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Original Article

In-Vitro Anti-Bacterial, Phytochemical and Antioxidant Activity of *Gezawa* (*Withania somnifera* (L.) Dunal) Extracts Against Human Pathogenic Bacteria

Yirgashewa Asfere Wuhibe*, Getaw Abera Zewdu, Getu Hailu W/Yohannes and Degisew Yinur Mengistu

Department of Biotechnology, College of Natural and Computational Science, Wolkite University, Wolkite Ethiopia

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Abstract

Withania somnifera (L.) Dunal is frequently used as traditional medicine for the treatment of numerous diseases. This study was aimed to assess bioactive compounds, antimicrobial activities and antioxidant studies on different part of *W. somnifera*. The plant parts were shaded dried and grinded to powders for biological activities. The ethanol, methanol and chloroform extracts were tested for antimicrobial activities. The extracts were subjected to a test of their antimicrobial properties by paper disc diffusion method. Minimum inhibitory concentrations (MICs) of the plant extracts against selected pathogens were also assessed using the agar dilution method. The highest potential was observed from methanol extract of the stem bark and ethanol extract of the leaf against *Staphylococcus aurous* with zone of inhibition 15.02 and 13.22 mm, respectively. Alkaloids, steroids, saponins, tannins, reducing sugars, phlobatanins, flavonoids, terpenoids, cardiac glycosides, anthraquinone, emodins and coumarins are result of phytochemical assessment. Strong antioxidant effect of free radical scavenging activity was recorded at 100µg/ml concentration for ethanol stem and root, methanol leaf extracts (72.22%, 80.84%, 74.24%) respectively. Chloramphenicol and DMSO were used as positive and negative controls. *W. somnifera* could show powerful antimicrobial and antioxidant properties.

Keywords: Antibacterial, Antioxidant, *In-vitro*, MIC, Paper disc diffusion, *Withania somnifera*

Introduction

Plants are mainly used in traditional medicines which play an essential role in health care. For the primary treatment more than half of the world's population relying mainly on traditional medicines [1]. Antimicrobial agents are essentially important in reducing the global burden of infectious diseases [2]. On the other hand, appearance and distribution of multidrug resistant strain in pathogenic bacteria have develop a significant public health threat as there are fewer, or even occasionally no, actual antimicrobial agents available for the infection affected by pathogenic bacteria [3]. Therefore, in the light of the proof of the fast global spread of resilient clinical isolates, the essential to find new antimicrobial agents is of dominant importance. However, the historical record of quick, varied spread appearance of resistance to newly familiarized antimicrobial agents have a short life expectancy [4]. A massive number of medicinal plants have been documented as valued resources of natural antimicrobial compounds as an alternative that can possibly be effective in the action of these problematic bacterial infections [5]. According to the World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs [6].

The therapeutic issues which produce a definite physiological action on the human body; include alkaloids, flavonoids, glycosides, value of plants is due to the presence of some chemical constituents within the plant tannins, gums, resins essential oils, fatty oils, carbon compounds, hydrogen, oxygen, nitrogen salts of some chemicals and secondary metabolites as well [7]. These compounds are believed to play a role in the plants defense against infection by working in synergy with intrinsic antimicrobials. It has therefore been suggested recently, that such compounds can potentially be used to

*Corresponding author: Department of Biotechnology, College of Natural and Computational Science, Wolkite University, Wolkite Ethiopia
Email Address: yirga.asfere@wku.edu

improve the efficacy of antibiotics against bacterial pathogens [8].

From ancient period medicinal plants have been used to cure different types of diseases. Since they are of great importance and commonly used by the local people, due include to which globally a lot of people are engaged in the trade of important medicinal herbs. Three quarters of the world population relies mainly on plants and plant extracts for health care. In China the allopathic believed drugs are mostly avoided because they use traditional medicine for non-toxicity [9]. In Africa, up to 80% of the population depends on traditional herbal medicine for primary health care, accounting for around 20% of the overall drug market according to the World Health Organization (WHO) [10]. Over the past 20 years, as source of new antimicrobial agents there has been a lot of study on plants. But motionless there is an instant need to recognize novel substances lively in the direction of pathogens with high resistance [11]. In diverse organisms numerous drug resistances are becoming common and resistance to antimicrobial agents is rising in a wide diversity of pathogens [8]. The indigenous people of Ethiopia have been using many plant species as traditional medicines long ago, including treatment of infectious diseases, but there has been paucity in data regarding their in vivo and in vitro efficacy.

