test tube separately containing Potassium ferricyanide. The solution was kept at 50^0 C for 20 minutes, and about 10 % of trichloroacetic acid was added to the mixture and allowed to spin at 3000rpm for 10mins. After spinning, 1.5 mL of sterile distilled water and 1 mL of 0.1% of Ferric chloride were dispensed into the solution. The absorbance of the solution was measured with spectrophotometer at 700 nm after 10mins incubation with the use of ascorbic acid as standard.

Total Antioxidant Activity

The total antioxidant activity was evaluated using method of Kanamaralapuda and Muddada (2017). About 1235 g of Ammonium sulphate, 45 mL of Sulfuric acid, and 0.0042 g of Sodium sulphate were added in 250 mL of distilled water and stirred thoroughly and regarded as the total antioxidant capacity. After stirring, 200 and 400 uL of the aqueous leaf extracts were mixed differently with 1 mL of the total antioxidant capacity. The absorbance of the mixture was taken at 695 nm after 15minutes using ascorbic acid as a guide.

RESULTS

Phytochemical analysis

Table 1 investigated the presence of phytochemical compounds in aqueous leaf extracts from *Helianthus annuus* (AqHa). The result showed that metabolites such as Alkaloids, saponins, flavonoid, glycosides, steroids, tannin, and terpenoids were present in the leaf extract. Out of the nine phytochemical compounds identified, only two (Phenol, Arthraquinones) were absent in the aqueous plant extract.

Table 1: Phytochemical analysis of Aqueous leaves Extract from *Helianthus annuus (*sunflower*)*

S/N	Phytochemical parameters	Aqueous Extract
1	Alkaloids	+
2	Saponins	+
3	Flavonoids	+
4	Steroid	+
5	Tannins	+
6	Phenol	-
7	Glycoside	+
8	Antraquinones	-
9	Trepenoids	+

Key: +**: present, -: absent**

Fourier Transform infrared Spectroscopy (FTIR):

The peak of FTIR analysis of the aqueous leaf extract ranged from 356.75 to 3759.69 cm^{-1}. The peak at 3759.69 cm^1 corresponds to hydroxyl (O-H) stretch that belong to polysaccharides and alcohol nutrient type. The peaks at 3440.00 and 3413.00 indicated the presence of O-H, H- bond associated with various polysaccharides while 2355.00 and 2927.66 cm^{-1} had a functional group of Methylene C-H stretch with nutrient type of fat and Amino related compound, respectively**.** The peaks at 2289.00 and 1735.00 cm^{-1} correspond to the stretching of the Esters carbonyl C=O C-C conjugated bonds, respectively indicating fat as nutrient type.

Moreover, an ester compound was observed at the peak of 1633.36 cm^{-1} without a nutrient type while the peak at 1574.66 cm^{-1} confirms with the presence of aromatic (CH) and nutrient type of lignin. The functional group of Glycosidic linkages C-O-C was detected at peaks 1383.36 and 1266.53 cm-^{1} with the presence of cellulose/polysaccharides whereas CH deformation functional group was observed at peak 1390.06 cm$^{-1.}$

