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Invitro Antibacterial Activity of Dodonaea Angustifolia and Withania Somnifera Plants Against Some Bacterial Human Pathogens

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Abstract
Medicinal plants are the wealthy source of various antibacterial agents. The objective of this study was to evaluated antibacterial activity of ethanol, methanol acetone, diethyl ether and hexane leave extracts of *Dodonaea angustifolia* and *Withania somnifera* using paper disc diffusion and broth dilution methods against six human pathogenic bacterial strains. The pathogenic bacteria such as *Escherichia coli, Shigella dysenteriae, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae* and *Salmonella typhi* were susceptible to ethanol, methanol and acetone extracts of the leaves of *Withania somnifera* followed by *Dodonaea angustifolia,*but hexane extract did not showed any activity. The maximum inhibition zone of 16.3±0.57mm was observed against E coli by ethanol extract of *Withania somnifera* and MIC of 3.125mg/ml against *Escherichia coli* and *Shigella dysenteriae by methanol extract. The methanol extract of Dodonaea angustifolia produced a pronounced inhibition of 13.1±0.37mm against Escherichia coli and ethanol extract of D. angustifolia* showed activity against *Shigella dysenteriae* 14.4±0.45mm and MIC of 6.25mg/ml against *I. and S. dysenteriae.* Four different antibiotics like Ciprofloxin, Tetracyclin, Kanamycin and Chloramphenicol were used as standard for the testing of antibacterial activity against six different human pathogens. Among the antibiotics Ciprofloxin showed maximum zone of inhibition ranging from 20-35mm followed by Kanamycin, Tetracycline and Chlormphenicol. The activities are attributed to the presence of some secondary metabolites present in the tested plants which have been associated with antibacterial activities. The obtained result forms a good basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds.
Keywords: Invitro antibacterial activity, Human pathogens, Plants

1. Introduction

Infectious diseases are an important cause of mortality and morbidity, in all regions of the world. The increasing emergence of antimicrobial resistance worsens the impact (Mulu *et al.* 2006; Olivier C, *et al.* 2010). It has been shown that risk of negative clinical consequences, mortality, and high treatment costs with drug-resistant bacteria is generally higher compared to patients infected with the same non-resistant bacteria (WHO 2003). Increased prevalence of resistant bacteria, together with lack and high cost of new generation drugs has escalated infection-related morbidity and mortality particularly in developing countries like Ethiopia (Mulu *et al.* 2006; Borkotoky *et al.* 2013).

This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection (Dean and Burchard 1996; Gonzalez *et al*. 1996). Examples include methicillin-resistant *Staphylococci, Pneumococci* resistant to penicillin and macrolides, vancomycin-resistant *Enterococci* as well as multi-drug resistant gram negative organisms (Norrby *et al.* 2005). There is an urgent need to control antimicrobial resistance by improved antibiotic usage and reduction of hospital cross-infection (Sung and Lee 2007). However, the development of new antibiotics should be continued as they are of primary importance to maintain the effectiveness of antimicrobial treatment (Marchese and Shito 2001).

The potential for developing antimicrobials from higher plants appears rewarding as it was lead to the development of a phytomedicine to act against microbes; as a result, plants are one of the bedrocks for modern medicine to attain new principles (Evans *et al.* 2002). Plant based antimicrobials represent a vast untapped source of medicine. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. Further continued exploration of plant derived antimicrobials is needed today (Hussain and Gorsi 2004).

In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants, to produce more cost effective remedies that are affordable to the population (Doughari 2006). Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century (Zaika 1975). It is estimated that today, plant materials are present in, or have provided the models for 50% Western drugs (Robbers *et al.* 1996). Many commercially proven drugs used in modern medicine were initially used in crude form in traditional or folk healing practices, or for other purposes that suggested potentially useful biological activity. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and

more affordable treatment. The medicinal plants around the world contain many compounds with antibacterial activity (Marjorie 1999). Many efforts have been made to discover new antimicrobial compounds from various sources such as microorganisms, animals, and plants. Systematic screening of them may result in the discovery of novel effective antimicrobial compounds (Costa et *al.* 2008).

The use of botanical medicines is generally on the rise in many parts of the world (Bbosa *et al.* 2007). The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of new anti-infective agents (Amani *et al.* 1998; Costa *et al,* 2008). Numerous experiments have been carried out to screen natural products for antimicrobial property (Nair and Chanda 2006). Medicinal plants possess immunomodulatory and antioxidant properties, leading to antibacterial activities. They are known to have versatile immunomodulatory activity by stimulating both non-specific and specific immunity (Pandey and Chowdhry 2006). The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant (Nascimento *et al.*2000).