Due to lesser side effects lesser toxicity, cheap and more effective compared to the synthetic drug agent the use of plant for treating infectious diseases has gradually increased in the world. *Withania somnifera* (L.) Dunal (kutilal) belongs to the family solanaceae. The plant has been found useful in the treatment of burns, wounds and dermatological disorders and gastrointestinal diseases, dysfunctions of the respiratory system, asthma, bronchitis, cancer and geriatric problems. *W. somnifera* is widely used in Ayurvedic medicine, the traditional medical system of India [9]. It is an ingredient in many formulations prescribed for a variety of musculoskeletal conditions (e.g., arthritis, rheumatism) and as a general tonic to increase energy, improve overall health and longevity and prevent disease in athletes, the elderly, during pregnancy. Many pharmacological studies have been conducted to examine the properties of *W. somnifera in* an attempt to authenticate its use as a multi-purpose medicinal agent. The anti-inflammatory properties of Linn have been investigated to validate its use in inflammatory arthritis, while animal module studies have been established, its use as an anti-stress agent [9]. Several studies have examined the antitumor and radio sensitizing effect of *W. somnifera*. Besides, photochemical screening, the extracts was also carried out to assess the presence of different photochemical in different extracts [10]. The present study is carried out to evaluate the antibacterial activity, phytochemical screening and antioxidant properties of *W. somnifera* plant extracts against human pathogenic bacteria and compare the activity of plants and selected antibiotics against the human pathogenic bacteria.

Material and Methods

Description of the Study Area

The study was conducted in the Biotechnology Laboratory of Wolkite University, which is found in Guragie Zone 158 km South of Addis Ababa, Ethiopia. The university has a total area of about 46 km^2. It has a moderate average temperature of 16 ^oC and the mean maximum and minimum annual temperature is 24.02 and 9.73 °C respectively. The mean annual rainfall is 780 mm.

Chemicals and Apparatus

Chemicals which are used in the study were ethanol, methanol, chloroform, distilled water, Nutrient agar, Broth agar, and Dimethyl Sulphoxide (DMSO), alcohol and Muller Hinton agar. The apparatuses which are used were, Petri dishes, analytical miller, polyethylene bag, water bath, round bottom flask, what man filter paper Number 1 (125mm) , Erlenmeyer flask, Incubator, shaker, oven, autoclave, slide, test tube, drop late, pipette, electrical balance, beaker, inoculation loop, forceps, cotton, pipette tips, aluminum foil, laminar flow hood, spreader, spatula, par-film, marker, refrigerator and cuvette.

Experimental Design

The research was designed based on the laboratory chemical analysis in a commercial Chemical relational database (CRD). The treatments were including one plant with three parts extracted by three solvents and tested on three pathogenic bacteria in three replications (1x3x3x3x3). Dimethyl sulfoxide (DMSO) was used as negative control and chloramphenicol was used as positive control.

Sample Collection and Identification of Plant Materials

Plant materials Fresh leaves, stem bark and root of *Withania somnifera* were collected from compound of wolkite University main campus Gubrea Ethiopia. The sample materials were properly identified by Mr. Belachew Garedew, Botanist at department of Biology, Wolkite University. Voucher specimen (No. WKU/Biol./VS/119) has been retained at the Department of Biology, Wolkite University for future reference. (Fig. 1).

Fig. 1 Plant of *Withania somnifera* (L.) Dunal in medicinal plant collection

Preparation of Crude Extracts from *Gezewa (Withania somnifera*)

The collected plant parts of *W. Somnifera* was separately washed thoroughly with tap water followed by sterile distilled water to remove debris and dust particles and cut into smaller sizes using a sterile knife. Then the leaves, roots and stem barks of the plants (Fig. 2 A, B, C) were dried under shade on a paper towel for two weeks with occasional shifting at room temperature. The resulting dry parts were then ground into fine powder with the help of suitable sterile grinder. Then it stored in sterile airtight containers according to [11].

Preparation of Crude Extracts using Organic

Twenty grams (20g) of the coarsely powdered plant materials (leaves, roots and stem barks) of *W. Somnifera was* suspended in 100ml of different solvents, i.e., methanol (98%), ethanol (99%) and chloroform (97%), separately in 250 ml conical flasks. As showed by [12]. The suspended plant materials were placed on a rotary shaker rotating at 190-220 rpm for 72hrs at room temperature [13]. Muslin cloth was then used to sieve the plant residue, the remainder thus obtained was further cleansed by filtration through Whatman No.1 sterile filter paper, and the resulting filtrates were collected as sources of crude extracts. The filtrates were dried in a water bath at 40 °C – 50 °C for 24 hours until the solvents were removed [14]. Next to the evaporation of solvents, the left over crude extracts were measured using a balance and the consequential weights documented. These crude extracts were further diluted with 10 ml of dimethyl sulphoxide (DMSO) and kept in sample vials with stoppers at 4 °C until they were used against the test pathogens [15].

Percentage Yield of Crude Extracts of *W. Somnifera* parts using Different Solvents

The percentage yield obtained from the plant parts was the amount of the crude extract recovered in mass compared with the initial amount of powdered plant materials used. It is presented in percentage (%) and was determined for each extraction solvent used.

Sterilization of Materials

All materials used were be sterilized. Glasses were cleaned with cleaners, washed appropriately with tap water and dried. They were then sterilized in the oven at 160 °C for 2 hrs. Inoculating loops were animated to redness in an exposed flame. All the media such as Nutrient Agar and Mueller Hinton Agar, distilled water and McCartney bottles used were sterilized in the autoclave at 121 °C and 15 psi for 15 minutes. In addition, the laboratory bench was always swabbed with 70% alcohol before and after each round of experiment [16].

