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*Research Paper*

# Assessment of Antimicrobial and Antioxidant Properties of Ethanolic Extracts of the Leaves of *Dysphania ambrosoides* (L.) *Tithonia diversifolia* (hemsly) A Gray *and Laggera alata* (D. Don)

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This research was designed to assess the antimicrobial and antioxidant properties of ethanolic extracts of the leaves of *Dysphania ambrosoides, Tithonia diversifolia and Laggera alata* on some pathogenic organisms. The phytochemical screening of the ethonolic extracts was conducted using standard methods. The antimicrobial activity of the ethanol extracts of the leaves of the plant species was tested against six pathogens namely; *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus and Candida albicans* was determined *in vitro* using the agar well diffusion method at concentrations ranging from 25 mg/ml to 200 mg/ml. Gentamycin and fluconazole were used as control. The minimum inhibitory concentration (MIC) of the extracts was also determined using standard methods. The extracts were screened for the presence of phytochemicals, and their inhibition of 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical was used to evaluate their free radical scavenging activity. Standard Rutin, Standard Gallic and Vitamin C were used as reference antioxidants. The phytochemical screening of the ethanolic extract of *Dysphania ambrosoides* revealed the presence of alkaloids, tannins, saponins, flavonoids, carbohydrates, steroids, cardiac glycosides, terpenes while *Tithonia diversifolia* and *Laggera alata* revealed all of the above except saponins and terpenes. The sensitivity test revealed that the zones of inhibition of *Dysphania ambrosoides* leaf extracts ranged between (6.00-16.00 mm) with the highest zone of inhibition being exhibited on *Bacillus subtilis* and the least on *Pseudomonas aeruginosa,* while the leaf extracts of *Tithonia diversifolia* ranged between (6.00-15 mm) with the highest zone shown on *Bacillus* subtilis and the least on *Pseudomonas aeruginosa.* Also, the zones of inhibition exhibited by antimicrobial activity of leaf extracts of *laggera alata* varied from (6.00-16.00 mm) with the highest zone being shown on *Salmonella typhi* and the lowest on *Pseudomonas aeruginosa*. There was no significant difference ($p>0.05$) between the extracts plant species with regard to their antimicrobial effects on the various test microorganisms. Also, the mean diameter zones of inhibition exhibited by the crude extracts of the plants decreased as the concentrations of the extracts deceased. Overall, the antibiotic drugs used as control exhibited better antimicrobial potential as compared to the plant extracts with zones of inhibition ranging from 13-29 mm for gentamycin and from 14-25 mm for fluconazole. The MIC of the ethanolic extracts of the plant species against the test microorganisms varies between 25-100 mg/ml. The antioxidant activity of the plant extracts were low compared to the control. However, the scavenging effect of *L. alata* was found to be greater than those of *T. diversifolia* and *D. ambrosoides*. Thus, the inhibition concentration at 50% ($IC_{50}$ ) ) was shown in increasing order 0.397±0.00 µg/ml <10.20±0.50 µg/ml <32.03±3.45 µg/ml <51.00±6.7 µg/ml <57.60±3.87 µg/ml <363.30±8.47 for *T. diversifolia, D. ambrosoides*, *L. alata*, Standard Gallic, Standard Rutin and vitamin C respectively. The findings of the present study suggest that the ethanolic extracts of the test plants possess significant antimicrobial and antioxidant activities as well as pharmaceutical potentials which make them potential candidates as natural chemoprophylactic agents. Studies are required to further elucidate antimicrobial and antioxidant potentials using *in vivo* biochemical and molecular biology techniques.

**Keywords:** Antimicrobial, Antioxidant, Ethanolic, Leaves Extracts, *Dysphania ambrosoides, Tithonia diversifolia, Laggera alata*

## INTRODUCTION

The use of plants in the management and treatment of diseases started with life. It has been observed that many plants do indeed have medicinal value and extracts from these plants have been used to make modern drugs. Various parts of plants such as stems, leaves, seeds, barks and roots are used to prepare the herbal medicine (Reshma, 2010). Also, plants contain phytochemical compounds with various bioactivities,

including, antibacterial, antifungal, antioxidant, anti-inflammatory and anticancer activities. Currently, about 25% of the bioactive components identified from plants are used as prescribed medicines (Gill *et al*., 2011). In effect, indigenous plants are reservoirs of various metabolites and provide unlimited source of important chemicals that have diverse biological properties. Phytochemical and secondary metabolites have been the subject for many research studies, because these compounds exhibit many pharmacological and biological activities.

In realization of the widespread use of herbal medicines across the globe, World Health Organization (WHO) in 1985 estimated that perhaps more than 80% of the world's population rely on plant based herbal medicines for their primary healthcare needs (Farnsworth and Soejarto, 1985). Herbal remedies have been used for thousands of years. According to United Nations report (Daes, 1993), United States government health agencies alone spent more than $8 million from 1986-1993 to collect thousands of medicinal plant species in more than twenty-two countries. This action must have been predicted on the need to source new drugs from plants. Masood, (1997) reported that the sale of medicinal plants have grown by nearly 25% in Indian over the past ten years, the highest growth rate in the world then. Germany is Europe's largest consumer of mecidicinal plants and spends £1.4 billion annually, France is the second (£116 million) and the United Kingdom third (£88 million).The demand for medicinal plants by pharmaceutical companies alone was estimated to be 25% of prescription drugs in United States of America-containing plant extracts or active principles prepared from higher plants (Farnsworth *et al*., 1986). Africans regard medicinal plants as sources of vital energy and to a great extent a participatory agent rather than a life object used in healing (Egunyomi, 2015). Hence this author further affirms that the use of herbs in Africa must be as old as the continent itself. The use of plants in Nigerian traditional practice as either extracts or infusion has wide spread practice in the treatment of common infections. In developing countries a large segment of the population still rely on folk medicine to treat serious diseases including infections, cancers, and different types of inflammations (Ramzi *et al*., 2009). It has become very obvious from various literatures, that many herbal remedies of old have since been adopted and adapted by conventional by western allopathic medicine, simply due to the fact that they are effective. Thus the inestimable value of medicinal plants to health care systems in the world has increasingly become appreciated (Egunyomi, 2015). Despite the overwhelming popularity and long term usage of herbal remedies through the world, there are still insufficient scientific studies to ascertain their safety and more effectiveness in the treatment of diseases especially in developing countries. It is regrettable to note that in Africa countries especially in Nigeria, the most populous black nation in the world medicinal plants are yet to be fully harnessed. It has been that in Nigeria less than 5% of the plant-derived drugs used in its health care.

