

ANTIBACTERIAL AND ANTIOXIDANT POTENTIALS OF LEAVE EXTRACTS OF *HELIANTHUS ANNUUS*

Oghenerobor Akpor, Tomilola Olaolu, Damilare Rotimi

ABSTRACT

Helianthus annuus has been widely used for its medicinal and nutritional properties. This study was aimed at assessing the ethyl acetate, n-hexane and methanol extracts of *Helianthus annuus* for antibacterial and antioxidant potentials. The phytochemical screening, total phenols, DPPH radical scavenging assay and nitric oxide radical scavenging activity were carried out following standard procedures. Preliminary screening of the antibacterial activities of the extracts was carried out on five bacterial species (*Bacillus subtilis*, *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae*), using the agar-diffusion method. Growth rate studies in presence of the extract was investigated on two bacterial species (*Bacillus subtilis* and *E. coli*). The methanol extract was observed to inhibit the growth of the five bacterial species while ethyl acetate and N-hexane extracts showed inhibition against *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. Extended lag periods of 5 – 6 h were observed when the *Bacillus subtilis* and *Escherichia coli* were grown in broth medium that contained the respective extracts. In broth medium with mixture of extract and ascorbic acid, there was no observed growth of the *Bacillus subtilis* and *Escherichia coli* throughout the 7 h incubation period. The total phenolics content of the extracts revealed concentrations of 6.66 ± 0.45 , 5.58 ± 0.11 and 6.06 ± 0.41 mg TAE.g⁻¹) for the methanol, N-hexane and ethyl acetate extracts respectively. The DPPH radical scavenging assay results displayed gradual increase in percentage inhibition from the lowest to the highest concentration across all the standard groups, a similar trend was observed with the extracts, the ethyl acetate extract showed highest percentage inhibition amongst the other extracts. All the extracts showed high reducing power ability. The nitric oxide scavenging ability of the extracts showed constant increase with increase in concentration. *Helianthus annuus*, it could be a good source of antimicrobial and antioxidant especially in a world where resistance to antibiotic has increasingly become a global concern.

Keywords: Antibacterial; antioxidant; growth inhibition; *Helianthus annuus*

INTRODUCTION

Plants are of great economic value all over the world as it has been as raw materials in the industries especially the pharmaceuticals, nutraceuticals and cosmetics (Eze et al., 2015; Ibrahim et al., 2014). The use of plants and its products as medicines is an age long pattern. Despite the advent of modern medicine, plant extracts and its constituents are being used traditionally as therapy in 70 – 80% of the world population to meet their health needs, most especially the rural dwellers in developing countries (Ibrahim et al., 2014; Subashini and Rakshitha, 2012). Most of these claims has not been scientifically proven as only few plants have been evaluated of their therapeutic effects.

Helianthus annuus has been noted for its medicinal and nutritional usage worldwide. *H. annuus* L. (*Asteraceae*) commonly referred to as sunflower is cultivated in

America, Africa, Asia and Australia. Its seed is an important source of edible oil all over the world (Ibrahim et al., 2014; Al-Snafi, 2018). The plant has a characteristic tap rooted plant with coarse toothed leaves, yellowish flower, rough-hairy stem and 1.5 – 3.5 m high (Eze et al., 2015; Al-Snafi, 2018). The seeds, roots, flowers and bark of *H. annuus* have been used for medicinal purposes (Al-Snafi, 2018). Analysis of the phytochemical analysis present in *Helianthus annuus* reveals that it contained phenolics, flavonoids, steroids, alkaloids, saponins, carbohydrates, tannins, phytosterols, triterpenoids and fixed oils. *Helianthus annuus* seed can be used as salad garnish, snacks and some bakery goods (Guo et al., 2017).