Fig. 2 *Withania somnifera* (L.) Dunal plant leaves (A), stems barks (B) and Roots (C) during drying time under shade

The Test Bacterial Species

The bacterial pathogens were gained from Ethiopian Public Health Institution (EPHI). Three species of pathogenic bacteria were used in this study. Two species from those that infect the skin (*Staphylococcus aureus* ATCC 29223 and *Pseudomonas aeruginosa* ATCC 27853) and one species (*Escherichia coli* ATCC 25922) from among the enteric bacterial species were used as experimental microbes in this study.

Preparation of Culture Media

Liquid broth media, nutrient agar, and Mueller-Hinton agar were prepared according to the manufacturer's instruction. All media were first autoclaved at 121 °C for 15 minutes before cultured bacteria.

Standardization of the Bacterial Cell Suspension used as Inoculum

All bacterial cultures were first grown in liquid broth media at 37 °C for 18 hours prior to inoculation onto the nutrient agar. Few colonies (4-5) of similar morphology of the respective bacterial species were transferred with a sterile inoculating loop to a liquid medium and incubated until adequate growth of turbidity equivalent to McFarland 0.5 turbidity standard was obtained [17].

Inoculation and Antibacterial Activities Using Paper Disks method

Inoculums' of the respective bacterial species were spread on to Mueller Hinton agar (MHA) plates using a sterile swab in such a way as to ensure thorough coverage of the dishes and the same thick lawn of growth achieved next to incubation. Sterile filter paper discs of 6.0 mm in diameter were cut and soaked with 30µl of each plant extracts from a stalk solution. The paper discs were then aseptically placed on Mueller–Hinton Agar (MHA) plates, inoculated with dense inoculums suspension of the test pathogens and the plates were then allowed to stay for 1–2hrs for pre-diffusion of the extracts at room temperature [18]. Finally, the plates were incubated at 37^{o}C for 18–24 hrs. At the final of the incubation time, the diameter of inhibition zone was measured by a caliper [19].

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined by using agar dilution method for the crude extracts. The ethanol, methanol and chloroform extracts of the different plant parts (stem bark, root and leaves) of *W. Somnifera* that showed significant antimicrobial activities in the previous test were selected for determination of MIC [20]. The sterilized media were allowed to cool at 50^{o}C and 18 ml of molten agar was added to test tubes, which contained 2ml of different concentrations of the crude extracts. The combination of the media (molten agar and crude extracts) and the experimental drugs were carefully mixed and decanted into pre-labeled sterile Petri-dishes on an equal surface. Extra Petri-dishes holding only the growth media were ready in the same way for contrast of the growth of the particular organisms. The concentrations of the extracts used in this test were 50, 25, 12.5, 6.125, 3 and 1.5 mg/ml. The plates were allowed to dry at room temperature. The suspensions of the respective pathogens whose densities were adjusted to 0.5 McFarland turbidity units (1.5 x 10^{8} CFU/ml) were inoculated onto the series of agar plates using a standard inoculating loop. Three loop full of suspension were moved into individual plate. The dishes were then incubated at 37 °C for 24 hrs. The lowest concentration, which inhibited the growth of the respective organisms, was taken as MIC.

Phytochemical Profiling of stem Bark, Leaf and Root of *W. somnifera*

The chemical examinations of the extracts were done in the ethanol, methanol and chloroform, of *W. somnifera* by standard technique with little modification was used to identify the bioactive compound metabolite (Alkaloids, steroids, saponins, tannins, reducing sugars, phlobatanins, flavonoids, terpenoids, cardiac glycosides, anthraquinone, emodins and coumarin) [21,31,32]

Test for Alkaloids

About 0.2 g of each of extracts was warm with 2% of H SO for two minutes. The response combination was sieve and added a few droplets of Dragendroff's reagent to all filtrate. Orange red precipitate shows the occurrence of alkaloids moiety.

Test for Tannins

A slight amount of each extract was combined with water and heated on water bath and filtered. A small drops of ferric chloride were added to each filtrate. A dark green solution shows the existence of tannins.

Test for Anthraquinone

0.5 g of individual extract was heated with 10% HCl for a minutes on water bath. The reaction combination was filtered and lets cooling. Equivalent volume of CHCl was added to each filtrate. Small drops of 10% ammonia was dropped to each combination and heated. Rose-pink color creation specifies the presence of anthraquinone.

Test for Glycosides

All Individual extract was hydrolyzed with HCl and neutralized with NaOH solution. A small drops of Fehling's solution were added to each mixture Creation of red precipitate shows the existence of glycosides.

Test for Reducing Sugar

All individual extract was shaken through distilled water and filtered. The remainders were boiled by few drops of Fehling's Solution for a minutes, an orange red precipitate shows the presence of reducing sugars.

Test for Saponins

About 0.2 g of each extract was mixed gently through 5 ml of distilled water and boiled. Foaming presence of creamy miss of minor bubbles indicate the presence of saponins.

Test for Flavonoids

0.2 g of each extract was added in diluted NaOH (0.1 N) and few drops of HCl were added. A yellow solution that turn colorless shows the existence of flavonoids.

