Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 4(2): 121-126
Received: 24-05-2015
Accepted: 27-06-2015

M. Nirmali Wickramaratne
Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka.

L.P. Gunatilake
Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka.

N.G. D. Anuradha
Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka.

A.N. Godavillathanna
Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka.

M.G.N. Perera
Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka.

I. Nicholas
Department of Natural Products, Medical Research Institute, Colombo 8, Sri Lanka.

Correspondence:
M. Nirmali Wickramaratne
Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka.

Antioxidant Activity and Antibacterial Activity of *Walidda antidysenterica*

M. Nirmali Wickramaratne, L.P. Gunatilake, N.G. D. Anuradha, A.N. Godavillathanna, M.G.N. Perera, I. Nicholas

Abstract
Traditional medical systems use plants in curing and preventing diseases as cancer, anti- aging, infections etc. *Walidda antidysenterica* is an endemic plant to Sri Lanka with many recoded traditional uses but yet to be investigated scientifically in detail. We investigated the antioxidant and antibacterial properties of the methanol extracts of *Walidda antidysenterica* leaves, seeds, trunk, bark and root. Antibacterial activity of the methanol extracts of *Walidda antidysenterica* determined using disk diffusion method against Methicillin sensitive Staphylococcus aureus (MSSA) (ATCC 25923***)***, Methicillin resistant Staphylococcus aureus (MRSA) (Bench***)*** showed the highest inhibition for leaves with zones of 22.9±0.2 and 23.6±0.4 mm against MSSA and MRSA respectively. The leaves showed the best antioxidant properties with a 65.77±1.99µg/ml, IC_{50} value for DPPH scavenging activity, total phenolic content of 32.56±1.08 and the best total reducing power of the extracts.

Keywords: Antioxidant activity, DPPH activity, total phenols, total reducing power, MRSA, MSSA, *Walidda antidysenterica*.

1. Introduction
Currently accepted modern allopathic medicine is known to have its roots in traditional medicine and therapies. Nearly 75% of the plant-derived prescription drugs used worldwide have been discovered following leads from traditional medical practices as Ayurveda and Deshiya Chikitsa [1, 2]. The metabolites which operate as effective warning signals and defense systems in plants have been clinically proved that they possess meaningful pharmacological properties which could be useful against human diseases as cancer, anti- aging, skin diseases, cardiovascular disease, immune deficiency disease etc [3].

Therefore researchers and pharmaceutical industries are involved in harnessing medicinally important natural products from plants that posses antioxidants, antitumor, anti- mutagenic, antimicrobial activities [4, 5]. Infections caused by microorganism are an arena where drugs are being developed throughout. Lately the treatment of such infections has become a challenge since some pathogenic bacteria such as *Staphylococcus aureus* have proved to develop resistance to a wide range of antimicrobial drugs [6]. *Staphylococcus aureus*, a gram-positive bacterium is one of the leading causes of infections in the hospital setting leading to bloodstream and surgical wound infections and also skin, bones and joint abscesses [7]. Methicillin Resistant *Staphylococcus aureus*' (MRSA) is identified as a strain that show resistance to different classes of commonly used antibiotics as macrolides, tetracyclines, aminoglycosides including powerful antibiotic as vancomycin [8, 9] making treatment of such infections very difficult. The infections caused by MRSA have become highly endemic and have emerged as a serious problem in many geographic areas [10]. Hence there is an urgent need of more efficacious drugs in treating such diseases. The search of new antibiotics or novel approaches is acknowledged by the WHO to overcome the growing problems associated with such infectious pathogens [11, 12].

Antioxidants are compounds that have the capability of delaying, inhibiting, or preventing the oxidative reactions by scavenging free radicals and diminishing oxidative stress. Free radicals and oxygen species such as hydroxyl radicals, superoxides and other singlet oxygen are generated in the human body under physiological conditions. These radicals are scavenged or its oxidative damages are inhibited by the natural defense system equipped in all cells with enzymes as superoxide dismutase glutathione peroxidase and catalase [13]. However excessive generation of such reactive species are associated with disease conditions as cardiovascular