Antimicrobial is a term used to describe substances which demonstrate the ability to kill or reduce the presence of microbes, such as bacteria and fungi. Besides the challenging side effects of conventional antibiotics to humans, there is an increase in drug resistant strains of human pathogenic bacteria and fungi all over the world. According to the United Kingdom (U.K) National Health Service (NHS) (2018), around 1-15 people have an allergic reaction to antibiotics especially penicillin and cephalosporins and in rare cases, an antibiotic can cause a severe and potentially life-threatening allergic reaction known as anaphylaxis. Hence the development of new therapies such as medicinal plants for decreasing the disease burden has become paramount in medical care system. Systematic screening of plants may result in the discovery of novel effective compounds which would tackle the problem of drug resistance (Parekh and Chanda, 2006).

The human body is exposed to free radicals, whose great reactivity and deleterious action on biological systems promote the aging and genesis of several diseases such as cardiovascular events and certain cancers (Pastre, 2005). The over production of reactive oxygen species like hydroxyl radical, superoxide anion radical, hydrogen peroxide radical can contribute to oxidative stress. Oxidative damage of proteins, DNA and lipid is associated with chronic degenerative diseases including diabetes, hypertension, coronary heart disease, cancer etc (Modi *et al*., 2010). Most of the reactive oxygen species are scavenged by endogenous defense systems. But these systems may not be completely efficient requiring them to depend on exogenous antioxidants from natural sources. Presently, there has been an amplified interest worldwide to identify antioxidant compounds which are pharmacologically effective or have low or no side effects for use in preventive medicine and the food industry. Generally antioxidants have been identified as major health beneficial compounds reported from varieties of medicinal plants and are sources for alternative medicine (Soro *et al*., 2012; Souleymane *et al*., 2018).The photographs of the plants under study are show in (Plates 1-3).

*Tithonia diversifolia* (Hemsley) A. Gray commonly known as Mexican sunflower or Mexican armica is a member of the family Asteraceae. It is an annual weed growing aggressively in abandoned lands, road- sides, river banks and cultivated farmlands (Linthoingambi and Mutum, 2013). Linthoingambi and Mutum, (2013) reported that the flower and leaves extracts of *Tithonia diversifolia* exhibited high degree of antimicrobial activity againgst some pathogenic fungal species. John-Dewole and Oni, (2013) evaluted the medicinal properties of the

**Plate 1.** *Tithonia diversifolia*

**Plate 2.** *Dysphania ambrosiodes*

**Plate 3.** *Laggera alata*

plant extracts *in vitro* by antimicrobial and antifungal asseys and reported that they exhibited growth inhibitory effects on *Staphylococcus aureus* and *Escherichia coli*. Montakarn *et al*. (2013) reported that the aqueous leaves extract of *Tithonia diversifolia* showed antioxidant property by inhibiting the chain reaction of lipid peroxidation, reducing the elevation of lipid profile and improves glucose metabolism. The decoctions of the various parts of *Tithonia diversifolia* are used for the treatment of malaria, diabetes mellitus, sore throat, liver, menstrual pains and treatment of wounds (Montakarn *et al*., 2013). An oral decoction of the leaves and stem are used for the treatment of hepatitis in Taiwan and gastrointestinal disorders in Kenya and Thailand. In Costa Rica, the dried leaves are applied externally on wounds, while in Cameroon; an infusion of the leaves is used for the treatment of measles (Elufioye, 2004).

*Dysphania ambrosioides*, formerly *Chenopodium ambrosioides*, known as wormseed, Jesuit's tea, Mexican-tea (Botanical Society of Britain and Ireland (BSBI) (2007). It is native to Central America, South America, and southern Mexico. As well as in its native areas, it is grown in warm temperate to subtropical areas of Europe and the United States and Africa. It is becoming an invasive weed. *D. ambrosioides* is an annual or short-lived perennial plant (herb), growing to 1.2 m (3.9 ft) tall, irregularly branched, with oblong-lanceolate leaves up to 12 cm (4.7 in) long. The flowers are small and green, produced in a branched panicle at the apex of the stem (Grieve, 1998). *D. ambrosioides* is used as a leaf vegetable, herb, and herbal tea for its pungent flavor. The fragrance of *D. ambrosioides* is strong but difficult to describe. A common analogy is to turpentine or creosote. It has also been compared to citrus, savory, and mint. Humans have died from overdoses of the essential oil (attributed to the ascaridole content). Symptoms include severe gastroenteritis with pain, vomiting, and diarrhoea (Steven and Sergei, 2003). The essential oils of *D. ambrosioides* contain terpene compounds, some of which have natural pesticide (Tampion, 1977).

*Laggera alata* (Asteraceae) is distributed mainly in the tropical Africa and Southeast Asia. This plant has been used as folk medicine in China for over 300 years, especially for the treatment of some ailments associated with hepatitis (Jiangsu New Medical College, 2009). Most studies concerning *L. alata* have focused on its folk use and phytochemical analysis (Bohlmann *et al*., 1985; Zheng *et al*. 2003; Wu *et al*., 2012). Zheng *et al*. (2003) examined the anti- inflammatory activities of *L. alata* and confirmed its potent inhibitory effects in models of acute and chronic inflammation. The principal chemical components isolated from *L. alata* are isochlorogenic acid (Wu *et al*., 2012). Worldwide use of natural products including medicinal plants has become more and more important in primary health care especially in developing countries. Many pharmacological investigations are carried out to identify new drugs. Structure for the development of therapeutic agents for the treatment of human diseases is ongoing (Newman *et al*., 2003). Therefore, the aim of this study is to assess the antioxidant and antimicrobial properties of leaf extracts of *Dysphania ambrosoides, Laggera alata and Tithonia diversifolia*.

## MATERIALS AND METHODS

### Collection of plant materials

The leaves of *Dysphania Ambrosoides, Tithonia diversifolia and Laggera alata* were collected from the Federal College of Forestry, Jos, compound and identified in the herberium of Plant Science and Technology,University of Jos with Voucher numbers; UJH17000273,UJH17000274 and UJH17000275 respectively as arranged above. The leaves were rinsed with distilled water and dried for about two to three weeks

under shade and then pulverized using mortar and pestle and then blended. The pulverized plant materials were kept in an airtight cellophane bag until use.

**Preparation of plant extracts**

Thirty grams (30 g) of the pulverized plants were macerated in 300 mls of 70% ethanol for 48 h. The mixtures were filtered and the filtrates were evaporated to dryness using rotary evaporator at 70°C. The extracts were collected in airtight bottles and stored in desiccators prior to use (Ardzard *et al*., 2009).

**Percentage of extracts yielded**

The percentage yield of the extract was then calculated using the formula described by Muhammad *et al*. (2016).

$$\%\text{Yield} = \frac{W_2 - W_1}{W} \times 100$$

Where:
$W_1$ = weight of empty beaker.
$W_2$ = weight of empty beaker + final dried extract.
W = weight of sample before extraction

**Sterilization and disinfection**

The test tubes that were used were sterilized by autoclaving at 121°C for 15 min. Wire loop and cork-borer was sterilized by flaming in Bunsen burner until they become red. After sterilization all the equipment were cooled before use. The work bench surfaces were disinfected using dettol, this was done by sprinkling the disinfectant on the surface and wiped with cotton wool. Hands were washed with soap and dettol and properly rinsed with clean water before and after work (Ardzard *et al*., 2009).