Test for Phlobatanins

0.5 g of each extract was added in distilled water and filtered. The filtrate was heated to boil with 2% HCl solution. Red precipitate indicates the existence of phlobatanins.

Test for Steroids

2 ml of acetic anhydride was added to the mixture of 0.5 g of each extract and concentrated H2 SO4 (2 ml). The color alteration from violet to blue or green in certain samples shows the existence of steroids.

Test for Terpenoids

0.2 g of each extract was combined with 2 ml of chloroform and concentrated H2SO4 (3 ml) was wisely poured to create a layer. The creation of a reddish brown coloration at the interface shows positive results for occurrence of terpenoids.

Test for Cardiac Glycosides

2 ml of the crude extracts, 1 ml of glacial acetic acid and 5% ferric chloride was added. A small drops of concentrated H2SO4 were added. Occurrence of greenish blue colour shows the existence of cardiac glycosides.

Test for Coumarins

About 3 ml of 10% NaOH was added to 2ml of each solvent extracts. Formation of yellow colour shows the existence of coumarin.

Test for Emodins

About 2 ml of NH3OH and 3 ml of benzene 4 was added to each crude extracts. Presence of red color shows the presence of emodins.

Antioxidant Assay

The hydrogen atom or electron donation abilities of the corresponding extract were measured from the bleaching of the purple-colored methanol solution of 1, 1-diphenyl-1-picrylhydrazyl DPPH) along with standard querceitin. Experiments were carried out according to the method of Blois [22]. The solution was stand for 30 min in dark and then absorbance was measured at 517 nm. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH solution absorbance shows the rise of the DPPH radical-scavenging action [23]. Scavenging of free radicals with DPPH as percent radical scavenging activities was calculated as follows.

$$\%DPPH = \frac{control\ absorbance - extract\ absorbance}{control\ absorbance}$$

Data Analysis

Mean values of zone of inhibitions were analyzed using ANOVA for significant difference with the help of SPSS version 20 statistical software package in Microsoft windows 7 operating system. The data were further subjected to Tukey's HSD analysis. All the tests were done in triplicates. Data were expressed as mean ± standard deviation and statistical significance was calculated. Values corresponding to $P< 0.05$ were considered as statistically significant.

Results and Discussion

Percentage Yield of Crude Extracts

Methanol crude extracts, chloroform crude extracts and ethanol crude extracts of *Withania Somnifera* leaf, stem barks and roots were obtained from the extraction of 20g powders of the plant parts using ethanol, methanol and chloroform extracting solvents as shown in Table 1. As indicated in the tables, the yield (amount) of the crude extracts ranged from 1.7% to 23.40% from the three parts of *Withania Somnifera*. MCE of the leaves of *W. Somnifera* gave the maximum yield (23.40%) and the lowest yield was obtained from chloroform extract of the stem bark of *W. Somnifera* (1.7%).

The outcome obviously revealed that the percentage yield of the crude extracts of the various plant parts differ from solvent to solvent. This could be attributed to the difference in polarity and extracting potential of methanol, ethanol and chloroform. As Ghosh (2009) reported, most antimicrobial agents that have been identified from plants are soluble in organic solvents and this reveals the better efficiency of methanol, ethanol and chloroform as extracting solvent. As indicated from the table the bioactive ingredients are not found uniformly throughout the plants and that some plant parts tend to have more bioactive compounds than the others [24].

Table 1 The percentage yields of the crude extracts of the leaves, steam barks, and roots of *Withania somnifera* (L.) Dunal

Weight in (g) and Percentage Yield in (%) of Crude Extracts						
Plant part	Ethanol		Methanol		Chloroform	
	Weight (g)	Yield (%)	Weight (g)	Yield (%)	Weight (g)	Yield (%)
Leaf	1.23	12.3	2.34	23.4	1.25	12.5
Stem bark	0.62	6.2	1	10	0.17	1.7
Root	4.82	48.2	0.74	7.4	1	10

Anti -bacterial Activities of Crude Extracts as Measured by the Paper Disc Method

In this study, the anti-bacterial activities of the ethanol, methanol and chloroform crude extracts of the stems, roots and leaves of *W. somnifera* were evaluated using paper disc method. Zone of inhibition formed was measured after incubation and the mean diameters were accomplished.

A total of nine crude extracts (ethanol, methanol and chloroform) were prepared from *W. somnifera* and antibiotics where tested for anti-bacterial activities against the test organisms *(E. coli, S. aureus* and *P. aeruginosa).* The anti-bacterial activities of the different extracts of *W. somnifera* against the three bacterial species are presented in tables 2 – 4.

Anti-bacterial Activities of Crude Extracts of the leaf of *W. somnifera* against the Test Organisms

The antibacterial activities of the crude extracts of the leaf were tested on *E. coli, S. aureus* and *P. aeruginosa.* As indicated in Table 2 below, ethanol leaf extract shows, the highest inhibition zone against *S. aureus* (13.22mm) and chloroform leaf extracts shows, the lowest inhibition zone against *P. aeruginosa* (3.22mm). Table 2 clearly shows that all leaf extracts at 30µl show significant antibacterial activities against all the three tested organisms. The zone of inhibition for *S. aureus* (13.22 mm) was significantly higher ($p<0.05$) than those observed for *E. coli and P. aeruginosa* and this bacteria show more susceptible than the others.