diseases, aging, neurodegenerative diseases Alzheimer's disease, mutations and cancers [14]. Both natural and synthetic antioxidants are being used as drugs in controlling and preventing above mentioned diseases. Synthetic antioxidants such as butylatedhydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butylhydroquinone have been widely used industrially as supplementary drugs and also in enhancing the nutritional value and the shelf life of food products [15]. The use of these synthetic antioxidants has been questioned due to their potential health risks and toxicity. [16, 17]
In the recent past the trend towards the use of natural antioxidants from plant sources have been increased due to the safety and cost effectiveness of the same [18, 19]. Many research groups therefore have taken the responsibility of screening and quantification of the antioxidant activities of the medicinal plant [20] and some of them are already being used in treating many diseases. [4, 5] Use of antioxidant in the treatment of cancer has also shown many clinical benefits as inhibiting, or delaying carcinogenesis [21, 22].
Natural antioxidant compounds fall under the category of ascorbic acid, tocopherols, simple phenolics, phenolic acids, anthocyanins, flavonoids and tannins [23]. Even though chemists have investigated many plants for their antioxidant activities and elucidated the structures of thousands of structures yet there are many more plants to be investigated and many compoundts to be identified. Phenolic compounds are considered to be the most abundant class of antioxidants hence the regular dietary intake is estimated to be 10 times higher than that of vitamin C and 100 times higher than vitamin E [24].
Walidda antidysenterica (Wrightia antidysenterica) is an endemic plant to Sri Lanka, of the family *Apocynaceae* and is common grown in lowlands of the country. However, little or no scientific studies have been done yet on the therapeutic potential of *Walidda antidysenterica* despite of many traditional medicinal uses are recorded on the plant (Table 1). A number of medicinal preparations containing roots of this plant are used in the traditional medical system and Ayurveda medical system in Sri Lanka in the treatment of blood disorders, chronic fever, jaundice and hemorrhoids [25].

Table 1: Medicinal value of *Walidda antidysenterica* [25]

Part used	Prescribed for
Roots	Hemorrhage
Flower	Gonorrhea, ulcers in genital organs, ailments after delivery
Roots	Epilepsy, chronic fever, cough, Hemorrhoids, Jaundice
Bark and leaves	Tonsillitis, Bronchitis
Bark	Snake bites
Latex	Tooth and gum ailments

Flowers are reported to be used in the treatment of ailments such as gonorrhea, ulcers in genital organs, and post delivery ailments. Bark and leaves are used in the treatment of tonsillitis, bronchitis, and snakebites [26, 27]. However various other species of *Wrightia* and related genera (*W. racemosa, W. tomentosa, W. tinctorica*, *W. jawanica*, *W. religeosa* and *Holorhena antidysenterica*) have been investigated for their biological activities [28, 31]. It is reported that these species carry many medicinally important secondary metabolites including alkaloids (Wrightine (Conessine) and Wrightiamine), steroids (Lupeol and Stigmasterol) and flavones (Wrightiadione) etc. [26]. Conessine is reported to be isolated from the bark of *Holarrhena antidysenterica* and related species and has shown helminthic, appetizing and antidiarrhoeal properties [30]. Wrightiamine is reported to exhibit cytotoxic activity against Vincristine- (VCR)-resistant murine leukemia P388 cells [32].
Present study was performed to investigate the antioxidant and antibacterial potential of *Walidda antidysenterica* and this would be the first report on such properties of the plant. We evaluated the antioxidant properties of the plant's seeds, leaves, root, bark and trunk by investigating, the DPPH radical scavenging activity, total phenolic content and total reducing power and the antibacterial activity was investigated against MRSA and MSSA using disk diffusion assay.

2. Materials and Method
All chemicals, reagents and solvents used in this study were of analytical grade purchased from Sigma or Sigma- Aldrich. Ferric chloride ($FeCl_3$); Folin-Ciocalteu's reagent; 2,20-diphenyl-1-picrylhydrazyl (DPPH); trichloroacetic acid (TCA); sodium carbonate (Na_2CO_3); sodium hydroxide (NaOH); disodium hydrogen phosphate (Na_2HPO_4); Potassium ferricyanide [$K_3Fe(CN)_6$]; methanol. UV-Vis spectrophotometer (Varian DU 60).

2.1 Plant Material and Extraction
Walidda antidysenterica plants were collected from Colombo and Gampaha districts in Sri Lanka. After identification of the plant a voucher specimen was deposited at the Botany Department, Bandaranayke Memorial Ayurvedic Research Institute, Nawinna, Maharagama, Sri Lanka. The plant was separated into parts as leaves, seeds, bark, trunk and roots and was air-dried separately
Dried plant material (200 g) of each part was coarsely powdered and extracted using methanol at 25 °C for 48 h. The extract was filtered and then evaporated under reduce pressure using a rotary evaporator.