The seed oil and shoot have been used as stimulant, antioxidant, anti-inflammatory, cathartic, diuretic, anti-hypoglycemic, antitumor, anti-asthmatic, antipyretic, antimicrobial and for stomach problem purposes. The seed

is essential for diuretic, cough, throat and lung infections treatment (Eze et al., 2015; Subashini and Rakshitha, 2012). In Venezuela, *H. annuus* flower and seed serve as cancer treatment. Reports has also shown crushed leaves serve as medicinal covering on swelling, sores, snakebites and spiderbites (Jiraungkoorskul, 2016; Al-Snafi, 2018). This study was therefore aimed at assessing the antimicrobial and antioxidant properties of ethyl acetate, hexane and methanol extracts of *Helianthus annuus* leaves.

Scientific hypothesis

Helianthus annuus extracts has antibacterial and antioxidant properties.

MATERIAL AND METHODOLOGY

For preparation of the plant extracts, the leaves of the *Helianthus annuus* were collected from the Teaching and Research Farm of Landmark University, Omu Aran, Kwara state. After collection, the leaves were washed with clean tap water to remove dirt and other unwanted materials, before sun-drying for five days. Following drying, the leaves were pulverized, and known quantities were placed in beakers containing the respective extracts. Extraction and concentration of the extracts were carried out as describes by earlier workers (Eze et al., 2015; Ibrahim et al., 2014; Subashini and Rakshitha, 2012).

For preliminary evaluation of the antibacterial potential and determination of the minimum inhibitor concentrations (MIC) of the extracts, the agar diffusion method. The bacterial species used were *Staphylococcus aureus*, (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* and *Klebsiella* sp. The *Escherichia coli* and *Klebsiella* sp were laboratory stock cultures of the Department of Microbiology, Landmark University, Omu-Aran, Nigeria. Prior to use, the respective isolates were streaked on sterile nutrient agar plates to ascertain their purity, before subculturing in sterile peptone water. Two bacterial species (*Bacillus subtilis* and *Escherichia coli*) were used for the growth rate studies. Growth rate studies was carried out liquid medium, with four different composition, which were: nutrient broth only, nutrient broth + ascorbic acid, nutrient broth + extract, nutrient broth + extract + ascorbic acid. In 100 mL sterile nutrient broth, 5 mL of MIC of the respective extracts against a known bacterial species was added and incubated in an orbital shaker (S15200) at 37 °C at 120 rpm. Prior inoculation and every 1 h interval for 7 h incubation period, 5 mL of sample was aseptically withdrawn from each flask for determination of optical density at wavelength of 720 nm.

Growth rate was calculated as:

$$Growth\ rate\ (d^{-1}) = \frac{\ln(C1) - \ln(C0)}{t1 - t0}$$

Where:

C0 and C1 represent initial and final absorbance, respectively; t0 and t1 represent initial and final time, respectively.

All experimental setups were carried out in duplicates.

The phytochemical screening methods used were protocols of Trease and Evans (2002) and Sofowora (1993). Total phenols content was determined by the method of McDonald, Prenzler and Antolovich (2001), DPPH radical scavenging assay was done using the protocol of Shimada et al., (1992). Nitric oxide radical scavenging activity was carried out by the method of Panda et al., (2009).

All chemicals and reagents used for the study were of analytical grade. Also, all experiments were carried out in duplicate.

Statistic analysis

Statistical analysis was carried out using the SPSS Statistical Software. Data were presented as means of duplicates analysis and error bars calculated as standard errors of means. The One-Way Analysis of Variance (ANOVA) was used in the determination of comparison of means. Statistical significance was determined at *p*-value of 0.005.

RESULTS AND DISCUSSION

Phytochemical profile of the extracts

Ten phytochemical tests were carried out on the ethyl acetate, methanol and N-hexane extracts, only alkaloids, flavonoids, saponins, tannins, phenols, cardiac glycosides and terpenoids were detected out of the ten while anthraquinones, phlobatannins and steroids were not detected. Flavonoids, tannins and phenols were detected in all the extracts while terpenoids and saponins were only detected in the methanol extract (Table 1).