Table 2 Antibacterial activities of crude extracts of the leaf of *Withania somnifera* (L.) Dunal against the test organisms (Mean ± SD, N=3).

Test bacteria	Amount used in (µl)	Zone of inhibition in mm				
		Crude extracts			P. control	N. control
		ELE	MLE	CLE	Chloram. (30µl)	DMSO
S. aureus	30	13.22 ±0.32 bA	10.12±0.12 cC	7.00 ±0.13 dA	20.66±0.22 aA	-
E. coli	30	8.22±0.44 bB	6.23±0.01 Cb	5.21±0.42 cB	10.23±0.23 aB	-
P. aeruginosa	30	5.00±0.12 bC	4.11±0.13 cC	3.22±0.62 dC	6. 11±0.34 aC	-

ELE = Ethanol leaf extract, MLE =Methanol leaf extract, CLE=chloroform leaf extract, chloramephenicol = positive control, DMSO= negative control, n= number of experimental replicates, SD = standard deviation, values with different superscripts on the same row(lower case) and values with different superscripts on the same column(upper case) are significantly different ($p<0.05$) - = no inhibition zone

Table 3 Antibacterial activities of crude extracts of the stem of *Withania somnifera* (L.) Dunal against the test organisms (Mean ± SD, N=3)

Test bacteria	Amount used in (µl)	Zone of inhibition				
		Crude extracts			P. control	N. control
		ESE	MSE	CSE	Chloramphenicol. (30µl)	DMSO
S. aureus	30	12.32 ±0.32 cA	15.02±0.12 bC	6.02 ±0.13 dA	16.06±0.22 aA	-
E. coli	30	9.42±0.44 cB	11.11±0.01 bB	3.00±0.42 dB	12.22±0.23aB	-
P.aeruginosa	30	7.10±0.12 bC	6.13±0.13 cC	3.20±0.62 dC	10. 05±0.34Ac	-

ESE = Ethanol stem extract, MSE =Methanol stem extract, CSE=chloroform stem extract, chloramephenicol = positive control, DMSO= negative control, n= number of experimental replicates, SD = standard deviation, values with different superscripts on the same row(lower case) and values with different superscripts on the same column(upper case) are significantly different ($p<0.05$), - = no inhibition zone

Table 4 Antibacterial activities of crude extracts of the root of *Withania somnifera* (L.) Dunal against the test organisms (Mean ± SD, N=3)

Test bacteria	Amount used in (µl)	Zone of inhibition				
		Crude extracts			P. control	N. control
		ERE	MRE	CRE	Chloram. (30µl)	DMSO
S. aureus	30	12.32 ±0.32 cA	15.02±0.12 bC	6.02 ±0.13 dA	16.06±0.22 aA	-
E. coli	30	9.42±0.44 cB	11.11±0.01 bB	3.01±0.42 dB	12.22±0.23 aB	-
P. aeruginosa	30	7.10±0.12 bC	6.13±0.13 cC	3.20±0.62 dC	10. 05±0.34 Ac	-

ERE = Ethanol leaf extract, MRE =Methanol leaf extract, CRE=chloroform leaf extract, chloramephenicol = positive control, DMSO= negative control, n= number of experimental replicates, SD = standard deviation, values with different superscripts on the same row(lower case) and values with different superscripts on the same column(upper case) are significantly different ($p<0.05$), - = no inhibition zone

Antimicrobial Activities of Crude stem Extract of *W. somnifera* Against the Test Pathogens

The antibacterial activities of the crude extracts of the stem were tested on *E. coli*, *S. aureus* and *P. aeruginosa.* As indicated in Table 3 below, methanol stem extracts show, the highest inhibition zone against *S. aureus* (15.02mm) and chloroform stem extracts show, the lowest inhibition zone against *E. coli* (3.01mm). Table 3 clearly shows that all stem extracts at 30µl show significant antibacterial activities against all the three tested organisms. The zone of inhibition for *S. aureus* (15.01 mm) was significantly higher ($p<0.05$) than those observed for *E. coli and P. aeruginosa* and this bacteria show more susceptible than the others.

Antimicrobial activities of crude extracts of the root of *W. somnifera* against the test organisms

As indicated in Table 4 below, methanol stem extracts show, the highest inhibition zone against *S. aureus* (15.02mm) and chloroform stem extracts show, the lowest inhibition zone against *E. coli* (3.01mm). All stem extracts at 30µl show significant antibacterial activities against all the three tested organisms. The zone of inhibition for *S. aureus* (15.02 mm) was significantly higher ($p<0.05$) than those observed for *E. coli and P. aeruginosa* and this bacteria show more susceptible than the others.

Crude Extracts of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) assay was employed to evaluate the effectiveness of the extracts that showed significant antimicrobial activities in the previous tests. MIC was determined for extracts that showed significant growth inhibition zone at 30µl of stalk solution. The experiment was done by the Agar dilution method. In agar dilution, the stalk solution was serially diluted to get 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.125 mg/ml, 3 mg/ml and 1.5 mg/ml concentrations. Then, each of the three test pathogens were added to the dilute ethanol, methanol and chloroform extracts of concentrations ranging from 1.5 mg/ml up to 50 mg/ml.