2.2 Antibacterial Activity
The methanol crude residue which were subjected to an antibacterial assay against Methicillin sensitive *Staphylococcus aureus* (ATCC 25923), Methicillin resistant *Staphylococcus aureus* (Bench) and (ATCC 25922) using the disk diffusion assay according to the modified method of Khan *et al*. [33]. The bacterial suspensions were adjusted with sterilized saline to obtain turbidities compared to that of McFarland standard (0.5M). The test samples were loaded to the disks of 6 mm diameter (2 mg/disk) and were placed on Mueller Hinton (MH) agar plates inoculated previously with the test bacteria. The plates were then incubated at 37 °C for 18 hours and the diameters of the zones of inhibition were measured. Commercial antibiotic discs- penicillin (10 µg) and erythromycin (15 µg) were used as the positive controls for MSSA and MRSA respectively. Hexane, chloroform and ethyl acetate were taken as the negative controls. The test was done in triplicate.
The two plant materials, which showed the highest inhibitions against the test bacteria, were subjected to a sequential solvent partitioning with increasing polarity using hexane, chloroform and ethyl acetate. ***The disk diffusion assay*** was ***performed on each fraction as mentioned above*** [33].

2.3 DPPH Radical Scavenging Activity
Free radical scavenging activity of the extracts was measured using 2, 20- diphenyl-1-picrylhydrazyl (DPPH) assay. The stock solution of DPPH at a concentration of 1 mg/ml was prepared and thereafter the DPPH stock solution was diluted with methanol until the absorbance was about 0.98±0.02 at

517 nm using the spectrophotometer. Extracts were prepared at different concentrations ranging from 0.5 mg/ml - 10 mg /ml. A volume of 3 ml of the DPPH solution (diluted) was mixed with 200 µl of the extract solutions and was incubated in the dark for 30 min at room temperature. Thereafter the absorbance of the mixture was measured at 517 nm. The control was prepared by using 200 µl of methanol instead of the extract sample and following the same procedure as above [34]. The scavenging activity was estimated based on the percentage of DPPH radical scavenged using the following equation:

$$\% \, Scavenging = \frac{Control\ Absorbance - Sample\ Absorbance}{Control\ Absorbance} \times 100$$

2.4 Total Phenolic Content

The total phenolic content (TPC) was determined spectrophotometrically using Folin-Ciocalteu's reagent. A volume of 1 ml of the extract sample (1 mg/ml) was mixed with 1 ml of Folin-Ciocalteu's phenol reagent. After 5 min, 10 ml of a 7% Na_2CO_3 solution was added to the reaction and thereafter the mixture was diluted with the addition of 13 ml of deionized distilled water. The mixture was kept in the dark for 90 min at 25 °C, and the absorbance was measured at 750 nm. The calibration graph was prepared using Gallic acid as the standard. The phenolic content in the extract was expressed as milligrams of Gallic acid equivalents in one gram of extract [34].

2.5 Total Reducing Power

The total reducing power (TRP) of the extracts was investigated by the Fe^{3+} reduction to Fe^{2+} in the presence of the extract. Formation of Fe^{2+} can be monitored, by measuring the Prussian blue color appearance in the reaction mixture at 700 nm. A volume of 500 µl of each fraction with of varying concentrations (0.375 - 6 mg/ml) was mixed with 1.25 ml of phosphate buffer (pH 6.6) and 1.25 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$] and was incubated for 30 minutes at 50 °C. Thereafter 1.25 ml of trichloroacetic acid (10%) was added to the mixture and centrifuged at 3000 rpm for 10 minutes. A volume of 1.25 ml of the upper layer of the mixture was pipetted out carefully and mixed with 1.25 ml of distilled water and 250 µl of 0.1% ferric chloride ($FeCl_3$) and the absorbance was measured at 700 nm. The reducing power is indicated by the increasing absorbance at 700 nm. Therefore higher the reducing power, higher the changers in the absorbance at 700 nm is observed. All tests were performed in triplicates and the graph was plotted using the average of the three determinations [35].