Antioxidant properties of the extracts

The total phenolics content of the extracts revealed concentrations of 6.66 ±0.45, 5.58 ±0.11 and 6.06±0.41 mg TAE.g⁻¹) for the methanol, N-hexane and ethyl acetate extracts respectively. These concentrations were calculated using the equation of calibration curve for tannic acid, *y* = 0.1471*x*, R² = 0.9922 and expressed as mg/g tannic acid equivalent TAE (Figure 1).

For the DPPH radical scavenging assay, gradual increase in percentage inhibition from the lowest to the highest concentration was observed across all the standard groups (rutin, vitamin E and vitamin C).

Table 1 Phytochemical profile of the extracts.

Extracts	Phytochemicals									
	Alk.	Fla.	Sap.	Tan.	Phe.	Car.	Ter.	Anthr.	Phlo.	Ste.
Ethyl acetate	+	+	-	+	+	-	-	-	-	-
Methanol	-	+	+	+	+	+	+	-	-	-
N-hexane	+	+	-	+	+	+	-	-	-	-

Note: Alk., Fla., Sap., Tan., Phe., Car., Ter., Anthr, Phlo and Ste. Represent Alkaloids, flavonoids, saponins, tannins, phenols, cardiac glycoside, terpenoids, anthraquinones, phlobatannins and steroids, respectively. ‘+’ and ‘-’ indicate detected and undetected, respectively.

A similar trend was observed with the extracts, with the ethyl acetate extract showing highest percentage inhibition amongst the other extracts (Figure 2). With respect to the reducing power ability of the extracts, while there was consistent increase with increase in concentration of the standard rutin, the extracts did not reveal that trend. Although all the extracts showed high reducing power ability, it was not dose-dependent (Figure 3).

The nitric oxide scavenging ability of the extracts showed constant increase with increase in concentration. This observation was irrespective of the extracts and standards. When compared with the standards, none of the extracts was observed to show substantial nitric oxide scavenging ability (Figure 4).

Antimicrobial potential of the extracts

The antibacterial potential of the extracts revealed the methanol extract showing inhibition against the five isolates tested. The hexane extract was not observed to inhibit growth of *Staphylococcus* and *Klebsiella pneumoniae*. Only *Staphylococcus* showed inhibition to the ethyl acetate extract (Table 2).

In presence of the ethyl acetate extract, growth of the *E. coli* was observed to experience an extended lag period of 6 h in broth medium that contained the *Helianthus annuus* extract only. In medium that contained both the extract and ascorbic acid, the *E. coli* showed no growth throughout the period of incubation. However, in the control broth medium (contained neither extract nor ascorbic acid), and broth medium that contained ascorbic acid, *E. coli* growth was observed from 1 h incubation period.

Table 2 Zone of inhibition and minimum inhibitory concentrations of the extracts against selected bacteria species.

Test bacteria	Ethyl acetate	Hexane	Methanol
<i>Bacillus subtilis</i>	25 mm (1000 mg.L ⁻¹)	10 mm (3000 mg.L ⁻¹)	20 mm (3000 mg.L ⁻¹)
<i>Escherichia coli</i>	10 mm (1000 mg.L ⁻¹)	20 mm (2000 mg.L ⁻¹)	10 mm (3000 mg.L ⁻¹)
<i>Pseudomonas aeruginosa</i>	15 mm (2000 mg.L ⁻¹)	21 mm (2000 mg.L ⁻¹)	12 mm (1000 mg.L ⁻¹)
<i>Staphylococcus aureus</i>	-	-	10 mm (2000 mg.L ⁻¹)
<i>Klebsiella pneumonia</i>	10 mm (3000 mg.L ⁻¹)	-	12 mm (2000 mg.L ⁻¹)

Note: Values in mm represent zones of inhibition while values in brackets indicate minimum inhibitory concentration of the extracts. ‘-’ represents resistance to the extract.