Dodonaea angustifolia (Sapindaceae) a small tree or shrub, commonly up to 8 m high, widely distributed within and below the Afromontane zone and occurs throughout the tropics and subtropics (Friis, et al. 2011). It grows at the altitudes between 800 and 2650 m a.s.l. and in areas with a rainfall range of 500-1500 mm/year (Friis, et al. 2011). It grows in a variety of habitats and rapidly colonies open areas of recently cleared forests, invades overgrazed bush lands and fallow lands and is usually found in rocky and stony sites. It can withstand fires to an amazing degree (Beentje 1994). *Dodonaea* is an evergreen shrub and without thorns and buttresses. The diaspore is a winged nut about 1.5 cm in diameter. It produces large numbers of small seeds (about 100 seeds/g) and the seeds are dormant (Azene 1993). It could be used to reclaim land, and is also good as hedge species and for sand binding (Azene 1993). It is not much browsed except when there is a shortage of other palatable plants. According to Jansen (1981), *Dodonaea* is known to have a medicinal value as wound dressing for skin disease in cattle, and in humans as a cure for sore throats and for lowering fever. *Dodonaea angustifolia* is used as a traditional medicine in different countries. Stem or leaf infusions were used to treat sore throats, root infusion to treat colds and seeds (in combination with other plants) used to treat malaria. The leaves are used to treat itching, digestive system disorders, including indigestion, ulcers and diarrhea; and the powdered leaves were given to expel round worms. The plant is also used as antibacterial (Rojas *et al*. 1992).

Withania somnifera is an important medicinal plant, a small, woody shrub 60-200 cm high, in the Solanaceae family, which is described under many common names such as Ginseng and Ashwagandha. This plant is used in more than 100 formulations in Ayurveda, Unani and Siddha and is believed to be therapeutically equivalent to ginseng (Sangwan *et al.* 2004). The ethno pharmacological properties of the plant include adaptogenic, anti-sedative and anti-convulsion activities, and the plant is used to treat various neurological disorders, geriatric debilities, arthritis, stress and behavior-related problems. It has been reported that all of the major parts of *W. somnifera* such as the roots, fruits and leaves provide potential benefits for human health because of their high content of polyphenols and antioxidant activities (Alam *et al.* 2011).

Withania somnifera is traditionally used as a therapeutic agent for diarrhea, dyspepsia and gastrointestinal disorders (Acharyya *et al.* 2009). It has been reported that the antioxidant activities in a plant are dependent on some phytoconstituents such as the phenolic compounds, the anthocyanin and ascorbic acids as well as many other important constituents (Pietta *et al.* 2000*)*. *W. somnifera* is also used as a dietary supplement because it contains a variety of -nutrients and phytochemicals. A decoction of *W. somnifera* roots and leaves is used as a nutrient and health restorative by pregnant women and the elderly. *W. somnifera* thickens and increases the nutritive value of the milk when given to nursing mothers. Additionally, its fruits or seeds are used to curdle plant milk to make vegetarian cheeses (Facciola 1990*)*.

Traditional use of '*W. somnifera*' was to increase energy, youthful vigour, endurance, strength, health, nurture the time elements of the body, increase vital fluids, muscle fat, blood, lymph, semen and cell production. It helps counteract chronic fatigue, weakness, dehydration, bone weakness, loose teeth, thirst, impotency, premature aging emaciation, debility, and convalescence and muscle tension.

In Ethiopia, plant remedies are still the most important and sometimes the only sources of therapeutics for nearly 80% of human and more than 90% in livestock population. Estimated floras of 6500 to 7000 species of higher plants are found in Ethiopia. Out of these 12% are endemic to the country (Tadeg *et al.* 2005). Despite their vital role in catering for the health of human and livestock population, large part of the knowledge of ethno medicinal plants is on the verge of irreversible loss and declining to deterioration due the oral passage of herbal heritage from generation to generation rather than in writings (Mesfin *et al.* 2009). Environmental degradation, agricultural expansions, cultivation of marginal lands and urbanization are also posing a significant threat to the future wellbeing of human and animal populations that have relied on these resources to combat various ailments for generations (Lulekal *et al.* 2008: Devi *et al.* 2009).

2. Materials and Methodology

2.1 Description of the study area

The study was conducted on some medicinal plants collected from Goba districts of Bale zone, Oromia Regional State, South Eastern Ethiopia. Goba district is located at 445 km south east of Addis Ababa. The area is situated at $7^{O}00'$ N and $39^{O}58'$ E Latitude and longitude respectively. The area has a typical vegetation type of undifferentiated Afromontane forests in Ethiopia and has a mean annual rainfall and temperature of 1218.64 mm and 10.26 oC, respectively. The economic activities of the local people are primarily based upon mixed farming that involves pastoralism and cultivation of crops such as wheat and barley.