3. Results

3.1 Antibacterial activity of the methanol extracts of *Walidda antidysenterica*

Walidda antidysenterica leaves, roots and the bark showed inhibitions against MRSA and MSSA, where no inhibitions were shown against *E.Coli*. *Walidda antidysenterica* stem, flowers and seeds did not show inhibitions against the three test bacteria (Table 2). The highest inhibition zones against MRSA and MSSA were exhibited by the leaves with mean inhibition zones of 23.6 ± 0.4 mm and 22.9 ± 0.2 mm followed by the roots with mean inhibition zones of 17.1 ± 0.7 mm and 16.2 ± 1.9 mm respectively. The lowest inhibition zones of 10.8 ± 2.2 mm and 11.7 ± 0.4 mm were exhibited by the bark against MRSA and MSSA respectively.

The controls: penicillin, erythromycin and ampicillin have yielded inhibition zones of 38.9 ± 0.2 mm, 34.1 ± 0.8 mm and 19.4 ± 0.5 mm against MSSA, MRSA and *E.Coli* respectively. No inhibition zones were exhibited by the negative control.

Table 2: Zones of inhibition of the test bacteria by the Crude methanol extracts of Leaves, Bark, Stem, roots, flowers and seeds of *Walidda antidysenterica*

	Zones of inhibition of the bacteria (mm)*		
	Methicillin-sensitive *S. aureus*	**Methicillin-resistant *S. aureus***	***Escherichia coli***
Positive Control	38.9 ± 0.2	34.1± 0.8	19. 4 ± 0.5
Leaves	22.9 ± 0.2	23.6 ± 0.4	-
Bark	11.7 ± 0.4	10.8 ± 2.2	-
Stem	-	-	-
Roots	16.2 ± 1.9	17.1± 0.7	-
Flowers	-	-	-
Seeds	-	-	-
Negative Control	-	-	-

*The values include the diameter of the disk (**6**mm) and expressed as Mean ± SD of 3 replicates**.**

-Absence of inhibition

3.2 MSSA and MRSA activity of the solvent extracts of the leaves and the roots

The methanol extracts of the leaves and the roots, which exhibited the highest inhibition, were further extracted using hexane, chloroform and ethyl acetate sequentially and tested for their MSSA and MRSA activity (Table 3). Hexane, chloroform and ethyl acetate extracts of leaves and the chloroform and ethyl acetate extracts of roots showed inhibitions against MRSA and MSSA. Hexane extract of roots did not show inhibitions against the test bacteria (Table 3). The highest inhibition zones against MRSA and MSSA were exhibited by the chloroform extract of leaves with mean inhibition zones of 26.9 ± 0.7 mm and 25.2 ± 0.5 mm respectively. The ethyl acetate extract of the leaves exhibited inhibition zones of 24.9 ± 0.4 mm and 25.6 ± 0.8 mm against MSSA and MRSA respectively.

The lowest inhibition zones of 17.7 ± 1.4 mm against MRSA and 9.4 ± 0.9 mm against MSSA were exhibited by the hexane extract of leaves and the ethyl acetate extract of roots respectively. The controls: penicillin and erythromycin have yielded inhibition zones of 38.9 ± 0.2 mm and 34.1 ± 0.8 mm against MSSA and MRSA respectively. No inhibition zones were exhibited by the negative controls.

3.3 DPPH Scavenging Activity

The free radical scavenging activity of the extracts as estimated by DPPH assay, showed that the leaves posses the best activity of all the extracts with an IC_{50} of 65.77 ± 1.99

µg/ml. The IC_{50} values of leaves, seeds, trunk, bark and roots were 65.77 ± 1.99, 88.22 ± 0.11, 102.85 ± 0.89, 264.8 ± 1.03 and 309.89 ± 2.90 µg/ml respectively (Figure 1, Table 4).