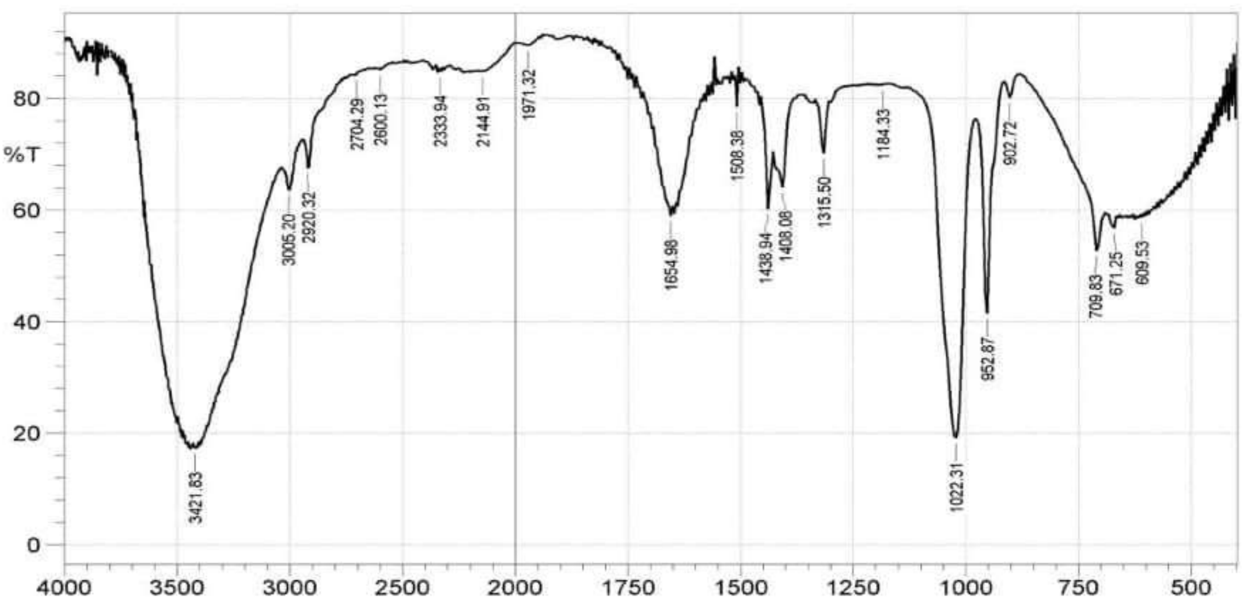

Figure 1: FTIR spectrum of the aqueous leaf extracts from *Helianthus annuus* (AqHa).

Table 2: FTIR spectrum and peak values of the aqueous extract leaves of *H. annuus (*sunflower)

Wave number cm-^{1}(peak values)	Assigned Functional Group	Possible Nutrient Type
3759.57	OH stretch,	Polysaccharides/Alcohols
3440.00	O-H stretch, H-bonded	Various polysaccharides
3413.00	O-H stretch, H-bonded	Various polysaccharides
2355.00	Methylene C-H stretch	Fat
2927.86	Methyne C-H stretch	Amino-related component
2289.00	Ester carbonyls C=O	Fat
1735.60	C=C Conjugated bonds	Fat
1633.36	Ester	-
1574.66	Aromatic/CH deformation	Lignin
1383.36	Glycosidic linkages (C-O-C)	Cellulose/Polysaccharides
1390.06	CH deformation	Cellulose/Polysaccharides
1322.80	Glycosidic linkages (C-O-C)	Outer surface-suberin/cutin
1266.53	Glycosidic linkages (C-O-C)	Cellulose/Polysaccharides
1257.82	C-C, C—O, C=O	Lignin
1032.60	C-O stretch	Starch
1030.54	Glycosidic linkages (C-O-C)	Cellulose/Polysaccharides
541.21	Deformation and P-O stretch	Phosphate anion
524.53	Deformation and P-O stretch	Phosphate anion
454.42	Fingerprints	
388.57	Fingerprints	
370.23	Fingerprints	
356.75	Fingerprints	

Antibacterial potential of aqueous leaves extract of *Helianthus annuus* against food pathogens

The aqueous extract demonstrated antimicrobial activity against a range of microorganisms, including both Gram-positive and Gram-negative bacteria.

Figure 2 shows the antimicrobial activities of aqueous extract of *Helianthus annuus* leaves against some food organisms. The zone of inhibition ranged between 7 – 20 mm. *Staphylococcus aureus* had the highest zone of inhibition (13 mm), followed by *Escherichia coli* (11 mm) while *Pseudomonas* spp. and *Klebsiella* spp. had 8 mm each. The least value (7 mm) was observed by *Staphylococcus spp.*

Antioxidant potential of the aqueous leaves extract of *Helianthus annuus* (sunflower)

Figure 3 also revealed the antioxidant potential (Hydrogen peroxide, reducing power and total antioxidant activity) of the aqueous extract leaves. At the concentration of 200 ul, the hydrogen peroxide, total antioxidant activity and reducing power scavenging assay ranged from 42 – 56%. The highest antioxidant activity was observed at concentration of 400 ul with Hydrogen peroxide scavenging assay (87 %), total antioxidant activity (54%) and reducing power scavenging assay (83%), respectively while concentration of 200 ul had hydrogen peroxide (56 %), total antioxidant activity (42 %) and reducing power of 49 %.