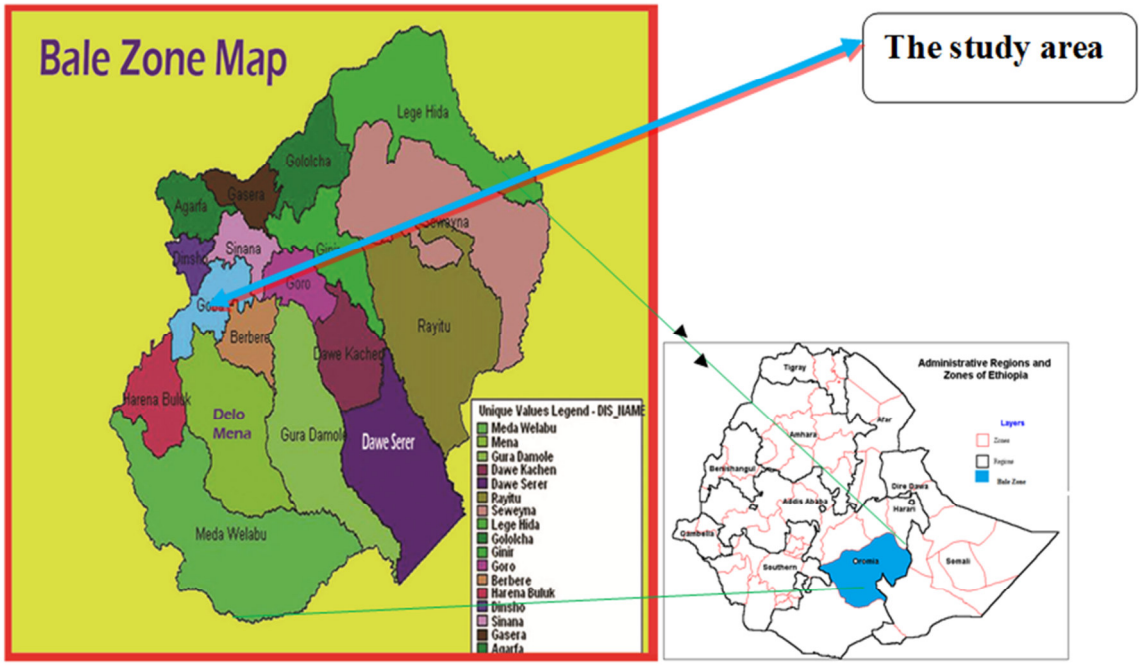

Figure 1 Map of study area

2.2. Collection and identification of plant materials

Two medicinal plants *Dodonaea angustifolia (*Kitkita) and *Withania somnifera (*Giziewa) were collected from Bale Zone, Goba district Oromia region, Ethiopia. The taxonomic position of the plants was identified and authenticated by plant experts from National Herbarium in Addis Ababa University. Leaves from the study plants were taken in a large quantity and repeatedly washed under tap water to remove any debris and were air dried under shade for fifteen days.

2.3. Preparation of plant's crude extracts

The preparation of crude extracts of plants under this study was conducted following the methods used by Tadeg *et al*. (2005) using different solvents. Five hundred grams of leaves from each plant was taken for extraction procedure and ground in a mortar and pestle separately under aseptic condition. Twenty grams of each powdered plant material were extracted with apparatus with 250 ml of ethanol, methanol, diethyl ether, hexane and acetone separately by maceration for 48 h with frequent agitation on orbital shaker for continuous two days and the resulting liquid was filtered (Whatman No. 3 filter paper, Whatman Ltd., England). Extraction was repeated five times and the filtrates of all portions were combined in one vessel. The organic solvent was removed by evaporation using Rota vapor (BU¨CHI Rota-vapor R-205, Switzerland) at 40 ˚C. The resulting dried mass was then powdered, packed into a glass vial until use. Finally, the gram yield of dried residue of each plant extracts were calculated. The concentrated extracts were stored at 4°C for the next antimicrobial study. Dried residues were dissolved in 100 % dimethyl sulfoxide (DMSO) to obtain a stock concentration of 100 mg/ml, which was kept at 4 °C until use.

2.4. Preparation of test organisms

The test organisms including Escherichia coli, Salmonella typhi, Shigella dysentery, Pseudomonas aerugenosa, Staphylococcus aureus and Klebsiella pneumoniae were obtained from Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia. These microorganisms were suspended in nutrient broth and subculture into fresh nutrient agar medium and kept at 4°C until use. The inoculums preparation was standardized by inoculating bacterial strains from the exponential phase and standardized with 0.5 McFarland turbidity standard prepared by

adding a 0.5 mL aliquot of 1.175% w/v $BaCl_2.2H_2O$, added to 99.5 mL of 0.18 mol/L H_2SO4 (1% v/v).