**Preparation of stock test concentration from the plant extracts**

Ethanolic extracts were weighed to about 2.0 g and dissolved in 10 mls of distilled water (Ardzard *et al*., 2009).

**Source of clinical isolates**

Already characterized clinical isolates were obtained from Pharmaceutical microbiology laboratory, University of Jos, Nigeria. The organisms that were used included *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtili, Salmonela typhi, Candida albicans and Staphylococcus aureus*.

**Standardization of test organisms**

All inoculums were standardized using the Mcfarland nephelometer method (Albert *et al*., 1991). The protocol for preparing this solution is as stated in the (Table 1). The reaction gives rise to turbid solutions at room temperature and were kept on the work bench for use. To prepare test organisms 0.5 ml of already prepared nutrient broth was pipette into a sterile test tube aseptically and the pure culture of the particular test organism were inoculated into it until the microbes' suspension match the turbidity of the standard solution. And this microbe's suspension corresponds to $1.5\times10^8$/microbe's suspension per millilitre.

**Preparation of media**

Nutrient Agar was prepared according to manufacturer's instruction (Oxoid CM0003, 28 g in 1liter of distilled water). 28 g of nutrient agar was weighed into a conical flask, 500 ml of distilled water was added and capped . The medium was shaken to dissolution and sterilized at 121°C for 15 min. The media was allowed to cool to 45°C and about 20 ml of the sterilized medium was poured into sterile Petri dishes and allowed to cool and solidify. The plates were labeled with the test organisms and the names of the plants (each plate with a test microbe). The microbes were spread evenly over the surface of the medium with the aid of a sterile wire loop (Ardzard *et al*., 2009).

**Phytochemical Screening**

The presence of some basic secondary metabolites in the pulverized plant materials were determined using standard methods (Sofowara, 1993; Evans, 2002, Prashant *et al*., 2011).

**Test for alkaloids**

To 3 ml of each extract mixture in a test tube, 1ml of 1% HCl was added and to 2 ml of extract mixture 2 drops of Mayer's, Wagner's and Dragendroff reagents were added separately. A creamy white (Mayer), a reddish brown (Wagner) and an orange brown (Dragendroff) precipitates in the ethanol extracts were taken as evidence of the presence of alkaloids (Evans, 2002).

**Test for tannins**

Two (2) drops of 5% $FeCl_3$were added to 1ml of extract in

separate test tubes and the appearance of a dirty-green precipitate was considered as indication for the presence of tannins.(Evans, 2000).

**Fronting test for saponins**

Two milliliters (2 ml) of the extract in a test tube was vigorously shaken for 2 min and the presence of frothing indicated the presence of saponins (Prashant *et al*., 2011)

**Shinoda test for flavonoids**

1 g of magnesium power and 1-5 drops of conc. HCl was added to 3 ml of the extract in separate test tubes. The appearance of red colour in the extracts was an indication of the presence of flavanoids (Sofowara, 1993).

**Detection of carbohydrates**

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

**Benedict's test**

Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

**Test for Anthraquinones**

Borntrager's test was used for the detection of anthroquinones. A 0.5 g of each extracts was taken into a dry test tube and 5ml of chloroform was added and shaken for five minutes. The extract was filtered and the filtrate shaken with an equal volume of 10% ammonia solution. A pink, violet or red colour in the ammoniacal layer (lower layer) indicates the presence of free anthraquinones.

**Determination of antimicrobial activity**

The antimicrobial effects of ethanolic leaf extracts of *Dysphania Ambrosoides, Tithonia diversifolia and Laggera alata* was determined individually using the agar well diffusion method according to Perez *et al*. (1990) and Olukoya *et al*. (1993). Nutrient agar plates were swabbed using sterile wire loop with the broth culture of the respective bacteria and fungi isolates. Four wells (6 mm in diameter) were made equidistance to each of the plates using a sterile cork borer and one well was made at the center of the medium. Nutrient agar was added to seal the bottom. Up to 100 µl (0.1ml) of each concentration of the extracts (200 mg/ml, 100 mg /ml, 50 mg/ml, 25 mg/ml) were respectively introduced into the well using sterile Pasteur pipette and 0.1ml the control (fluconazole and gentamicin, 1.7 mg/ml) was introduced into the well at the centre. They were allowed to diffuse at room temperature for 2 h and the plates were incubated at 37°C for 24 h. Diameters of the inhibition zones were measured. The antimicrobial activities of the extracts were expressed as the mean diameter zone of inhibition (in mm) measured using a transparent ruler.

**Determination of minimum inhibitory concentration (MIC)**

The MIC was carried out using broth dilution method by Cowan and Steel, (1985). MIC is the lowest concentration of the various extracts that inhibit the growth of the microbial isolates. Tube 1 contained only 5 mls of the stock concentration. Five milliliters of each extract was added into each of the test tubes (numbered 2) using Pasteur pipette. The content was mixed thoroughly so as to achieve even dilution and distribution of the extract within the broth. Five milliliters of the mixture was withdrawn from tube 2 and transferred into the tube numbered 3 and evenly mixed. This was repeated for all the tubes until they were exhausted. Finally, 5mls of the mixture was discarded from the last tube in the set. This process was done for all sets of the tubes. 0.1 ml inoculums of the test organism was taken and inoculated into each tube (numbered 1-4) using the micropipette. The tubes were thoroughly mixed and incubated at 37°C for 24 h, after which they were examined for visible turbidity. Tubes which showed turbidity were tubes which had microbial growth while those which were clear (no turbidity) do not have microbial growth. The minimum inhibitory concentration (MIC) was reported as the lowest concentration that prevented visible growth (Cheesbrough, 2000).

**Determination of antioxidant activity**

The ability of each extract to scavenge DPPH radicals was measured according to standard. 3 ml of each plant extracts at a concentration of 100 µg/ml was mixed well with 1 ml of (0.1 M of 2, 2-diphenyl-1-picryl-hydrazyl; DPPH) in methanol and distributed in test tubes. The mixture was shaken and left for 30 min in the dark at room temperature. Absorbance was measured at 517 nm against the blank using UV/VIS spectrophotometer (Model-ST-UV-7558). Standard Gallic, Standard Rutin and Vitamin C were used as reference antioxidants.

Controls contain only solvent and DPPH without any extract. The experiments were carried out in triplicate. The antioxidant activity of the leaf extract was expressed as IC50 and compared with standard. The IC 50 was defined as the concentration of extract that inhibit the formation of DPPH radical by 50% (Josephinol *et al*., 2017).