Table 3: Zones of Inhibition of the test bacteria by the hexane, chloroform, and ethyl acetate solvent extracts of the leaves and roots crude methanol extract of *Walidda antidysenterica.*

	Zones of inhibition of the bacteria (mm)*	
	Methicillin-sensitive *S. aureus*	**Methicillin-resistant *S. aureus***
Positive Control	38.9 ± 0.2	34.1 ± 0.8
Leaves- Hexane	17.2 ± 2.7	17.7 ± 1.4
Leaves- Chloroform	25.2 ± 0.5	26.9 ± 0.7
Leaves- Ethyl acetate	24.9 ± 0.4	25.6 ± 0.8
Roots- Hexane	-	-
Roots- Chloroform	24.3 ± 0.9	25.4 ± 0.8
Roots- Ethyl acetate	9.4 ± 0.9	-
Negative Control	-	-

*The values include the diameter of the disk (***6*** mm) and expressed as Mean ± SD of 3 replicates**.**
-Absence of inhibition

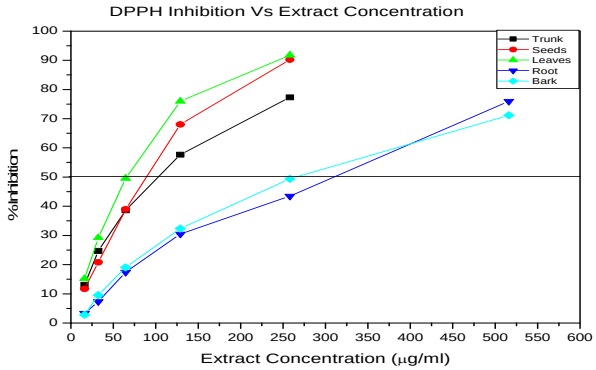

Fig 1: The plot of % inhibition of DPPH radicals against concentration of the extract of seed, leaves, root, bark, and trunk of Walidda antidysenterica.

3.4 Total Phenolic Content

The TPC of the methanolic extracts of the *Walidda antidysenterica* seeds, bark, toot, leaves and trunk measured using Folin-Ciocalteu's method and expressed as milligrams of Gallic acid equivalents/gm was 18.38 ± 0.23, 22.53 ± 0.31, 23.37 ± 0.29, 32.56 ± 1.08 and 47.52 ± 0.18 mg/g extract respectively. The highest TPC was observed in the trunk of the plant and the lowest was observed in the seeds of the plant (Figure 2, Table 4).

Table 4: Total Phenolic content (mg/g extract) as Gallic Acid equivalents and IC50 of DPPH radical scavenging Activity of the leaves, seeds, trunk, bark and roots of *Walidda antidysenterica*

Extract	Total Phenolic Content mg/g extract	DPPH Scavenging activity IC_{50} (µg/ml)
Seeds	18.38 ± 0.23	88.15 ± 0.11
Bark	22.53 ± 0.31	265.53 ± 1.03
Roots	23.37 ± 0.29	309.89 ± 2.9
Leaves	32.56 ± 1.08	65.77 ± 1.99
Trunk	47.52 ± 0.18	102.85 ± 0.89

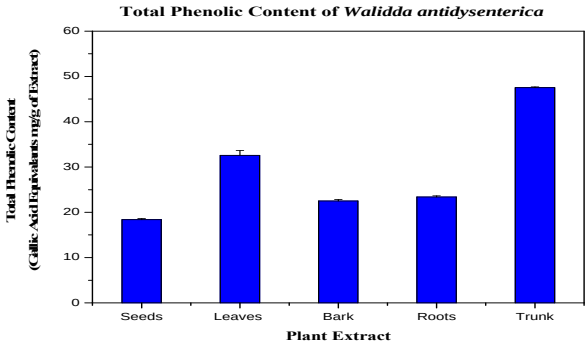

Fig 2: Plot of total phenolic content as Gallic acid equivalents in milligrams per gram extracts of Walidda antidysenterica seeds, leaves, bark, roots and trunk as determined using Folin-Ciocalteu's reagent.

3.5 Total Reducing Power

The reducing power was determined based on Fe $^{3+}$ being reduced to Fe $^{2+}$ in the presence of the extract and measuring the color change at 700 nm spectrophotometrically. The Absorbance increase at 700 nm indicates extent of the reducing power and higher the absorbance better reducing capacity the extract posses. The reducing power of seeds > leaves > trunk > bark > root (Figure 3).