2.5. Antimicrobial Assay

2.5.1. Antibacterial sensitivity testing using disc diffusion method

The antibiotic susceptibility testing, stock concentrations of (100mg/ ml) plant crude extracts were prepared in DMSO. A circular antibiotic assay disc of 6 mm diameter was prepared from the Whatman filter paper No.3 and sterilized by autoclaving for 15 min at 121° C. The sterile discs were impregnated with 50µl of the reconstituted extract and were dried completely at 37 °C overnight. A sterile cotton swab was dipped into a homogenous suspension of test organism with adjusted 0.5 McFarland turbidity standards. The test pathogenic microorganisms were swabbed gently by cotton swab onto Muller Hinton Agar (MHA) and were then allowed to dry for half an hour. The discs were aseptically placed over plates of Muller Hinton Agar (MHA) (Haniyeh *et al*. 2010). The plates were incubated in an upright position at 37 °C for 24 hours and the zone of inhibition was measure (in mm diameter). Inhibition zones with diameter less than 12 mm will consider as having low antibacterial activity. Diameters between 12 and 16 mm was consider moderately active, and these with >16mm was consider highly active (Indu *et al*. 2006). The test organisms were tested for their sensitivity against the standard antibiotics, Ciprofloxacin (35 µg), Chloramphenicol (30 µg) Tetracycline (30 µg) and Kanamycin (20µg) by the disc diffusion method (Bauer *et al*. 1966).

2.5.2. Minimum Inhibitory Concentration (MIC) assay methods

The minimum inhibitory concentration (MIC) was determined by comparing the various concentrations of plant extracts which have different inhibitory effect and selecting the lowest concentration of extract showing inhibition (Agatemor 2009). The minimum inhibitory concentration (MIC) was determined for extracts that showed inhibition zone of $\geq$ 7 mm diameter and for extract that inhibited the growth of all tested bacteria at concentration of 200 mg/ml. The test was performed by using standard tube dilution (serial dilution) method using nutrient broth as diluents. Accordingly, the plant extract was prepared by double serial dilution from 200 mg/ml to obtain 1:2, 1:4, 1:8, 1:16, 1:32, and 1:64 in order to get 100, 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.78 mg/ml concentration of extract respectively using 50% DMSO. 1 ml of each extracts were dissolved in sterile test tubes which containing 9 ml of nutrient broth. Then, 0.1ml of the test organism was inoculated to the each tube. One tube was used as the control (broth + extract). The tubes were incubated at 37°C for 24 h and the presence of growth was evaluated by comparing the optical density (OD) of each well before and after incubation. When the difference of OD value (after incubation-before incubation) of the test (broth + extract + organism) was greater than that of the control (broth + extract) at each concentration, it was considered as presence of turbidity or growth of bacteria. The lowest concentration, at which there was no turbidity, was also regarded as MIC value of the extract.

2.6 Data Analysis

Data on mean inhibition zone produced by each plant extract and MIC on various bacteria were entered in to Microsoft excels spreadsheet and SPSS (Statistical Package Software for Social Science version 16). Values are given as mean± SD.

3. Results

3.1 Antibacterial activity of the plant extracts

The crude extracts study plant such as Withania somnifera and Dodonaea angustifolia were tested for antibacterial activity on six human pathogens. The solvents that were used in this study produced an overall yield of plant crude extracts that were ranging from 0.8 to 2.2 gm from different plants (Table.1).

In-vitro antimicrobial activity of crude extracts of plants under this study were evaluated against human pathogenic bacteria such as Escherichia coli, Salmonella typhi, Shigella dysenteriae, Pseudomonas aerugenosa, Staphylococcus aureus and Klebsiella pneumonia. The results obtained in the present study revealed that the tested two medicinal plants (Withania somenifera and Dodonaea angustifolia) extracts possess a potential antibacterial activity.

Table 1:The yield of plant crude extracts by using different solvents

Plant species	Parts used (gm)	Extraction type	Yield in grams (Mean in mm)
Withania somnifera	20 Leaves	Methanol	1.5
		Ethanol	1.25
		Diethyl ether	0.8
		Acetone	1.6
		Hexane	1.3
Dodonaea angustifola	20 Leaves	Methanol	2
		Ethanol	2.2
		Diethyl ether	1.3
		Acetone	2.1
		Hexane	1.3

3.1.1 The antibacterial activity of Withania somnifera crude extracts

The antibacterial activity of *W. somnifera* crude extracts was assayed by disc diffusion method. The methanol and ethanol leaves extract of *W. somnifera* showed considerably a higher mean antibacterial activity as compared to other solvents. The highest antibacterial activity was exhibited on *Escherichia coli* (16.3±0.57 mm) by ethanol extract, followed by *Shigella dysenteriae* (12.5±0.5 mm) and a moderate inhibition of *Klebsiella pneumoniae* (10±1.0 mm) and the least activity against *Salmonella typhi* (6.1±0.76 mm). The methanol extracts showed a strong inhibitory activity against *S. typhi* (14.8±0.76 mm), followed by *Shigella dysenteriae* with a zone of inhibition 11±0.57mm and a moderate inhibition against *Staphylococcus aureus* (9.8±0.28 mm), and *P. aerugenosa* (8.8±0.76mm). With Methanol, a minimum zone of inhibition of *W. somniera* (6.5±0.5 mm) was exhibited by *E. coli*.