**Calculation**

% scavenging of the DPPH free radical was measured using the equation below:

$$\text{DPPH Scavenging activity } (\%) = \frac{A_{Control} - A_{Test}}{A_{Conrtol}} \times 100$$

$$\%ISAE = \frac{A_0 - A_1}{A_0} \times 100$$

Where $A_O$ is the absorbance of the control and $A_1$ is the absorbance of samples. ISAE = Inhibition standard antioxidant and extracts

**Statistical analysis**

One way Analysis of Variance (ANOVA) with a statistical significance level set at $p<0.05$ was carried out. Duncan Multiple Range Test (DMRT) was used to test the significance at 5% confidence level.

**RESULTS**

The percentage yield of the ethanolic extracts of leaves of the plant species are presented in (Figure 1).The extraction of the compounds was conducted using maceration technique and the parameter considered was based on extraction yield calculated in percentage. The highest percentage yield (17.0%) was obtained from ethanolic extract of *D. ambrosoides* followed by (13.2%) from *T. diversifolia*, while the least (8.5%) was obtained from *L. alata*. The Phytochemical Components of the ethanolic extracts of *Dysphania Ambrosoides, Tithonia diversifolia and Laggera alata* was investigated on saponin, alkaloid, tannins, anthroquinones, flavonoid, carbohydrate, terpenes, cardiac glycosides and steriods. Table 2 showed that ethanolic leaf extract of *Dysphania ambrosoides* possessed alkaloids, tannin, saponins, flavonoid, carbohydrates, steroids, Cardiac glycosides and terpenes. *Tithonia diversifolia* ethanolic leaf extracts possessed tannin, alkaloids, flavoniods, carbohydrates, steroids,cardiac glycosides and terpenes, while *Laggera alata possessed tannin, alkaloids, flavoniods, carbohydrates and steroids.* Table 3 shows the antimicrobial activity in terms of zones inhibition of the different concentrations of *Dysphania ambrosoides, Tithonia diversifolia and Laggera alata* against the test organisms. All the test organisms were susceptible to the plant species extracts at different degrees and their susceptibility was concentration dependent. *Staphylococcus aereus, Escherichia coli, Bacillus subtili, Pseudomonas aeruginosa and Salmonella typhii and Candida albicans* showed visible zones of inhibition at 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml of the ethanolic extracts. The best *in vitro* antimicrobial activity at 200 mg/ml was by *L. alata* (16.00 mm) against *Salmonella typhi* followed by *T. diversifolia* (15.00 mm) against *Bacillus subtilis* and *D. ambrosoides* (14.0 mm) against B. *subtilis* and *S. aureus* respectively. The minimum antimicrobial potential was exhibited by the ethanolic extracts of the three plants with zone of inhibition of 6mm at 25 mg/ml. *Candida albicans* was found to be more susceptible to the extracts of *D. ambrosoides* and *T. diversifolia* with uniform zone of inhibition of 13 mm than that of *L. alata* 10.0 mm at 200 mg/ml. At low concentration the standard antibiotic drugs compared favourably to the extract at high concentrations as shown in (Table 3).

Figure 2 summarizes the results of the minimum inhibitory concentration (MIC) of ethanolic extract of *Dysphania ambrosoides, Tithonia diversifolia* and *Laggera alata* on the test organisms. The ethanolic extracts of the three plant species exhibited the lowest uniform MIC of 25 mg/ml against *S. typhi* and *B. subtilis* for *D. ambrosoides,* against *S. typhi, B. subtitis* and *E. coli* for *Tithonia diversifolia* and against *S. typhi and B. subtilis for L. alata,* while the moderate and highest MIC of the extracts of the same plants that inhibited some of the microorganisms were 50 and 100 mg/ml respectively. The results of the antioxidant curves of the extracts of the three plant species and the standard antioxidants are presented in (Figures 3-8). The inhibition concentrations at 50% ($IC_{50}$) of *D. ambrosoides, T. diversifolia* and *L. alata* is 51.00±6.7 µg/ml, 363.30±8.47, 32.03±3.45 µg/ml respectively, while those of Standard Gallic, Standard Rutin and Vitamin C are 10.20±0.50 µg/ml 57.60±3.87 µg/ml 0.397±0.00 µg/ml respectively.

**DISCUSSION**

In this study the percentage yield of the different plant species, antimicrobial and antioxidant potentials of *Dysphania ambrosoides, Tithonia diversifolia and Laggera alata* were explored. While the antimicrobial potential of the ethanolic extracts of the plant species was investigated through diamete zone of inhibition and minimum inhibitory concentration, the antioxidant potential of the selected plants was evaluated through DPPH assay. Also, in this study 5 bacterial isolates including two gram-positive (*S. aureus and B. substilis*) and three gram-negative *(E.coli, P. aeroginosa and S.*

**Table 1.** Protocol for test tubes for Mcfarland nephelometer in10 ml.

| Test tube number | 1%barium chloride (ml) | 1% sulphuric acid (ml) | Corresponding microbes suspension ($\times 10^8$/ml) |
|---|---|---|---|
| 0.5 | 0.05 | 9.95 | 1.5 |

Source: Albert *et al*. (1991).

**Table 2.** Phytochemical component of ethanolic extracts of *Dysphania Ambrosoides,Tithonia diversifolia and Laggera alata*.

| Phytochemical components | *Dysphania Ambrosoides* | *Tithonia diversifolia* | *Laggera alata* |
|---|---|---|---|
| Alkaloids | +++ | ++ | ++ |
| Tannins | ++ | +++ | +++ |
| Saponins | ++ | - | - |
| Flavonoids | +++ | +++ | +++ |
| Carbohydrates | ++ | ++ | +++ |
| Steroids | ++ | + | +++ |
| Anthraquinones | - | - | - |
| Cardiac glycosides | + | + | - |
| Terpenes | + | + | - |

- = absent, + = slightly present, + = moderately present, +++ = very present

**Table 3.** Zones of inhibition produced by different concentrations of ethanolic extracts of *Dysphania ambrosoides*, *Tithonia diversifolia* and *Laggera alata and the* control against the test organisms.