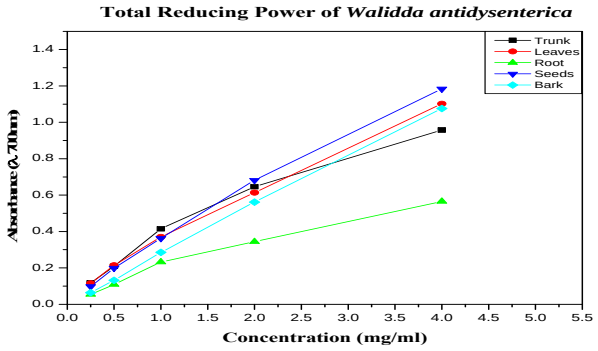

Fig 3: The plot of absorbance at 700 nm against the concentration of extract of Walidda antidysenterica seeds, leaves, bark, roots and trunk when treated with 1% potassium ferricyanide [K_3Fe (CN) $_6$] and 0.1% ferric chloride ($FeCl_3$) in phosphate buffer at pH 6.6 in determining the total reducing power

4. Discussion

Natural products and their derivatives still remain as the best source for new leads for the development of new pharmaceutical agents. Use of herbal medicines is still popular in most countries as they are readily available at an affordable cost. There are many resources in the plant kingdom that posses many medicinal values and yet to be discovered.

The misuse of antibiotics has resulted in the development of new pathogenic strains of bacteria, which are resistant to the available antibiotics. The spread of infections due to Methicillin-resistant Staphylococcus aureus (MRSA) has become a major problem especially in the hospital environments and have pose a great difficulty in the management of such infections as selecting an appropriate and effective antimicrobial agents. Glycopeptide derivatives, Vancomycin and teicoplanin are considered to be last resort for the treatment of MRSA infections.

Pharmaceutical companies are focused on the development of antibiotics capable of treating such antibiotic-resistant bacterial strains. In recent years, researches have reported on several active compounds isolated from medicinal plants that

could be used as leads for the development of new antibiotics [36]. In our study we investigated the antibacterial activity (anti-MRSA and anti- MSSA activity) of crude extracts and the solvent partitioned fractions of *Walidda antidysenterica* by disc diffusion method and the antioxidant properties of the crude extract of each plant part.

This study clearly exhibits that *Walidda antidysenterica* posses antibacterial activity specifically MRSA and MSSA activity. The methanol extract of the leaves showed the best activity with inhibition zone**s** against MRSA and MSSA to be 23.6 ± 0.4 mm and 22.9 ± 0.2 mm respectively. The chloroform solvent extracted fraction of the methanol leave extract showed even more improved activity with inhibition zone of inhibition zones of 26.9 ± 0.7 mm and 25.2 ± 0.5 mm for MRSA and MSSA respectively.

Free radicals are known to play a major role in a wide variety of diseases such as cancer, anti- aging, skin diseases, cardiovascular disease, immune deficiency disease etc. Antioxidants are used to fight against free radicals in curing or controlling such diseases [14]. They exert their action either by scavenging the reactive oxygen species or retarding oxidative reactions [37].

The electron donation ability or the radical inhibiting capacity of plant extracts can be measured using 2, 20-diphenyl-1-picrylhydrazyl radical (DPPH) which has a purple-color and under goes decoloration upon being scavenged by any other substance. The change in absorbance measured at 517 nm is directly proportional to the degree of color change, which is also proportional to the concentration and efficiency of the antioxidants. In most cases the higher inhibition of radicals are associated with the presences of phenolic compounds, ascorbic acid, flavonoids etc [36].

Our experiment shows that the leaves posses the best DPPH scavenging property with the IC_{50} value of 65.77 ± 1.99, trunks to have 309.89 ± 2.90 µg/ml which was the poorest of the extracts. The results reveal that the scavenging activity of the leaves were > seeds > bark > root > trunk.

Phenolic compounds are considered as secondary metabolites of plants and are rich in such substances. They are widely used in the food industry as they posses the ability of retarding oxidative degradation of food thereby improving the nutritional value and the shelf life of the food products [38]. Phenolic compounds can act as reducing agents, hydrogen donors and singlet oxygen quenchers. Therefore these compounds have the capability of exhibiting antioxidant activity. Phenolic compounds also have the ability of chelating metal ions that could trigger oxidation reactions [39]. The results obtained through this experiment indicated that the TPC of the *Walidda antidysenterica* seeds was the lowest 20.37 ± 2.96 mg/g, and trunk was the highest with 56.39 ± 1.43 mg/g extract. However there was no direct correlation observed between the IC_{50} of DPPH scavenging activity and the total phenolic content of the plant extracts indicating that there are other phytochemicals other than phenolic compounds that could be influencing the activity. It is also important to note that compounds, as sugars and ascorbic acid are not responsive to the Folin-Ciocalteu's method but does influence in the DPPH scavenging activity [40].