Table 5 The minimum inhibitory concentration (MIC) of crude extracts of leaves, stem barks and roots of *W. somnifera* (L.) Dunal *against* the selected bacterial test organisms in mg/ml

Test organisms	plant parts	MIC of the three crude extracts (mg/ml)		
		Ethanol	Methanol	Chloroform
E. coli	Leaf	25	12.5	6.5
	Stem bark	3	3	6.5
	Root	-	-	-
S. aureus	Leaf	1.5	1.5	6.5
	Stem bark	3	3	3
	Root	12.5	12.5	12.5
P. aeruginosa	Leaf	25	12.5	12.5
	Stem bark	25	12.5	50
	Root	-	-	-

The results are shown in table 5. The MIC obtained from *W. somnifera* using Agar dilution method for different part extracts are shown in Table 5 as follows. The MIC value of the different extracts of *W. somnifera* indicated that the highest activity was recorded against *S. aureus* (1.5 mg/ml) in ethanol and methanol extracts of the leaf (Table 5). The lowest activities were obtained against stem chloroform extracts against *P. aeruginosa* (50ml/gm).

In general there are many factors, including the plant part, geographical source, soil conditions, harvest time, moisture content, drying method, storage conditions and post-harvest processing which effect type and level of biological activity exhibited by any plant material. For example, during grinding the relatively high temperatures can denature chemical constituents and the extraction solvent, secondary metabolites extracted from plants solvent tissue can be affected by temperature and time period [25].

Natural material as a source of new antibacterial *E. coli* and *P. aeruginosa* shows more resistant than *S. aureus.* According to many reports, against microorganisms traditional herbs show effectiveness, so the base of modern medicine is plants [26]. The current study was conducted to investigate the antimicrobial properties of one medicinal plant i.e. common human pathogenic bacteria including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Among all the selected plants *W. somnifera* showed increased zone of inhibition against *S. aureus.* Against other tested microorganisms we also observed that the *W. somnifera* activity was optimal but minimum activity was observed against *P. aeruginosa*. This finding in agreement with previous work conducted by [27] which reported that ethanolic extract of *W. Somnifera* leaves showed antibacterial activities against bacterial strains including the *S. aureus* our findings are in line with his observation. *W. somnifera* showed higher antimicrobial activity. Our finding is supported by these results and evaluates the traditional use of the plant in therapeutic use against microbial infection.

Phytochemical Profiling of stem bark, leaf and root of *W. somnifera*

The preliminary phytochemical screening of the stem, leaf and root of *W. somnifera* revealed the presence of bioactive secondary metabolite alkaloids, saponins, glycosides, steroids, terpenoids, cardiac, emodins, tannins, phlobatanins, Anthraquinin, flavonoids, coumarins and reducing sugar (Table 6). These bioactive secondary metabolites showed the medicinal value of *W. somnifera* which is used to treat different human causing bacteria.

Table 6 Qualitative analysis of Phytochemical Profiling of stem bark, leaf and root of *Withania somnifera* (L.) Dunal**.**

Name of phytochemicals	Extract	Plant Parts		
		Leaf	Root	Stem bark
Test for Alkaloids	Ethanol	+	-	+
	Methanol	+	+	+
	Chloroform	+	-	+
Test for Saponins	Ethanol	+	-	+
	Methanol	+	-	-
	Chloroform	-	-	-
Test for Tannins	Ethanol	+	+	+
	Methanol	+	-	+
	Chloroform	-	-	+
Test for Steroids	Ethanol	+	+	+
	Methanol	+	-	-
	Chloroform	-	-	-
Test for Cardiac	Ethanol	+	+	-
	Methanol	+	+	-
	Chloroform	+	+	+
Test for Glycoside	Ethanol	-	+	-
	Methanol	+	+	-
	Chloroform	-	-	-
Test for Flavonoids	Ethanol	+	+	+
	Methanol	-	-	-
	Chloroform	-	-	-
Test for Anthraquinone	Ethanol	+	+	-
	Methanol	-	-	-
	Chloroform	-	-	-
Test for Reducing sugar	Ethanol	+	+	-
	Methanol	+	+	-
	Chloroform	+	+	-
Test for phlobatanins	Ethanol	+	+	+
	Methanol	-	-	+
	Chloroform	-		
Test for Terpenoids	Ethanol	+	+	+
	Methanol	+	+	+
	Chloroform	+	+	+
Test for Emodins	Ethanol	-	-	+
	Methanol	+	-	-
	chloroform	-	-	-
Test for Coumarins	Ethanol	+	-	+
	Methanol	-	-	-
	chloroform	-	-	-

Antioxidant Assay

Table 7 DPPH radical scavenging activities of leaf crude extract of *Withania somnifera* (L.) Dunal *for* Antioxidant Assay