| Test organism | Extract Concentration (mg/ml) | Mean Zone of inhibition (mm) exhibited by plant extract | | | |
|---|---|---|---|---|---|
| | | *D. ambrosoides* | *T.diversifolia* | *L. alata* | Gentamycin/ Fluconazole |
| *Salmonella typhi* | 200 | $10.0^{a}$ | $14.0^{d}$ | $16.0^{f}$ | $22.0^{c}$ |
| | 100 | $9.5^{a}$ | $11.0^{a}$ | $10.0^{a}$ | $18.0^{i}$ |
| | 50 | $9.0^{a}$ | $10.0^{a}$ | $9.0^{a}$ | $15.0^{d}$ |
| | 25 | $7.0^{ab}$ | $8.0^{ab}$ | $7.0^{ab}$ | $13.0^{ed}$ |
| *Bacillus subtilis* | 200 | $14.0^{d}$ | $15.0^{d}$ | $13.0^{ed}$ | $25.0^{e}$ |
| | 100 | $12.0^{ed}$ | $13.0^{ed}$ | $11.0^{ef}$ | $23.0^{c}$ |
| | 50 | $10.0^{a}$ | $7.0^{ab}$ | $9.0^{a}$ | $19.0^{i}$ |
| | 25 | $9.0^{ab}$ | $6.0^{cd}$ | $8.0^{ab}$ | $16.0^{f}$ |
| *Staphylococus aureus* | 200 | $14.0^{d}$ | $12.0^{ed}$ | $12.0^{ed}$ | $26.0^{e}$ |
| | 100 | $12.0^{ed}$ | $9.0^{a}$ | $9.0^{a}$ | $23.0^{c}$ |
| | 50 | $10.0^{a}$ | $8.0^{ab}$ | $8.0^{ab}$ | $20.0^{h}$ |
| | 25 | $9.0^{a}$ | $7.0^{ab}$ | $7.0^{ad}$ | 17.0i |
| *Escherichia coli* | 200 | $11.0^{ef}$ | $13.0^{ed}$ | $13.0^{ed}$ | $21.0^{h}$ |
| | 100 | $9.0^{a}$ | $10.6^{a}$ | $10.0^{a}$ | $17.0^{i}$ |
| | 50 | $8.6^{ab}$ | $8.0^{ab}$ | $8.0^{ab}$ | $15.0^{d}$ |
| | 25 | $7.0^{ab}$ | $7.0^{ab}$ | $7.0^{ab}$ | $13.0^{ed}$ |
| *Pseudomonas aeruginosa* | 200 | $10.0^{a}$ | $10.0^{a}$ | $10.0^{a}$ | $29.0^{g}$ |
| | 100 | $9.0^{a}$ | $9.0^{a}$ | $8.0^{ab}$ | $26.0^{e}$ |
| | 50 | $8.0^{a}$ | $8.0^{ab}$ | $7.0^{ab}$ | $22.0^{c}$ |
| | 25 | $6.0^{cd}$ | $6.0^{cd}$ | $6.0^{cd}$ | $20.0^{h}$ |
| *Candida albicans* | 200 | $13.0^{ed}$ | $13.0^{ed}$ | $10.0^{a}$ | $25.0^{e}$ |
| | 100 | $10.0^{a}$ | $10.0^{a}$ | $8.0^{ab}$ | $21.0^{h}$ |
| | 50 | $9.0^{a}$ | $9.0^{a}$ | $7.5^{ab}$ | $18.0^{i}$ |
| | 25 | $7.0^{ab}$ | $7.0^{ab}$ | $7.0^{ab}$ | $14.0^{d}$ |

The data with same letter in the same column are not significant. Control = (Gentamycin) against bacterial isolates, Control = (Fluconazole) against *Candida albicans.*

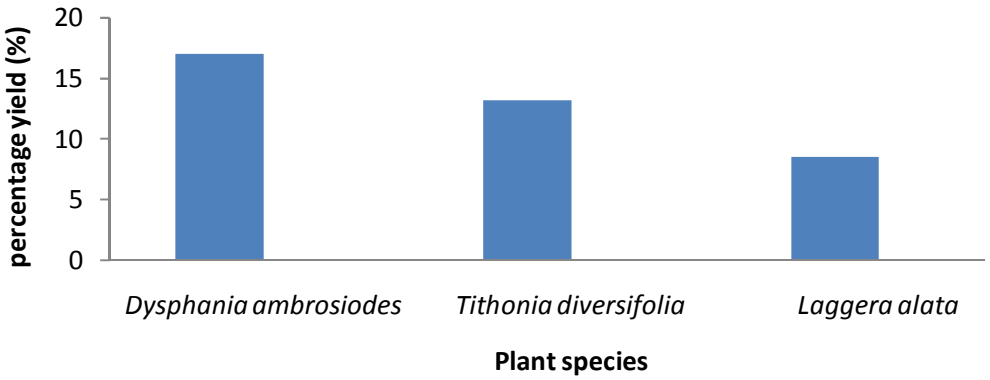


**Figure 1.** The percentage yield (%) of the ethanolic extracts of the leaves of *Dysphania Ambrosoides, Tithonia diversifolia and Laggera alata.*

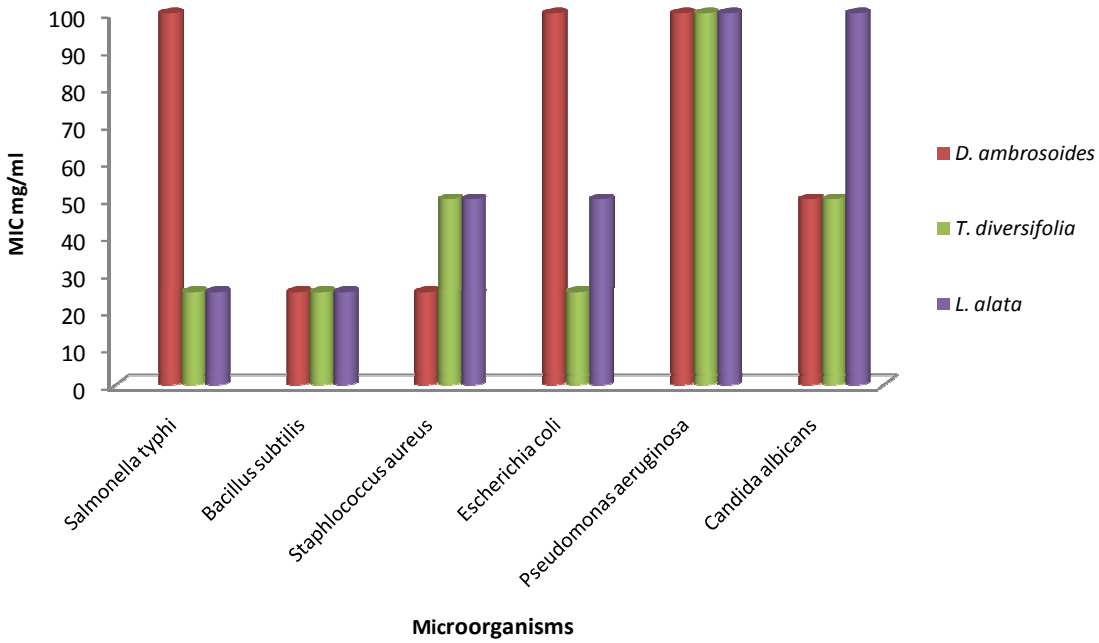


**Figure 2.** Minimum inhibitory concentration (MIC) (mg/ml) of ethanolic extracts of *Dysphania ambrosoides, Tithonia diversifolia* and *Laggera alata* on the test organisms.