Acetone extracts of *W. somnifera* were exhibited a maximum zone of inhibition against *Salmonella typhi* (11.9±0.35 mm) followed by *Staphylococcus aureus* (10.5±0.5mm) and minimum activity against *Pseudomonas aerugenosa* (5.4±0.5 mm). Diethyl ether extracts showed inhibitory activity against only three pathogens. The maximum inhibiton was detected on *Salmonella typhi* (7.9± 0.17mm) followed by *Klebsiella pneumoniae* (6.2±0.68 mm) and least activity against *E. coli* (4.8±0.76mm). Hexane extract didn't show any antibacterial activity against test pathogenic bacteria. (Table .2)

Table 2: The effect of the different extracts of the leaves of *Withania somnifera* against the bacterial test organism (Zones of inhibition in mm; Mean± SD mm))

Test organisms	Mean Inhibition zone of leaves extract of **W. somnifera in mm* (Mean± SD mm)				
	Methanol	Ethanol	Diethyl Ether	Acetone	Hexane
Escherichia coli	6.5±0.5	16.3±0.57	4.8±0.76	-	-
Salmonella typhi	14.8±0.76	6.1±0.76	-	11.9±0.35	-
Shigella dysenteriae	11±0.57	12.5±0.5	7.9±0.17	7.3±0.57	-
Staphylococcus aureus	9.8±0.28	8.6±0.52	-	10.5±0.5	-
Pseudomonas aeruginosa	8.8±0.76	6.1±0.36	-	5.4±0.5	-
Klebsiella pneumonia	8.8±0.28	10±1.0	6.2±0.68	5.8±0.28	-

-= implies no inhibition zone detected*; ** = a crude extract at concentration of 100mg/ml was used for assay.

3.1.2. The antibacterial activity of Dodonaea angustifolia crude extracts

The methanol extract of D. angustifolia produced a pronounced inhibition zone of 13.1±0.37mm in diameter against E. coli, followed by K. pneumoniae (10.9±0.3mm) and S. typhi (9.7±0.64 mm). The methanolic extracts exhibited the least inhibitory activity against S. dysenteriae and S. aureus with mean inhibition zone of 7.2±0.46mm and 7.7±0.45mm, respectively. The prominent zone of inhibition from the ethanol extract of D. angustifolia against Shigella dysenteriae was 14.4±0.45mm followed by K. pneumoniae (13.9±0.35mm), .S. typhi (12.9±0.51mm) and 11.5±0.51mm against .E. coli. Moderate inhibitory activity was noticed against S. aureus (10±0.15mm) followed by P. aerugenosa (8.5±0.55 mm) The diethyl ether extract produced a maximum zone size of 12.1±0.3mm against P. aerugenosa and a moderate activity of 8±0.2mm against S. dysenteriae and 7.9±0.35mm against K. pneumoniae and minimum inhibitory activity against E. coli with a zone size of 5.6±0.52mm. Acetone extract of D. angustifolia inhibited S. aureus with a highest zone of inhibition 11.9±0.25 mm and minimal inhibition was 6.8±0.2 mm and 5.7±0.32mm against S. dysenteriae and P. aerugenosa. No good antibacterial activity was excreted by the Hexane extracts (Table 3).

Table 3: The effect of the different extracts of the leaves of *Dodonaea angustifolia* against the bacterial test organism using disc diffusion method (**Zones of inhibition; Mean± SD mm**)

Test Organisms	Mean Inhibition zone of leaves extract* *Dodonaea angustifolia* (Mean± SD mm)				
	Methanol	Ethanol	Diethyl Ether	Acetone	Hexane
Escherichia coli	13.1±0.37	11.5±0.51	5.6±0.52	-	-
Salmonella typhi	9.7±0.64	12.9±0.36	6.2±0.62	-	3.7±0.26
Shigella dysentery	7.2±0.46	14.4±0.45	8±0.2	6.8 ±0.2	-
Staphylococcus aureus	7.7±0.45	10±0.15	-	11.9±0.25	-
Pseudomonas aeruginosa	-	8.5±0.55	12.1±0.32	5.7±0.32	-
Klebsiella pneumonia	10.9±0.3	13.9±0.35	7.9±0.35	6±0.2	-

-= implies no inhibition zone detected*; *= a crude extract of at concentration of 100mg/ml was used for assay.