Previous reports suggested that the reducing properties are an indication of the capacity to exert antioxidant action by donating a hydrogen atom to break the free radical chain [23]. The reducing power assay monitors the reduction of Fe^{3+} ferricyanide complex to its Fe^{2+} form. Measuring the absorbance at 700 nm this change can be monitored and a higher absorbance indicates an increase in the reducing ability in the presence of a reductant. Our Results show that the reducing capability of the leaves were > seeds > bark > root > trunk.

The overall results show that all plant parts analyzed show good DPPH scavenging activity, the total phenolic content and the total reducing power supporting the traditional uses of this plant in treating many aliments.

5. Conclusion

This finding provides valuable scientific and quantitative information on the MRSA and MSSA activity and the antioxidant properties of *Walidda antidysenterica* for the first time to the best of our knowledge. These results strongly supports its use in the traditional medical system as an antibacterial agent and antioxidant agent. However further investigations on the isolation and identification of bioactive compounds would help to develop more potent drugs by the pharmaceutical industry.

6. References

1. Wakdikar S. Global health care challenge: Indian experiences and new prescriptions, Electr. J. Biotechnol 2004; 7:217-223.
2. Weragoda PB. The traditional system on medicine in Sri Lanka. J Ethnopharmacol 1980; 2(1):71-3.
3. Cragg GM, Newman DJ, Weiss RB. Coral reefs, forests and thermal vents: the worldwide exploration of nature for novel antitumor agents, Semin. Oncol 1997; 24:156-163.
4. Middeleton E, Kandaswamy C, Theoharides TS. The effects of plant flavonoids on mammalian cells: Implications for inflammation heart disease and cancer. Pharmacol Rev 2000; 52:673-751.
5. Ishikawa Y, Morimoto K, Hamasaki T. Falvoglaucin a metabolite of *Eurotiumchevalieri* its antioxidation and synergism with tocopherol. J Am Oil Chem Soc 1984; 61:1864-1868.
6. Batabyal B, Biswas S, Kundu GKR. Methicillin-resistant *Staphylococcus aureus*-A breif review, I. Res. J. Biological Sci 2012; 1(7):65-71.
7. Jones ME, Karlowsky JA, Draghi DC, Thornsberry C, Sahm DF *et al.* Epidemiology and antibiotic susceptibility of bacteria causing skin and soft tissue infections in the USA and Europe: a guide to appropriate antimicrobial therapy. Int J Antimicrob Agents 2003; 22:406-419.
8. Ness T. Multiresistant bacteria in ophthalmology. Ophthalmologe 2010; 107:318-322.
9. Centre for Disease Control and Prevention (CDC) Staphylococcus aureus resistant to vancomycin. JAMA, 2008, 288-825.
10. Ayliffe GA. The progressive intercontinental spread of methicillin-resistant Staphylococcus aureus. Clin Infect Dis 1997; 1:74-79.
11. Ahmed MO, Elmeshri SE, Abuzweda AR, Blauo M, Abouzeed YM *et al.* Seroprevalence of brucellosis in animals and human populations in the western mountains region in Libya December 2006- January, 2008. Euro Surveill, 2010, 15.
12. Ahmed MO, Alghazali MH, Amri SG, Abuzweda AR. Detection of Inducible Clindamycin Resistance (MLSBi) among Methicillin-resistant Staphylococcus aureus (MRSA) from Libya. Libyan J Med 5, 2010.
13. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition 2002; 8(10):872-9.