*typhi*) were used for the estimation of antimicrobial activity of the test plant species. Furthermore, one fungal isolate, *C. albicans* was selected for the antifungal potential of the plant extracts. The maceration technique was used for the extraction of the compound from the plant materials in this research because it provides remarkable percentage extraction and it is more cost effective than other techniques (Ajaib *et al*., 2014). The difference in percentage yield observed among the three plants' extracts could be attributed to the fact that the percentage extraction increases progressively along with the solvent's polarity, that is the % extraction yield is directly proportional to the solvents' polarity index. It is also possible that extraction yield with polar solvents such as ethanol employed in this study could be more as compared to non polar solvent. Similar result was reported by Krishnananda *et al*. (2017). These workers observed that methanol, a polar solvent, gave a better extraction yield as compared to non polar solvents such as ethyl acetate and hexane. Thus crude ethanol maceration extraction method could be selected for further exploitation of antimicrobial activity/screening. This present findings showed that the ethanolic extracts of the plants possess phytochemical constituents such as

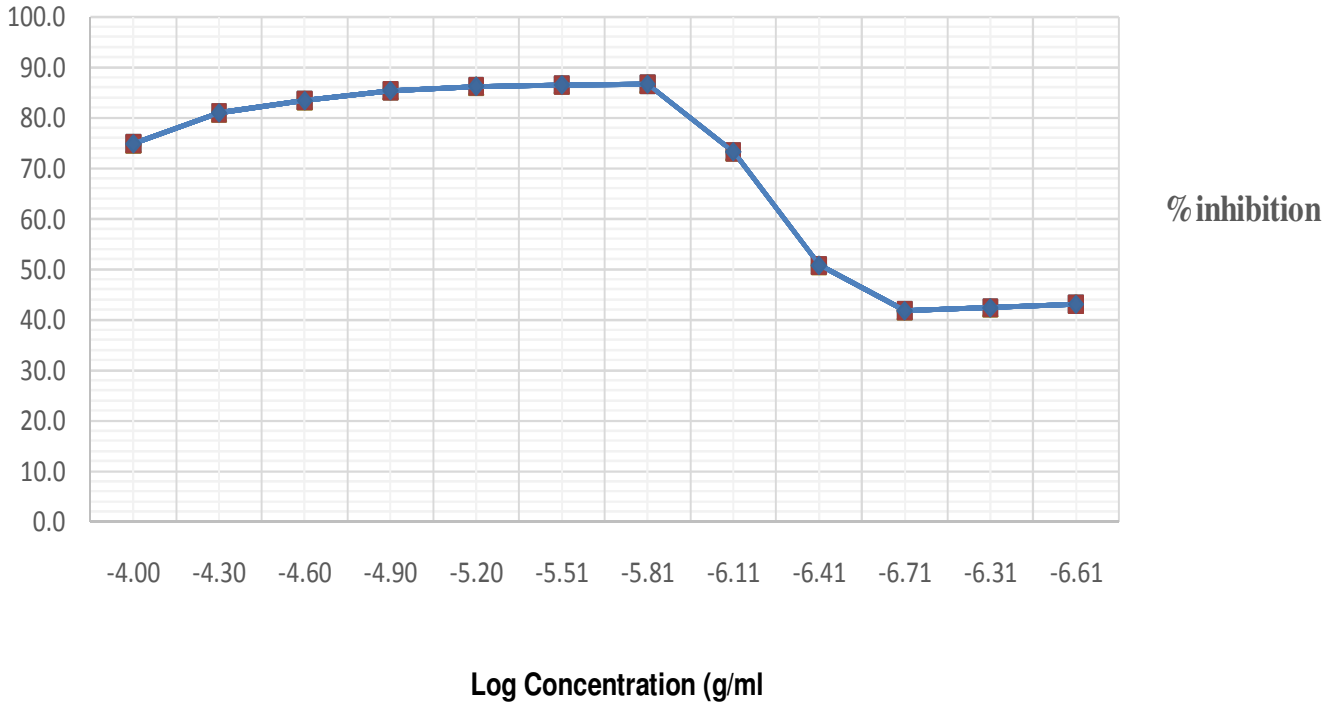


**Figure 3.**Antioxidant curve of ethanolic extract of *Dyshania ambrosoides.*

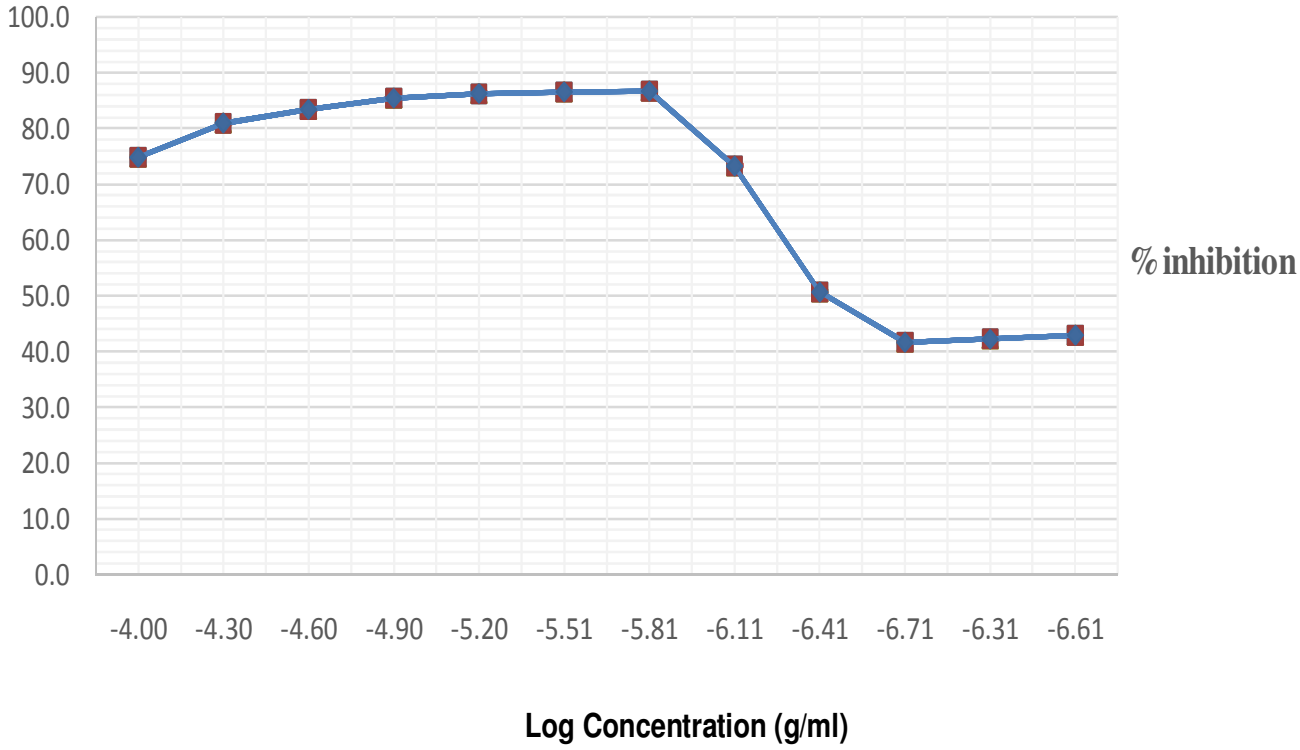


**Figure 4.** Antioxidant activity of ethanolic extract of *Tithonia diversifolia.*

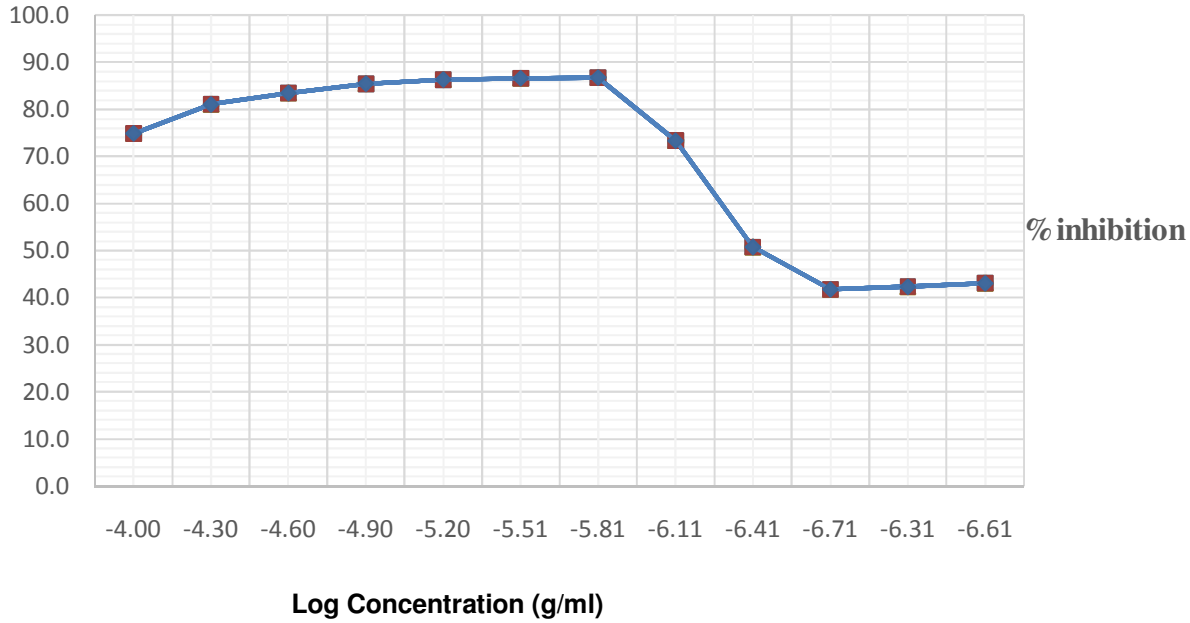


**Figure 5.** Antioxidant activity of ethanolic extract of *Laggera alata.*

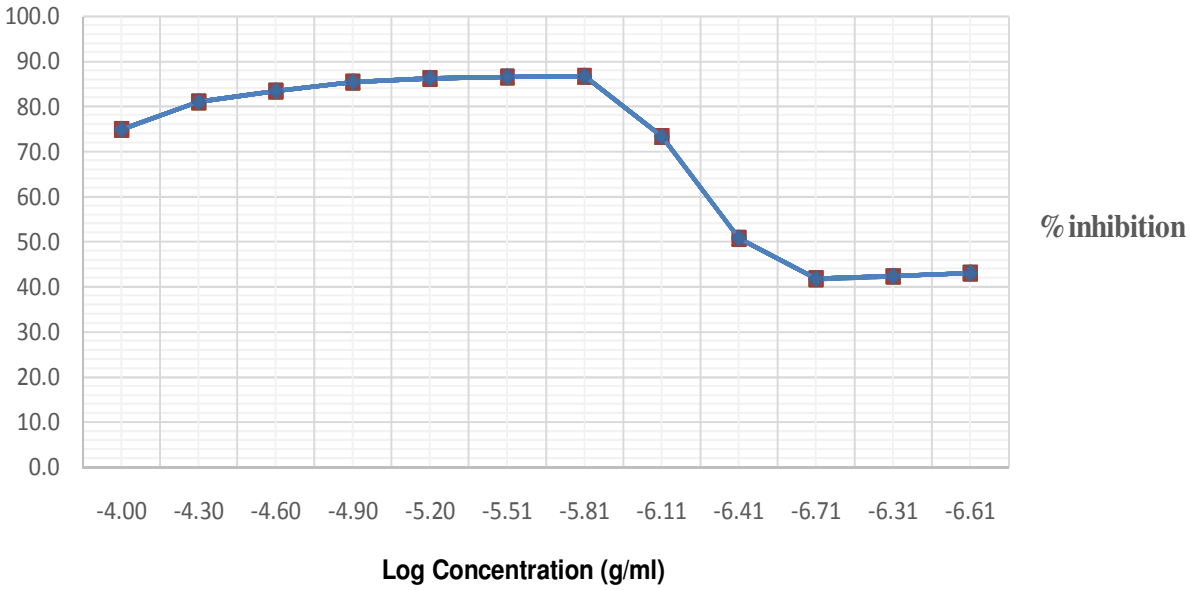


**Figure 6.** Antioxidant activity of Standard Gallic.

of tannins, flavonoids, saponins, alkaloids, carbohydrates, steroids, cardiac glycosides and terpenes. The medicinal values of the leaf extracts of the tested plant species may be related to their constituent phytochemicals. According to Agbafor and Nwachukwu, (2011) phytochemicals are bioactive compounds found in plants that work with nutrient and dietary fibre to protect against diseases. Similarly, Varadarajan *et al*. (2008) stated that secondary metabolites and other chemical constituents medicinal plants accounted for their medicinal value. Furthermore, phytochemical compounds have pharmacological effects and have been the basis of chemical synthesis of drugs used in modern medicine (Okogun, 1996). The result of the study agrees with similar research done by John-Dewole and Oni, (2013); Kela *et al*. (1999); Menut *et al*. (2002) and Okugun(1996).