3.1.3 Inhibitory Zones of test pathogens with Standard Antibiotics (Positive control)

Four different antibiotics such as Ciprofloxin, Tetracyclin, Kanamycin and Chloramphenical were used as standard and as positive control for the testing of antibacterial activity of six different human pathogens. The Ciprofloxin showed maximum zone of inhibition ranging from 20-35 against all pathogens; Kanamycin showed average zone of inhibition 20mm, Tetracycline exhibited ranging from 8-18mm and Chlomphenicol showed least inhibition against all test pathogens (Table.)

Table 4: The inhibition zone of antibiotics against human pathogens

Test organisms	Zone of inhibition in mm			
	Ciprofloxin	Kanamycin	Tetracycline	Chlromphenicol
Escherichia coli	30	20	15	10
Salmonella typhi	35	20	15	10
Shigella dysenteriae	32	20	13	10
Staphylococcus aureus	31	20	10	5
Pseudomonas aeruginosa	30	15	8	5
Klebsiella pneumonia	20	15	20	11

3.2. Minimum Inhibitory Concentration of Plant Extracts (MIC)

The Minimum Inhibitory Concentration assay was employed to evaluate the effectiveness of the plant extracts to inhibit the growth of bacterial test organisms. The extracts of the four medicinal plants were subjected to the concentrations ranging from 0.78 mg/ml to 100mg/ml. In the antibacterial activity test, five different solvents were used for their *in vitro* antibacterial test among which only the best three solvents namely methanol, ethanol and acetone has selected for MIC test.

3.2.1. Minimum Inhibitory Concentration (MIC) of *Withania somnifera* leaf extracts against bacterial test organism (in mg/ml)

The methanol extract of *W. somnifera exhibited the lowest MIC at 3.12mg/ml against E. coli and S. dysenteriae followed by S. typhi and Pseudomonas aeruginosa at a concentration of 6.25mg/ml.* The ethanol extract exhibited MIC at 3.12 mg/ml concentration against *S. dysenteriae* and *K. pneumoniae* and at concentration of 6.25 mg/ml against *E. coli.* The ethanol extract also displayed its MIC at concentration of 12.5 mg/ml against *S. typhi* and *S. aureus* .The MIC of acetone extract of *W. somnifera* was 6.25 mg/ml against the *E. coli* and *S. typhi* followed by *S. dysenteriae* at 25 mg/ml and *S. aureus at* 50mg/ml (Table.5).

3.2.2. Minimum Inhibitory Concentration (MIC) of *Dodonaea angustifolia* leaf extracts against bacterial test organism in mg/ml

The methanol extract of *D. angustifolia showed* MIC activity at 6.25 mg/ml concentration against *E. coli and S. typhi* followed by *S. dysenteriae* and *K. pneumoniae* at 12.5 mg/ml concentration. The ethanol extracts showed strong MIC activity at 1.56 mg/ml against *S. dysenteriae* and *against S. typhi at 6.25 mg/ml concentration followed by S. aureus* and *Pseudomonas aerugenosa at 12.5 mg/ml. The acetone extract of D. angustifolia exhibited a MIC at12.5 mg/ml against S. dysenteriae followed by S. aureus at 25 mg/ml and at 50 mg/ml against P. aerugenosa and K. pneumoniae (Table 6).*

Table 5: Minimum Inhibitory Concentration (MIC) of *Withania somnifera* leaf extracts against bacterial test organism in mg/ml

Withania somnifera	Con mg/ml	*Escherichia coli*	*Salmonella typhi*	*Shigella dysenteriae*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Klebsiella pneumonia*
Methanol	1.56	-	-	-	-	-	-
	3.12	**	-	**	-	-	-
	6.25	+	**	+	-	**	-
	12.5	+	+	+	-	+	-
	25	+	+	+	**	+	-
	50	+	+	+	+	+	-
Ethanol	1.56	-	-	-	-	-	-
	3.12	-	-	**	-	-	**
	6.25	**	-	+	-	-	+
	12.5	+	**	+	**	-	+
	25	+	+	+	+		+
	50	+	+	+	+	**	+
Acetone	1.56	-	-	-	-	-	-
	3.12	-	-	-	-	-	-
	6.25	**	**	-	-	-	-
	12.5	+	+	-	-	-	-
	25	+	+	**	-	-	-
	50	+	+	+	**	-	-

** = Minimum Inhibitory concentration, + = Positive inhibition observed, - = No activities

Table 6: Minimum Inhibitory Concentration (MIC) of *Dodoneae angustifolia* leaf extracts against bacterial test organism in mg/ml