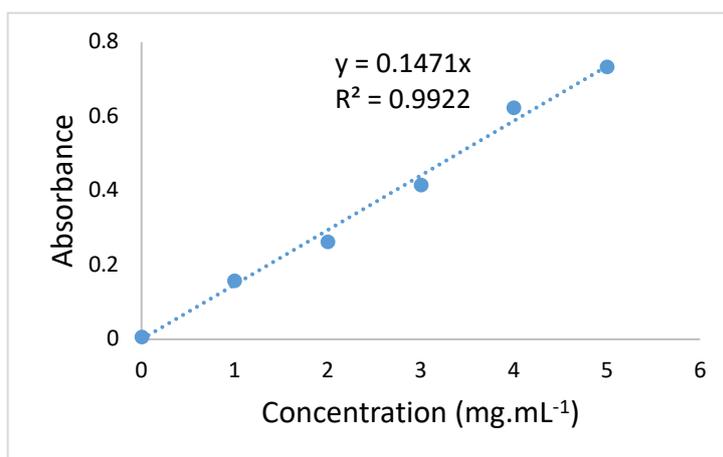


Figure 1 Calibration curve for total phenols.

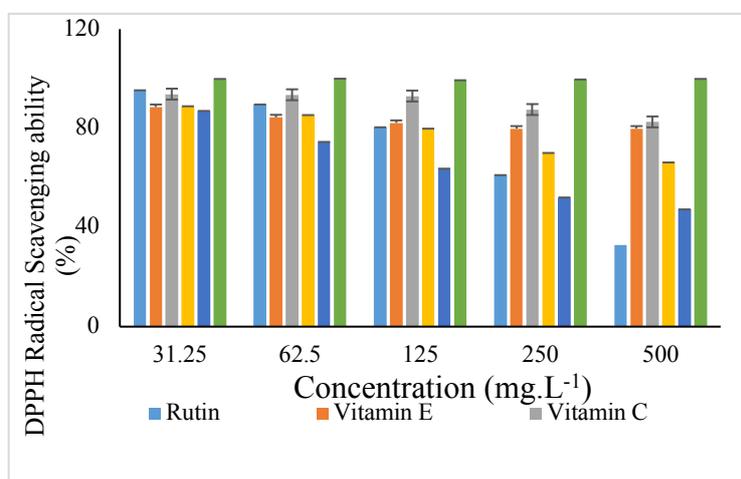


Figure 2 Free radical scavenging ability of extracts using DPPH.

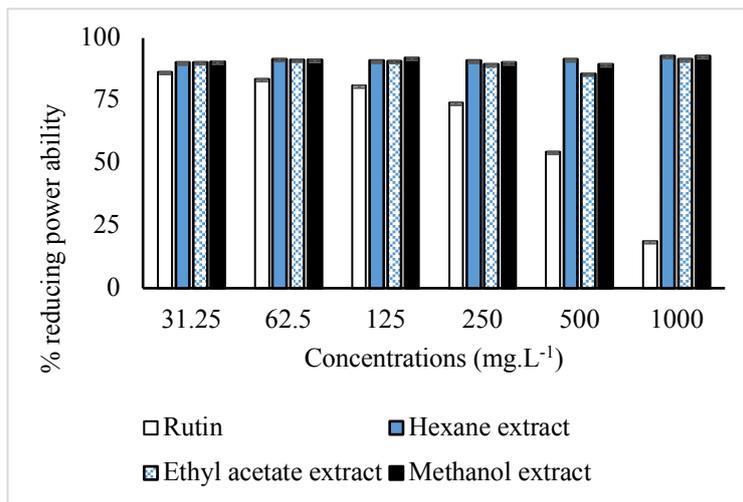


Figure 3 Reducing power assay of the extracts.