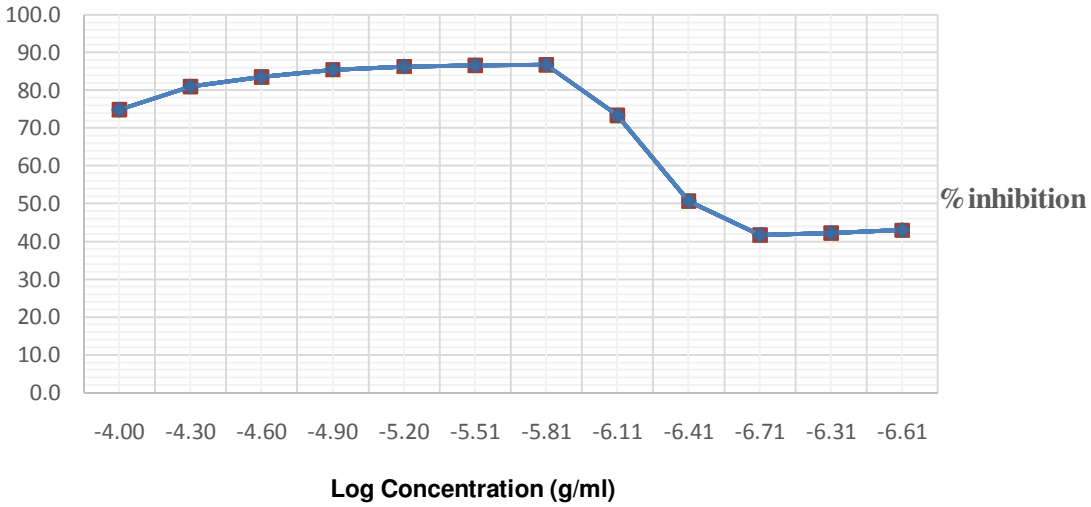


**Figure 7.** Antioxidant activity of Standard Rutin.

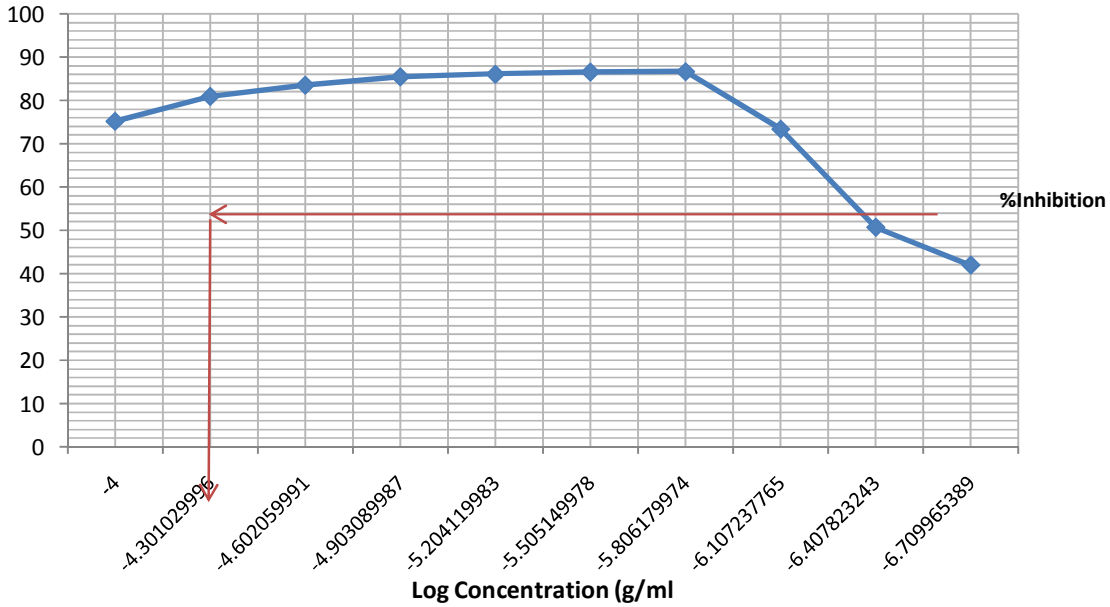


**Figure 8.** Antioxidant activity of Vitamin C.

Thus the presence of these phytochemicals in the plant extracts could be responsible for the the observed pharmacological effects and their medicinal use in traditional medicine. The results of the present study are similar to the work of John-Dewole and Oni, (2013) who reported that the extracts of *T. diversiflolia* showed growth inhibitory effects on *Staphylococcus* and *Escherichia coli,* while Muzychikina and Tolstikov,(1998) reported that anthraquinones possess bactericidal effects. The findings of the present study have shown that extracts of the plants exhibited good antimicrobial activities although not as good as the antibiotic drug which exhibited higher inhibitory effects against the tested organisms than the the extracts of the plant species. According to Ramesh and Monohar, (2010) zone of inhibition that exhibited more than 15 mm was considered as highly active for extracts. Following this statement the best *in vitro* antimicrobial activity was *L.alata* with zone of inhibition of (16 mm) against *S. typhi* suggesting that the plant can be utilized for the treatment of typhoid fever. The findings of the study revealed that all the tested organisms used were susceptible to the ethanolic extracts at various concentrations. Thus suggesting that medicinal potential of the plants is con-

centration dependant. The susceptibility of the ethanolic leaf extracts of the plant species is in agreement with the work of many workers (Liasu and Ayandele, 2008), which showed that the ethanolic extracts had varying degrees of activity against a wide range of pathogenic microbes. And this result also justifies the traditional claim that the plants have medicinal potency. The antimicrobial plants species against the tested microorganisms as observed in the present study is supported by the report of Ahmad and Mohammed, (1998) who stated that the use of higher plants and preparations made from them to threat infections is longstanding practice in large part of the population, especially where there is dependence on traditional medicine for a variety of ailments. It is interesting to note that at low concentration the standard antibiotic drugs compared favourably to the extract at high concentrations thus revealing the potency of the ethanolic extracts of the plant species as feature herbal candidate to treat infections caused by the tested microorganisms. The minimum inhibitory concentrations of the different extracts in relation to their susceptibility to the tested microorganisms is quite satisfactory as most of the extracts had comparatively similar concentration of standard antimicrobial, which confirms the presence of bioactive components of medicinal plants (Ramesh and Manohar, 2010).

The results of the antioxidant assay also revealed that all the plants investigated have satisfactory antioxidant potentials. Although, the scavenging effects of *L. alata* is greater than *T. diversifolia* and greater than *D. ambrosoides*, they were low compared to the control. The inhibition concentration at 50% ($IC_{50}$) was shown in increasing order 0.397±0.00 µg/ml <10.20±0.50 µg/ml <32.03±3.45 µg/ml <51.00±6.7 µg/ml <57.60±3.87 µg/ml <363.30±8.47 for *T. diversifolia, D. ambrosoides, L. alata,* Standard Gallic, Standard Rutin and vitamin C respectively. Free radical damage and oxidative stress are the major reasons for liver tissue damage. The antioxidant enzymes are first-line defense against such demage and thus provide protection against deteriorating outcome (Gaurav *et al*., 2008). The antioxidant activity of the extracts may be attributed to the presence of identified phytochemicals. Flavonoids, tannins, phenolic compounds, saponins, glycosides and steroids are major group of compounds that acts as primary antioxidants or free radical scavengers (Potterat *et al*., 1997). Similarly terpenoids, as vitamins, acts regulators of metabolism and play a protective role as antioxidants (Soetan, 2008). Thus, the antioxidant property of the extract may be strong contributing factor to the applications of plants in the management and treatment of various diseases.

## Conclusion

The presence of identified phytochemicals makes the leaves of *Dysphania ambrosoides, Tithonia diversifolia and Laggera alata* pharmacologically active. Their antimicrobial and antioxidant activities may be responsible for their usefulness in the management and treatment of various diseases.

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