Dodoneae angustifolia	Con mg/ml	*Escherichia coli*	*Salmonella typhi*	*Shigella dysenteriae*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Klebsiella pneumonia*
Methanol	1.56	-	-	-	-	-	-
	3.12	-	-	-	-	-	-
	6.25	**	**	-	-	-	-
	12.5	+	+	**	-	-	**
	25	+	+	+	-	-	+
	50	+	+	+	**	-	+
Ethanol	1.56	-	-	**	-	-	-
	3.12	-	-	+	-	-	-
	6.25	-	**	+	-	-	-
	12.5	**	+	+	-	-	**
	25	+	+	+	**	**	+
	50	+	+	+	+	+	+
Acetone	1.56	-	-	-	-	-	-
	3.12	-	-	-	-	-	-
	6.25	-	-	-	-	-	-
	12.5	-	-	**	-	-	-
	25	-	-	+	**	-	-
	50	-	-	+	+	**	**

** = Minimum Inhibitory concentration, + = Positive inhibition observed, - = No activities (bacterial growth observed)

4. Discussions

Ethno botanical investigations have been found to offer important clues in the identification and development of traditionally used medicinal plants into modern drugs. Contribution of the field has also been reflected in the current study. Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal was the *in vitro* antibacterial activity assay (Samy and Ignacimuthu, 2000). Many reports were available on the antiviral, antibacterial, antifungal, anthelminthic, and

anti-inflammatory properties of plants (Palombo and Semple, 2001, Kumarasamy *et al*., 2002). Traditional medicine comprises of therapeutic practices that have been in existence, for hundreds of years, before the development and spread of modern medicine and are in use today (WHO, 1991).

In the present study, crude extracts have been eluted from the leaves of two different Plants viz. *Withania somnifera* and *Dodonaea angustifolia* using five different solvents such as methanol, diethyl ether, ethanol, acetone and hexane. So far extraction of crude compounds from different medicinal plants has been conducted by using different organic solvents in many researches. The medicinal plants in general can produce highly potent inhibitory compounds against various human pathogenic microorganisms. The results of current study were an indication of such understandings. The yield of the extract that was obtained by different solvents considerably differs in two of the medicinal plants (Table 1).

In the present study, among the solvents used to extract the biologically active substances from two medicinal plants, ethanol and methanol were the best solvents, followed by acetone and least by diethyl ether and hexane (Table 2 to 5). This indicates that the extraction of medicinal plants with different solvents may produce different *in vitro* inhibitory result which based on the potential of the solvents used to extract the biologically active constituents (George *et al*., 2010). In the present study, most commonly ethanol and methanol leaf extracts of *W. somnifera* and *D. angustifolia* showed the strongest activity against *E. coli, S. aureus, S. typhi,* and *S. dysenteriae* compared with other three solvents and plant based products have been effectively proven for their utilization as source for antimicrobial compounds. In related study, methanol extracts of *W. somnifera* exhibited an appreciable inhibitory activity against all the strains of *Neisseria gonorrhoea* while, only the methanol extract of *W. somnifera* was effective against *C. albicans* (Ragavendra *et al*., 2006).

The methanol and ethanol leaf extracts of *W. somnifera* showed significant antibacterial activity against most of bacterial human pathogens evaluated in the present study. The highest antibacterial activity exhibited was against *E. coli* (16.3±0.57 mm) by ethanol extract, followed by *S. dysentriae* (12.5±0.5 mm) and a moderate inhibition against *K. pneumoniae* (10±1.0mm). In a study by (Rinku *et al*., 2012), the ethanolic leaves extract of *W. somnifera* displayed relatively higher antibacterial activities against bacterial strains including the *S. aureus* which is in concordance with result of our study. Sundaram *et al*. (2011) demonstrated that *W. somnifera* has the potent antibacterial activity against Gram-negative bacteria, particularly *S. typhi* and *E. coli.*

In the present study, the methanol extract exhibited the second with inhibition zone of 14.8±0.76 mm against *S. typhi*, followed by *S. dysentriae* with a zone of inhibition of 11±0.57mm. A different study reported that the antibacterial activity of the methanolic extracts of *W. somnifera* leaves against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus mutans* and *Candida albicans*, with zones of inhibition of 38, 36, 15, 38 and 32 mm, respectively (Jain and Varshney, 2011).

Pavithra and co-workers (2011) reported that the methanol extracts of *Mollugo cerviana* inhibited the growth of *S. aureus* and *E. coli* with zones of 7.33±0.57 mm and 11±1mm, respectively while chloroform extracts were ineffective against these bacterial strains. Our study showed that the methanolic extract of *W. somnifera* to have a strong inhibitory activity against test pathogens which is in concordance with other studies. The decrease of antibacterial activity of *W. somnifera* against test pathogens in the current study may be attributed to the difference in the initial plant extract used and extraction method used the difference in the strains of test pathogens or due to unexplained reasons. According to Mirjalili *et al.* (2009), the most important compounds, withaferin and withanolides were isolated from methanolic extraction of the roots of *W. somnifera* which might have contributed to the inhibition of the test pathogens evaluated in our study.