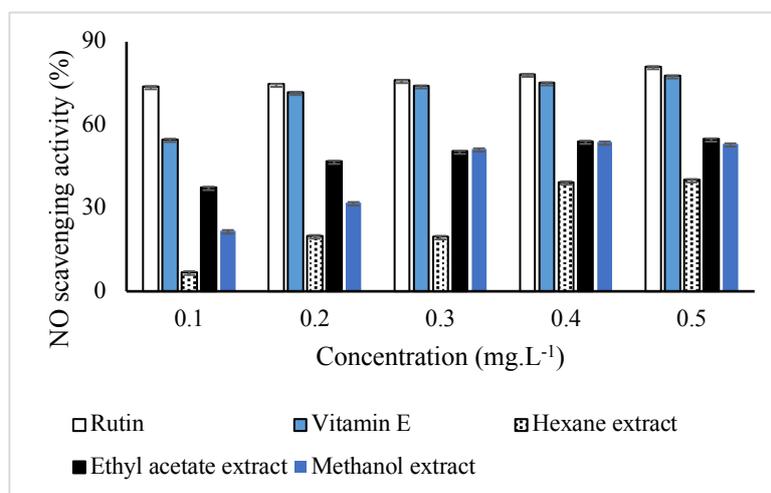


Figure 4 Nitric oxide scavenging assay of the extracts of the methanol extract.

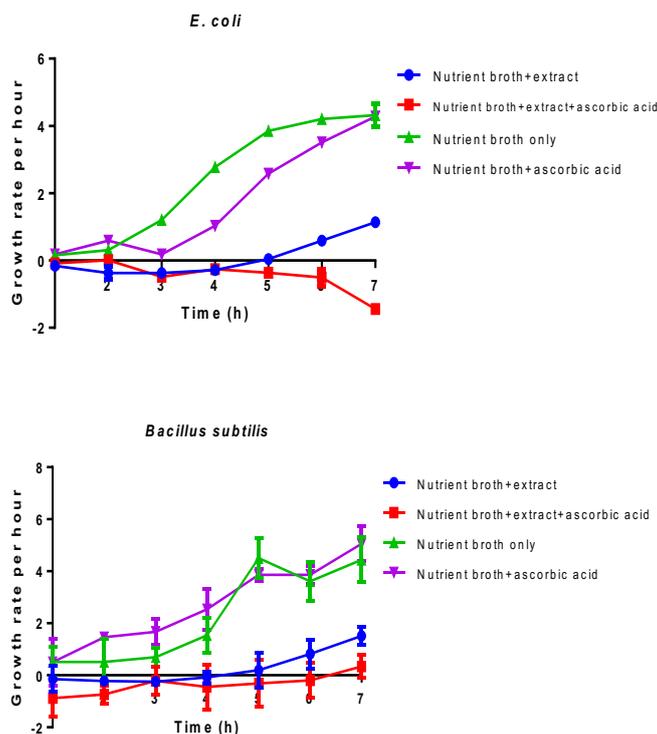


Figure 5 Growth profile of the bacteria species in presence of the ethyl acetate extract.

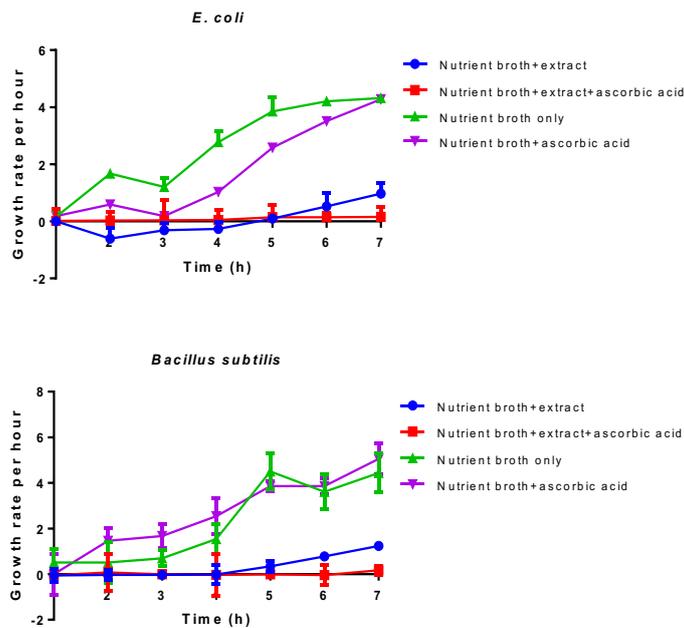


Figure 6 Growth profile of the bacteria species in presence of the hexane extract.