Ethanol Extract		Methanol Extract		Chloroform Extract	
Conc. (µg/ml)	%DPPH	Conc. (µg/ml)	%DPPH	Conc. (µg/ml)	%DPPH
10	1.68	10	2.64	10	1.62
20	2.44	20	6.68	20	2.42
30	6.66	30	12.24	30	4.42
40	18.8	40	14.42	40	16.62
50	26.34	50	19.68	50	18.88
60	44.34	60	48.82	60	22.22
70	48.44	70	66.42	70	52.14
80	50.88	80	70.04	80	62.24
90	64.56	90	72.42	90	66.62
100	72.25	100	74.24	100	68.44

Table 8 DPPH radical scavenging activities of stem bark crude extract of *Withania somnifera* (L.) Dunal *for* Antioxidant Assay

Ethanol Extract		Methanol Extract		Chloroform Extract	
Conc. (µg/ml)	%DPPH	Conc. (µg/ml)	%DPPH	Conc. (µg/ml)	%DPPH
10	1.22	10	2.44	10	1.12
20	2.24	20	7.48	20	2.24
30	4.42	30	8.94	30	3.42
40	16.42	40	12.42	40	4.44
50	18.34	50	22.68	50	34.82
60	34.42	60	44.22	60	38.34
70	42.42	70	48.42	70	46.44
80	54.44	80	60.00	80	54.32
90	58.6	90	62.42	90	70.42
100	72.22	100	64.24	100	66.67

Table 9 DPPH radical scavenging activities of root crude extract of *Withania somnifera* (L.) Dunal *for* Antioxidant Assay

Ethanol Extract		Methanol Extract		Chloroform Extract	
Conc. (µg/ml)	%DPPH	Conc. (µg/ml)	%DPPH	Conc. (µg/ml)	%DPPH
10	2.24	10	1.46	10	1.24
20	3.32	20	4.22	20	2.26
30	4.32	30	8.22	30	4.54
40	14.42	40	18.12	40	16.44
50	46.60	50	24.22	50	24.42
60	50.00	60	38.44	60	34.42
70	56.66	70	56.24	70	64.44
80	66.64	80	62.12	80	68.22
90	76.66	90	68.22	90	71.34
100	80.84	100	72.22	100	78.78

The preliminary phytochemical screening of the stem, leaf and root of *W. somnifera* revealed the presence of bioactive secondary metabolite alkaloids, saponins, glycosides, steroids, terpenoids, cardiac, emodins, tannins, phlobatanins, Anthraquinin, flavonoids, coumarins and reducing sugar (Table 6). These bioactive secondary metabolites showed the medicinal value of *W. somnifera* which is used to treat different human causing bacteria. DPPH radical scavenging assay was done to check the antioxidant activity. Different crude extracts of *Withania somnifera* showed activity at different level (Table 7-9). The highest anti radical activity was found in methanol crude extracts and ethanol crude extracts of leaf, which is almost near to the activity shown by standard querceitin. Chloroform crude extracts of leaf showed moderate activity. The low anti radical activity was found in methanol crude extracts and ethanol crude extracts of stem bark. The low anti radical activity was found in methanol crude extracts and ethanol crude extracts of Root. While chloroform crude extracts of root was the least active among entire solvents. The present finding showed that the leaf, stem bark and root of *Withania somnifera* can be taken in good quantity in order to reduce the risk of various types of diseases causes due to free radicals. This finding also in lies with the work of [28, 29]. Free radical damage of nervous tissue may contribute to neuronal loss in cerebral ischemia and may be involved in normal aging neurodegenerative diseases, e.g. especially schizophrenia, Parkinson's, Alzheimer's and other diseases [30]. The pharmacological activity of *W. somnifera* was confirmed from the antimicrobial and antioxidant assay of crude extract various solvents.

Conclusion

In general, the antimicrobial performance of the three parts of *W. somnifera* crude extracts by using different organic solvents was confirmed on different gram negative and gram positive bacteria (Table 2, 3, 4). The antioxidant activity (Table 7, 8, 9) and percentage yields (Table 1) also carried out and showed positive results specially Ethanol and Methanol crude extracts of all plant parts. The phytochemical test result also revealed the presence of different bioactive compounds (Table 6). Therefore, we can confirm that based on the result obtained, *W. somnifera* could prove to be a good natural source of a potent antimicrobial and antioxidant agent.

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Author's Contribution

All authors have worked equally for this work.