The Acetone extracts of *W. somnifera* exhibited the maximum zone of inhibition against *S. typhi* (11.9±0.35mm) followed by *Staphylococcus aureus* (10.5±0.5mm) and minimum activity against *P. aerugenosa* (5.4±0.5mm). Similar investigations have been reported where acetone extracts showed a pronounced inhibitory effect on the growth of pathogenic bacteria (Abdullahi *et al.,* 2010). The methanol and Ethanol extract of *W. somnifera* exhibited the lowest MIC at 3.21mg/ml concentration against *Escherichia coli* and *Shigella dysentriae* and *K. pneumoniae*. Comparable results were observed from a study of Singh *et al*. (2013) where the MIC values of 31 µg/mL and 62 µg/mL were obtained against the pathogens *Nocardia asteroids* and *S. pyogenes* with *W. somnifera.* Study has indicated the presence of various flavonoids, including quercetin glycosides in the leaves of *W. somnifera (*Kandil *et al*., 1994*)* which have contributed to antibacterial activity noticed in this study. In other study, a number of withanolide steroidal lactones have been isolated from the leaves of *W. somnifera* (Glotter *et al*., 1973) which exhibit antibacterial, anti-fungal and antitumor properties (Devi *et al*., 1993).

The result of the present study showed that the plant extracts of *D. angustifolia* exhibited antibacterial activity against some of the common pathogenic bacteria. The prominent zone of inhibition from the ethanol extract of *D. angustifolia* against *S. dysenteriae* was 14.4±0.45 mm and against *K. pneumoniae* was 13.9±0.35mm followed by *Salmonella typhi* 12.9±0.51 mm. Previous study showed that ethanolic extract of *D. angustifolia* exhibited a highest zone of inhibition (28.33 mm) against *S. aureus* with MIC 12.5µg/ml (Amabeoku *et al*., 2001), a result higher than the size of inhibition zone in our study. The results of this study showed that the extracts from *D. angustifola* was found to have significant antibacterial activity against both the

selected Gram positive and Gram negative bacteria. A similar finding has been reported by (Kaithwas *et al*., 2008) on ethanol extracts of *Aloe. vera*.

The methanol extract of *D. angustifolia* produced a pronounced inhibition zone of 13.1±0.37mm against *E. coli*, followed by *K. pneumoniae* with a zone of inhibition of 10.9±0.3 mm and *S. typhi* 9.7±0.64mm. According to Alexandru *et al*. (2007), the crude extract of *D. angustifolia* has antibacterial activity against both *S.aureus* and *E.coli*. The methanolic extract of *Dodonaea viscosa* showed activity against multiresistant Gram-positive bacteria (Mothana *et al*., 2010). In our study , the result clearly showed that this plant is effective against *E.coli*. The possible explanation for this difference in inhibitory activity might be the ecological difference on their distribution plants which might have contributed to variations in the concentration of the active ingredients.

Acetone extract of *D. angustifola* inhibited *S. aureus* with a highest zone of inhibition 11.9±0.25 mm. The methanol extract of *D. angustifolia* showed MIC activity at 6.25 mg/ml concentration against *E. coli* and *S. typhi* which is supported by work of (Teffo *et al*., 2010) where the minimum inhibitory concentration (MIC) of isolated compounds from *D. angustifola* against *S. aureus, E. faecalis, E. coli and P. aeruginosa* was found to be varied from 16 µg/ml to more than 250 µg/ml. Ethanol extract showed a very minimal MIC of 1.56 mg/ml against *S. dysenteriae* and *S. typhi* which is strongly supported by the results of Tegenu Gelana (2011) where the Acetone and ethyl acetate extracts of the leaves of *Z. scabra* showed best activity against *S. aureus* exhibited an MIC of 1.56mg/ml and 0.781mg/ml respectively. The least inhibition zone was observed for hexane extract against *Salmonella typhi* according to Tsuchiya *et al*., (1996).

5. Conclusion

From the above results it could be concluded that the crude extracts of the two plants especially the ethanol and methanol revealed the fact that they have higher potential to produce broad spectral antibacterial activity with minimal concentration against a wide range of human pathogens. The extracts were good in inhibiting *Escherichia coli, Salmonella typhi, Shigella dysenteriae, P. aerugenosa* and in some instances *K. pneumoniae.* The results of this study provide an insight into the antimicrobial properties of the extracts of *Dodonaea angustifolia* and *Withania somnifera.* As well as it created an opportunity for selection of bioactive extracts for initial fractionation and further studies of these two medicinal plants in the antibacterial assays. This *in vitro* study demonstrated that these two folklore medicinal plants have good potential. This study gives an indication of the efficacy of the plants obtained from the traditional healers. The results from this study form a basis for further studies of the potent plants so as to isolate the compounds responsible for the antimicrobial activity. Various modern drugs were extracted from traditional medicinal plants through the use of plant material following the ethno botanical leads from indigenous cures used by traditional medical systems.

Competing Interests

The authors declare that they have no competing interests.

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