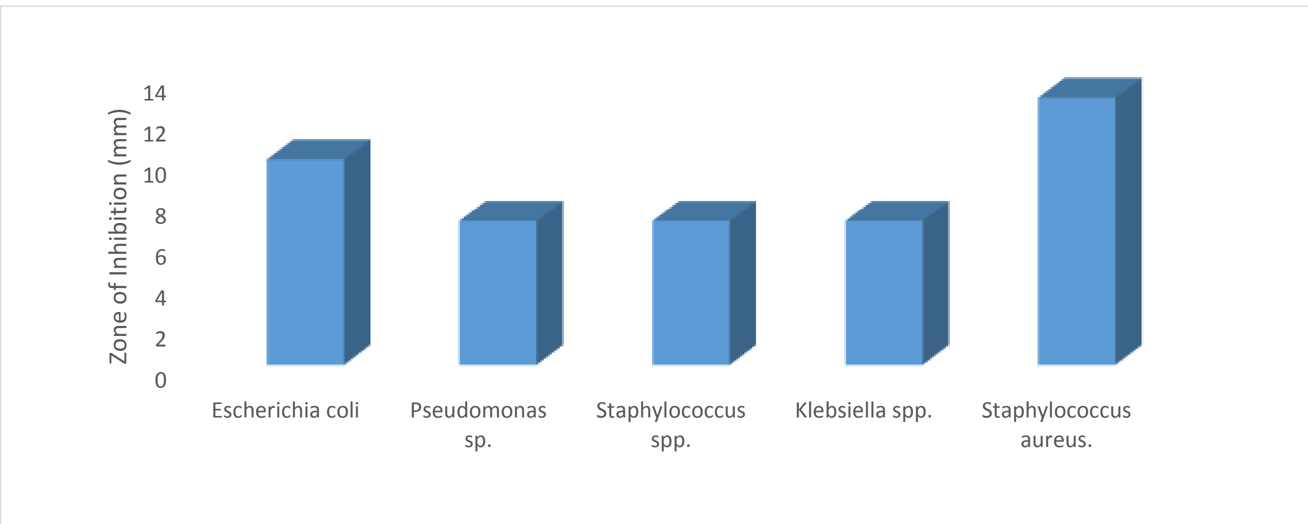

Figure 2: Antimicrobial potential of Aqueous leaves extracts of *Helianthus annuus* (sunflower) against some food pathogens

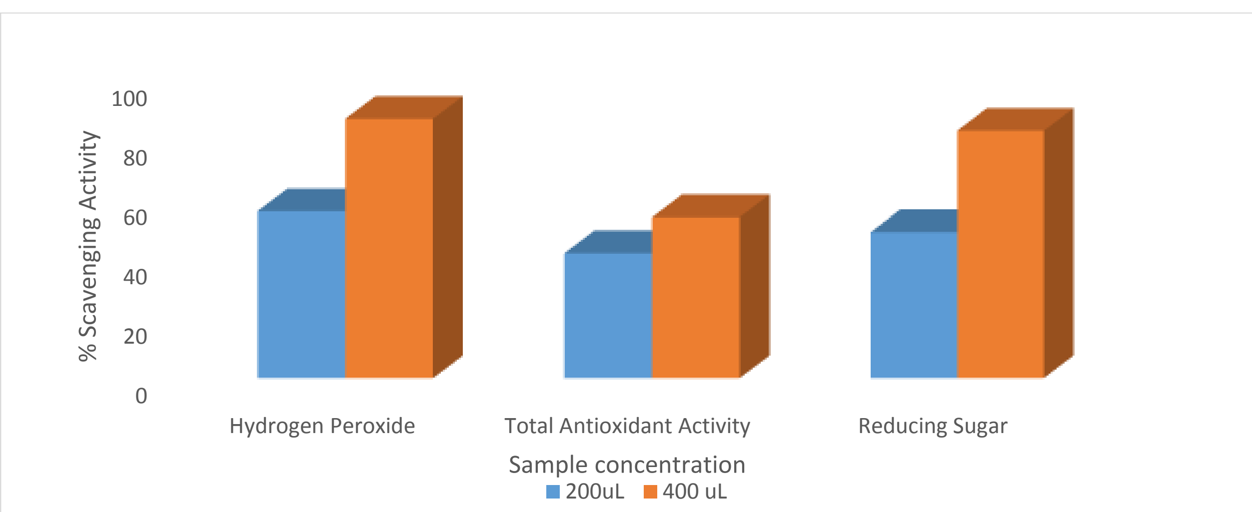

Fig. 3: Antioxidant potential of the aqueous leaves extract of *H. annuus* (sunflower)

DISCUSSION

The phytochemical analysis revealed the presence of various bioactive compounds in the aqueous extract of *Helianthus annuus* leaves, including flavonoids, alkaloids, saponins, and tannins. These compounds have been associated with numerous health benefits and contribute to the medicinal properties of the plant.