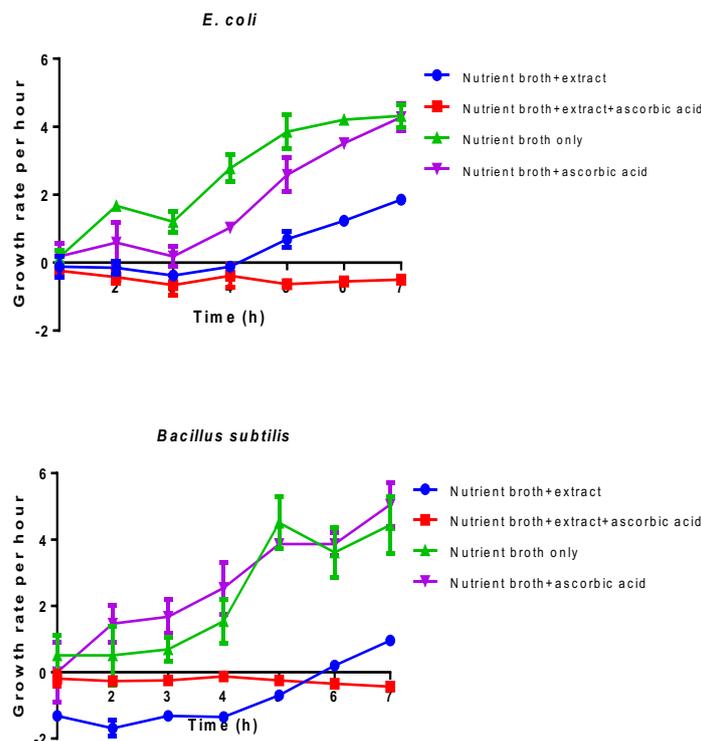


Figure 7 Growth profile of the bacteria species in presence Phytochemical components of the extracts.

Generally, growth rates of 1.14, 1.44, 4.32 and 4.27 were observed for the *E. coli* in media with nutrient broth + extract, nutrient broth + extract + ascorbic acid, nutrient broth only and nutrient broth + ascorbic acid, respectively (Figure 5). In the case of the *Bacillus subtilis*, extended lag periods of 5 and 7 h were observed in media with nutrient broth + extract and nutrient broth + extract + ascorbic acid. In media with nutrient broth only and nutrient broth + ascorbic acid, growth was observed from the first hour of incubation. At the end of 7 h incubation, growth rate of the *Bacillus subtilis* was observed to be 1.51 and 0.34 in media

with nutrient broth + extract and nutrient broth + extract + ascorbic acid, respectively (Fig. 5). Generally, growth rates of bacterial species were observed to be significantly higher in the absence of the ethyl acetate extract. No significant difference in growth rate was however observed in medium that contained only the extract or a combination of the extract and ascorbic acid ($p = 0.005$).

In presence of the hexane extract, growth rate of *E. coli* was observed to be minute at the end of incubation. This observation was irrespective of growth in media with nutrient broth + extract or nutrient broth + extract +

ascorbic acid. Extended lag of 4 h was observed when grown on nutrient both + extract but no lag period was observed in the other media compositions. Growth rates of 0.97, 0.15, 4.31 and 4.28 were observed for the *E. coli* in media with nutrient broth + extract, nutrient broth + extract + ascorbic acid, nutrient broth only and nutrient broth + ascorbic acid, respectively (Figure 6).