Conflict of Interest

Authors have declared that no conflict of interest is linked with this work

References

1. Doughari, JH, AM. El-mahmood and I. Tyoyina. Antimicrobial activity of leaf extracts of Senna obstusifolia. African J Pharmacy Pharmaco. 2008;2:344-346.
2. Abouzid SF, Bassuony AA, Nasib A, Khan S, Qureshi J, Choudhary MI. Withaferin a production by root cultures of withania coagulans. 2010, pp. 762-769.
3. Tegos G, Frank R, Stermitz L, Olga Kim L. Multidrug Pump Inhibitors Uncover Remarkable Activity of Plant Antimicrobials Antimicrobial Agents and Chemotherapy. 2002;46:3133-3141.
4. Selvamohan T, Ramadas VS, Shibla SK. Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria. J Pelagia Res Library. 2012;3:3374-3381.
5. Yirgashewa A, Ameha K, Dejene Z. In-vitro Antimicrobial Activities and Phytochemical Screening of Selected Plant Extracts against Some Medically and Agriculturally Important Pathogens. 2020;31:167-189.
6. Muskazli M, Nurhafiza Y, Nor A, Dalilah EN. Comparative study on the in vitro Antibacterial, Efficacy of Aqueous and Methanolic Extracts of Quercus infectoria Gall's Aganist Cellulosimicrobium cellulans. J Biological Sci. 2008;4:47-50.
7. Samy RP, Ignacimuthu S. Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats o India. J Ethno Pharmacol. 2000;69:63-71.
8. Ahmad I, Beg AZ. Antimicrobial and on 45 phytochemical studies Indian Medicinal plants against multi-drug resistant human Pathogens Ethnopharmacol. 2017;4:113-123.
9. Nagham DR, Mahmood FI. Study effect of medical plant extracts in comparison with antibiotic against bacteria. J Sci Innovative Res. 2011;2:843-845.
10. Swaminathan. Int J of Chem Tech Res. 2019;12:218-222.
11. Devmurari, VP. and NP. Jivani. Annals of Biological Res. 2010;1:10-14.
12. Thamacian Arulappan M, John Britto S, Ruchmani K, Mohan Kumar R. Antimicrobial and Antifungal activities of W. Somnifera(1.f.) sond against human pathogens. Int J Development Res, 2015;5: 3858-3859.
13. Thamacin Arulappan S. Britto J, Igance Iindo K. GC-MS analysis of ormocarpumsennoides (willd) dc. (Leaf) and bauhinia tomentose 1. (Leaf). European J Phytochem Compounds Present in W. Somnifera (1.f.) sond. (Tuber), of Biomed Pharma Sci. 2014; 2: 324-342.
14. Al-Hindawi MK, Al-Khafaji SH, Abdul-Nabi MH. Antigranuloma activity of Iraqi Withania somnifera. J Ethnopharmaco. 1992;l37:113-116.
15. Anbalagan K, Sadique J. Role of prostaglandins in acute phase proteins in inflammation. Biochem Med. 1984;31:236-245.
16. Anbalagan K, Sadique J. Influence of an Indian medicine (Ashwagandha) on acutephase reactants in inflammation Indian J Exp Biol. 1981;19:245-249.
17. Andallu B, Radhika B. Hypoglycemic, diuretic and hypocholesterolemic effect of winter cherry (Withania somnifera) root. Indian J Exp Biol. 2003;8:607-609.
18. Anonymous. Monograph: Withania somnifera. Altern Med, Rev. 2004;9:211-214.
19. Bhattacharya SK, Muruganandam AV. Adaptogenic activity of Withania somnifera: an experimental study using a rat model of chronic stress. Pharmacol Biochem Behav. 2003;75:547-555.
20. Dhuley JN. Nootropic-like effect of Ashwagandha (Withania somnifera). Phytother, Res. 2001;15:311-318.
21. Grigorescu M, E, Stanescu U. New data referring to chemistry of Withania somnifera species. Rev Med Chir Soc Med Nat Iasi. 1990;94:385-387.
22. Ghias Uddin, Sumaira Gul and Abdur Rauf. Preliminary Phytochemical Screening, in vitro Antimicrobial and Antioxidant Evaluation of Withania somnifera Dunal. World Applied Sci J. 2013;27:562-565.
23. Sharma H, Parihar L, Parihar P. Review on cancer and anticancerous properties of some medicinal plants. J Med Plants Res. 2011;5:1818-1835.
24. Joy P, Thomas J, Mathew S, Skaria B. Medical Plants. Page 55. Aromatic and Kerala Medicinal Plants Research Station, Kerala Agricultural University, India. 1998.
25. Chaurasia S, Panda S, Kar A. Withania somnifera Root Extract in The Regulation of Lead-Induced Oxidative Damage in Male Mouse. Pharmacological Res. 2000; 41: 6.
26. Bhatia R, Narain JP. The growing challenge of antimicrobial resistance in the South East Asia Region-are us losing the battle? Indian J Med Res. 2010; 132: 482-486.
27. Coates A, Hu Y, Bax R, Page C. The future challenges facing the development of new antimicrobial drugs. Nature Reviews Drug Discovery. 2002;1:895-910.
28. Giamarellou H. Multidrug-resistant Gram-negative bacteria: how to treat and for how long. Int J Antimicrobial Agents. 2010;36: S50–S54.
29. Savithramma, N., M. lingarao and D. Suhrulatha. Screening of medicinal plant for secondary metabolites. Middle-East J. Sci. Res. 2011;8:579-584.
30. Boucher HW, Talbot GH, Bradley JS, *et al*. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clinical Infectious Diseases. 2009;48:1-12.
31. Sofowora A. Medicinal Plants and Traditional Medicinal in Africa. Sunshine House, Ibadan, Nigeria, 1993, pp. 134-156.
32. Harborne JB. Phytochemical methods: A guide to modern technique of plant analysis, Champ man and Hall, London. 1998.