Most plants protect themselves by producing some chemicals that can also protect humans against diseases (Oti and Olivia, 2017). Phytochemicals are chemical compounds that naturally occur in plants which are responsible for some attributes such as smell, taste, colour and other organoleptic properties. They are classified into curative (or nutritive) such as alkaloid, saponnins, tannins, flavonoids, phenols, glycosides, isoflavones, cartenoids, sulfides, and non-curative (or non-nutritive) such as cyanide, oxalates, terpene and terpenoids. All the phytochemicals that were detected are known to have industrial and medicinal importance. Saponin is known to be an antioxidant or anti-cancer, and for the treatment of hypercholesterolemia. It is also used as a mild detergent in histochemistry staining to allow antibody access to intracellular proteins. Tannins exhibit antiviral, antibacterial and antitumor activities (Drabble and Nierentein, 2000). Certain tannins are also able to inhibit Human Immune Virus (HIV) replication selectivity. Although, the result from this study revealed the absence of phenols and glycosides which contradicts the study of Eze-Ilochi (2017) that reported the presence of steroids and glycosides in aqueous *Carica papaya* leaf. Glycosides are known to work by inhibiting the Na/ K pump (Manske, 2009).

Moreover, steroid play important role in cardio tonic activities and also possess both insecticidal and antimicrobial properties. They are very useful in the nutrition, herbal medicine and cosmetics industries. They are routinely used in medicine because of their profound biological activities.

In addition, metabolites like flavonoid has inherent ability to modify the body's reaction to allergies, virus and carcinogens (Erdman *et al*., 2007). They show anti allergic, anti-inflammatory, antimicrobial and anticancer activities. Alkaloid has the potency to correct serious disorders such as heart failure, cancer and blood pressure.

The FTIR revealed the specific chemical constituents in the extract as well as those that might be participating in the reduction and stabilization of the nanoparticles (Badmus *et al*., 2020). Also, chemical composition and functional groups could also be responsible for their antimicrobial effects. The FTIR peaks obtained in this study are closely related to the observed peaks in previous studies and this confirms that irrespective of their mode of synthesis, similar phytochemicals are responsible for the synthesis of the AgNPs by the plant extracts (Badmus *et al*., 2020).

The high antimicrobial activity of 11 mm zone of inhibition for *E. coli* and maximum 13 mm for *staphylococcus aureus*, *Pseudomonas* and *staphylococcus* spp. could be as a result in degrees of solubility of the active constituents in the aqueous leaf extract (Marjorie, 1999). The difference in values of antimicrobial activities recorded in this study could also be affected by the presence of oil, wax, resin, fatty acid or pigments, which have the capacity to block the active ingredient in the plant extracts from accessing the bacteria cell wall (Jigna *et al.,* 2006). Tannin, saponin, flavonoid and glycoside are major phytochemicals that contribute to the inhibition (Fowomola, 2012). The presence of antimicrobial compounds in the extract could potentially be used for the development of natural antimicrobial agents.

Based on the obtained result, leaves of sunflower extract possessed some potential phytochemicals that exhibit antimicrobial activity with plants extract being the most potent than water extract. Apparently, this findings suggested the potential use of leaf extract as therapeutic agent for the treatment of urinary tracts infection and diseases caused by food borne pathogens. The presence of flavonoid in the aqueous extract of *helianthus annuus* is an indication for the strong zone of inhibition.

The aqueous extract exhibited significant antioxidant activity, as evidenced by its ability to scavenge free radicals. The extract's strong DPPH radical scavenging activity suggests its potential in reducing oxidative stress and protecting against oxidative damage-related diseases.

Moreover, antioxidant potential measures the ability of the food or plant to prevent or slow down oxidative damage caused by free radicals in the body. Free radicals are unstable molecules that can damage cells and contribute to the development of diseases such as cancer, diabetes and heart diseases. Antioxidants are known to neutralize free radicals and protect the body from their harmful effects.

The importance of antioxidant potential of the leaf extract could help in identifying plants or plant based food product that can provide protection against chronic diseases and promote good health. This is also useful in formulating a balanced and nutritious diet that is rich in antioxidants.

The comprehensive analysis of the aqueous extract of Helianthus annuus leaves provides scientific evidence to support its traditional use in folk medicine. The presence of phytochemicals and their associated antioxidant and antimicrobial activities offer potential health benefits and therapeutic applications. These findings contribute to the growing body of knowledge on natural products and their potential as alternative sources of antioxidants and antimicrobial agents. However, further studies are needed to isolate and identify the specific bioactive compounds responsible for the observed activities, as well as to investigate their mechanisms of action.

In conclusion, the aqueous extract of Helianthus annuus leaves possesses a diverse range of phytochemicals and exhibits significant antioxidant and antimicrobial activities. These findings underscore its potential as a valuable natural resource for food and nutraceutical industries, and warrant further exploration for the development of novel therapeutic intervention.

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