For the *Bacillus subtilis*, extended lag periods of lag period of 4 and 6 h, were observed in nutrient broth + extract and nutrient broth + extract + ascorbic acid. Growth was however observed in media with nutrient broth only and nutrient broth + ascorbic acid from the first hour of incubation. At the expiration of the 7 h incubation period, growth rates of 1.24 and 0.16 were observed in media with nutrient broth + extract and nutrient broth + extract + ascorbic acid, respectively (Figure 6). Significantly higher growth rates were observed for the test bacterial species in medium that did not contain the hexane extract ($p = 0.05$).

In presence of the methanol extract of the *Helianthus annuus*, growth rates of 0.15, 4.31 and 4.24 were observed for the *E. coli* in media with nutrient broth + extract, nutrient broth only and nutrient broth + ascorbic acid, respectively. Throughout the 7 h period of incubation, growth was not observed in media with, nutrient broth + extract + ascorbic acid (Figure 7). For the *Bacillus subtilis*, as was observed for the *E. coli*, growth was not observed in media with nutrient broth + extract + ascorbic acid was observed throughout the incubation period. Minute growth rate of 0.96 was observed at the end of incubation in media with nutrient broth + extract (Figure 7). As was observed for the ethyl acetate and hexane extracts, significantly higher growth rates of the test bacterial species were observed in medium that did not contain the methanol extract ($p = 0.05$).

This study revealed antimicrobial activity of the extracts against most of the test bacteria investigated, with the methanol extract showing inhibition against all the test bacteria in solid media. When the test bacteria were grown in liquid media containing the extracts, growth inhibition and extended lag periods were observed. The presence of vitamin C in the liquid media did not impact negatively on the inhibitory properties of the extracts on the test bacteria species. Minimum inhibitory concentrations (MIC) of 1000 – 3 000 mg.L⁻¹ were observed for the ethyl acetate and methanol extracts while 2000 – 3000 mg.L⁻¹ was observed for the hexane extract. In a related study, *Helianthus annuus* seed oil, ethanolic stem extract have been established to possess anti-microbial properties against different microorganisms such as *Staphylococcus aureus* and *Candida albicans*. The MIC and MBC of the ethanol stem extract against *Staphylococcus aureus* and *Candida albicans* were 70 and 90 mg.mL⁻¹ and *C. albicans* were 50 and 70 mg.mL⁻¹ (Al-Snafi, 2018). In a report by Dwivedi (2014), *Helianthus annuus* extract have also been reported to inhibit the growth of bacteria, such as *Salmonella typhi*, *Staphylococcus aureus*, *vibrio cholera* and *Bacillus subtilis*. With respect to antifungal action, the extract has been indicated to display high antifungal action on *Rhizopus stolonifer*, *Aspergillus fumigates* and *Candida albicans* while *Fusarium oxysporum* is indicated to be

resistant (Subashini and Rakshitha, 2012; Dwivedi 2014; Eze et al., 2015).

In this study, flavonoids, tannins and phenols were observed to be present in all the extracts. It is indicated that plants of rich medicinal potentials usually contain polyphenols as their primary and most abundant secondary metabolite. Phenols are reported to neutralize free radicals and prevent the breakdown of hydroperoxides to free radicals thereby contributing to plants' antioxidant potential (Adawia, 2017). As observed in this study, when compared with the standard rutin, all the extracts showed similar abilities to reduce oxidized intermediates of lipid peroxidation processes. Reducing power ability of a sample signifies its potential antioxidant activity. A substance with strong antioxidant capacity can act as electron donor, hence can reduce oxidized intermediates of lipid peroxidation processes (Benslama and Harrar, 2016).

The findings of this study revealed total phenolics content of 5.58 – 6.66 mgTAE.g⁻¹ using tannic acid as standard with higher value recorded in the methanol extract. A similar report on total phenol content and antioxidant potential of green and yellow beans of *Phaseolus vulgaris* varieties showed values that ranged between 2.27 and 4.55 mg.g⁻¹, when catechin was used as standard (Weidner et al., 2017). The high phenolic content observed in the methanol extract has been reported in similar studies. In a study on the total phenolic content and antioxidant and antibacterial activities of *Pereskia bleo*, highest phenolic content was recorded in the methanol extract that chloroform and hexane extracts (Johari and Khong, 2019).