14. Koppula SB, Ammani K. Antioxidant findings of Araku environment medicinal plants using different assays. Drug invention today 2011; 3(8):203-205.
15. Williams GM, Iatropoulos MJ, Whysner J. Safety assessment of butylatedhydroxy anisole and butylatedhydroxy toluene as antioxidant food additives. Food Chem Toxicol 1999; 37:1027-1038.
16. Bauer AK, Dixon D, De Graff LM, Cho HY, Walker CR, Malkinson AM *et al.* Toll-like receptor 4 in butylatedhydroxytoluene-induced mouse pulmonary inflammation and tumorigenesis. J Natl Cancer Inst 2005; 97:1778-1781.
17. UNEP, OECD. 2, 6-di-tert-butyl-p-cresol (BHT) Screening Information Data Set: Initial Assessment Report (Paris: OECD), 2002.
18. Liu F, Ng TB. Antioxidative and free radical scavenging activities of selected medicinal herbs. Life Sci 2000; 66:725-735.
19. Park EJ, Pezzuto JM. Botanicals in cancer chemoprevention. Cancer Metastasis Rev 2002; 31(3-4):231-255.
20. Ramarathnam N, Ochi H, Takeuchi M. Antioxidant defense system in vegetable extracts. AOCS Press: Champaign, IL, 1997, 76-87.
21. Shureiqi I, Chen DJJ, Yang P, Newman RA, Brenner DE, Lotan R *et al*. A novel molecular target of nonsteroidal anti-inflammatory drug-induced apoptosis in colorectal cancer cells. J. Natl Cancer Inst. 1997; 92:1136.
22. Tsao AS, Kim ES, Hong WK. Chemoprevention of Cancer. CA Cancer J Clin 2004; 54:150-180.
23. Duh PD, Tu YY, Yen GC. Antioxidant activity of the aqueous extract of harngjyur (Chrysanthemum morifolium Ramat). Lebensmittel-Wissenschaft and Technol. 1999; 32:269-277.
24. Scalbert A, Johnson IT, Saltmarsh M. Polyphenols. Antioxidants and beyond 1,2,3 Am J Clin Nutr. 2005; 81:1(215-217).
25. Ashton M, Gunathilaka S, Soyza N, Dassanayaka MD. A field guide to the common trees and shrubs of Sri Lanka; Wildlife Heritage Trust, 1997.
26. Gibbon S. Anti-Staphylococcal plant natural products. Nat Prod Rep 2004; 21:263-277.
27. Andrews JM. Determination of Minimum Inhibitory Concentration. J Antimicrob Chemoth 2001; 48:S1, 5-16
28. Ayurveda Pharmacopoeia. Published by the Department of Ayurveda, Colombo, Sri Lanka (I), 2006, 1.
29. Ahameethunisa AR, Hopper W. Antibacterial activity of *Artemisia nilagirica* leaf extracts against clinical and phytopathogenic bacteria. BMC Complem. Altern M 2010;10:6.http://www.biomedcentral.com/1472-6882/10/6
30. Ballal M, Srujan D, Bhat KK, Shirwaikar A, Shivananda PG. Antibacterial activity of *Holarrhena antidysenterica* [Kurchi] against the enteric pathogens. Indian J. Pharmacol 2001; 33:392-393.
31. Balunas M, Kinghorn AD. Drug discovery from medicinal plants. Life Sci 2005; 78:431-441.
32. Jones WP, Kinghorn AD. Extraction of plant secondary metabolites. In: Natural Products Isolation, 2nd edn. Methods of Biotechnology Series, 20, 2005; Eds. Sarker, S. D., Latif, Z., Gray, A.I., Humana Press: Totowa, NJ. 2005, 323-351.
33. Khan RA, Salman MT, Shukla I. Anti microbial activity of Nigella sativa Linn. Seed oil against multi-drug resistant bacteria from clinical isolates, Natural product radiance 2008; 7(1):10-14.
34. Stanković MS. Total phenolic content, flavonoid concentration and antioxidant activity of marrubium peregrinum extracts. Kragujevac J Sci 2011; 33:63-72.
35. Fejes S, Blazovics A, Lugasi A, Lemberkovics E, Petri G, Kery A. In vitro antioxidant activity of Anthriscus cerefolium L. (Hoffm.) extracts. J Ethnopharmacol 2000; 69:259-265.
36. Rababah TM, Hettiarachchy NS, Horax R. Total phenolics and antioxidant activities of fenugreek, green tea, black tea, grape seed, ginger, rosemary, gotu kola, and ginkgo extracts, vitamin E, and tert-butylhydroquinone. J Agric Food Chem 2004; 52(16):5183-5186.
37. Jian-Ming Lü, Peter Lin H, Qizhi Yao, Changyi Chen. Chemical and molecular mechanisms of antioxidants: Experimental approaches and model systems J Cell Mol Med 2010; 14(4):840-860.
38. Maqsood S, Benjakul S, Shahidi F. Emerging role of phenolic compounds as natural food additives in fish and fish products. In: Critical Reviews in Food Sciences and Nutrition 2013; 53:2,162-179.
39. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radical Res 1995; 22:375-383.
40. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 1965; 16:144-158.