With respect to nitric oxide scavenging, all the extracts displayed ability to scavenge nitric oxide radical in a dose dependent manner. However, the standard rutin used inhibited the nitric oxide radical more than the extracts even at low concentrations when compared with the extracts. A similar observation has been reported by earlier investigators (Boora et al., 2014). Nitric oxide radical is a free radical that is obtained from the interaction of nitric oxide NO and oxygen or reactive oxygen species Its toxicity is greatly increased on its reaction with superoxide anion radical with the resultant formation of the highly reactive peroxy nitrite anion radical ONOO⁻ (Bhaskar and Balakrishnan, 2009; Amaeze et al., 2011).

DPPH radical is a stable radical that accepts electron or donate hydrogen when reacting with antioxidant compounds and reduced to yellow-colored diphenyl picrylhydrazine radical (Alara et al., 2017). The results showed that the different extracts had the ability to neutralize the free radicals' unpaired electron (Pavithra and Vadivukkarasi, 2015). The DPPH scavenging activities of the extracts were effective in the order: Ethylacetate > Methanol > Hexane. An increase in the percentage of DPPH inhibition caused by antioxidant might be due to the scavenging ability of radicals by hydrogen donation. It can also be seen that the ethylacetate extract was more active than that of the standards used rutin, vitamin E and Vitamin C at the same concentrations. A similar observation was reported by (Pavithra and Vadivukkarasi, 2015), where they showed that ethylacetate extract of *Helianthus annuus* possesses a strong antioxidant activity.

Resistance to antibiotic has increasingly become a global concern. The emergence of multidrug-resistant pathogens has threatened the clinical efficacy of many existing antibiotics (Westh et al., 2004). Therefore, many studies are carried out to discover novel antimicrobial compounds and their mechanisms of action for treating new and re-emerging diseases (Ullah et al., 2017). In this current study, the methanol, ethylacetate and the hexane extract showed inhibitory effect against *Escherichia coli* and *Bacillus subtilis*. This was similar to the findings of Al-Snafi (2018) where *Helianthus annuus* displayed strong inhibitory effects against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis* and *Candida albicans*.

CONCLUSION

This study investigated the antibacterial and antioxidant activities of ethyl acetate, hexane and methanol leaf extracts of *Helianthus annuus*. The findings reveal that all extracts of *Helianthus annuus* used in this current study contain flavonoids, tannins and phenols, displaying their rich phytochemical constituent. These extracts also displayed inhibitory activity against most test bacteria isolates used, as well as possess strong reducing power abilities. They also exhibited good free radical scavenging abilities (using DPPH) with ethyl acetate extract being the most effective in this regard. The result from this current study therefore shows the strong medicinal properties of *Helianthus annuus*, it could be a good source of antimicrobial and antioxidant especially in a world where resistance to antibiotic has increasingly become a global concern.

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Contact address:

*Oghenerobor Akpor, Landmark University, College of Pure and Applied Sciences, Department of Microbiology, PMB 100, Omuran 25110, Kwara State, Nigeria, Tel. +2348099189171,

E-mail: akpor.oghenerobor@lmu.edu.ng

ORCID: <http://orcid.org/0000-0002-4256-1549>

Tomilola Olaolu, Landmark University, College of Pure and Applied Sciences, Department of Biochemistry, PMB 100, Omuran 25110, Kwara State, Nigeria, Tel. +2348099189171,

E-mail: olaolu.tomilola@lmu.edu.ng

ORCID: <http://orcid.org/0000-0002-3760-5172>

Damilare Rotimi, Landmark University, College of Pure and Applied Sciences, Department of Biochemistry, PMB 100, Omuran 25110, Kwara State, Nigeria, Tel. +2348099189171,

E-mail: rotimi.damilare@lmu.edu.ng

ORCID: <http://orcid.org/0000-0003-3721-8065>

Corresponding author: *