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Comparative Study on the Antibacterial Activity of Four Medicinal Plants Leaves of Different Ages

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ABSTRACT: *Plants are the richest source of natural antimicrobial agents. The present study was carried out to evaluate the antibacterial effect of four important plants namely, Rauvolfia serpentina, Tagetes erecta, Brassica nigra, Ocimum tenuiflorum. Powdered leaf materials of all selected plants were extracted with methanol. The solvent extracts were evaporated for dryness using rotary evaporator. Dry residue was dissolved in methanol (1:10 w/v) and different volume of tested sample is applied for antibacterial activity. The antibacterial screening of the selected crude methanolic extracts were determined according to Kirby-Bauer's Disc diffusion method on the following bacteria- Staphylococcus aureus, Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa. The experiment showed that R. serpentina exhibited excellent antibacterial activity against tested bacterial organisms as compared to the standard Norfloxacin. Maximum of 21.60 ±0.40 mm zone of inhibition was recorded against gram positive bacteria S. aureus for the leaves aged >30 days (100µl) of R. serpentine and maximum of 18.90 ±0.84 mm was recorded for the leaves of T. erectra aged >30 days (100µg), whereas the least inhibitory zone was found 7.80± 0.45 mm for the leaves aged 5-15 days (15 µl). Again, maximum zone of inhibition 28.12±0.40 mm was found for the positive control, Norfloxacin. This result revealed that due to the increases of ages, antimicrobial activities of leaves also increases. S. typhi showed no sensitivity for any of the tested plant leaves extracts, whereas other gram-negative bacteria showed some degree of susceptibility. E. coli showed excellent susceptibility against R. serpentina. P.aeruginosa showed susceptibility against O.tenuiflorum. Although, it is resistant against the other leaves extracts. B. nigra didn't show any antimicrobial activity. Our results demonstrated that methanol extracts of these plants leaves have age and concentration dependent antibacterial activity against some of the tested organisms. Further studies should be undertaken to elucidate the exact mechanism of action of antimicrobial effect to identify the active ingredients which can be used in drug development program.*

KEY WORDS: *Brassica nigra, Ocimum tenuiflorum, Rauvolfia serpentina, Tagetes erecta, Zone of inhibition.*

I. INTRODUCTION

Human infections particularly those involving microorganism i.e. bacteria, fungus, viruses; they causes serious infections in tropical and subtropical countries of the world. In recent years, multiple drug resistance in human pathogenic microorganism has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of such diseases [1,2]. Plants are the richest source of natural antimicrobial agents. Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics [3]. Biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens [4]. Different extracts from traditional medicinal plants have been tested. Many reports have show the effectiveness of traditional herbs against microorganisms, as a result, plants are one of the bedrocks for modern medicine to attain new principles [5]. Until natural products have been approved as new antibacterial drugs, there is an urgent need to identify novel substances active towards highly resistant pathogens [6,7]. The medicinal plants are widely used because of its easy availability and cost effectiveness. The active principles of many drugs found in plants are secondary metabolites. The antimicrobial activities of plant extracts may reside in a variety of different components, including aldehyde and phenolic compounds [8]. Again, Scalbert review revealed that tannins can be toxic to filamentous fungi, yeasts, and bacteria. Condensed tannins have been determined to bind cell walls of ruminal bacteria, preventing growth and protease activity [9]. Alkaloid and its derivatives have activities against *Staphylococcus aureus* and methicillin-resistant *S. aureus* [10].

In our study, we observed the comparative study on the antibacterial effeicacy of the methanolic extract of leaves of four medicinal plants; *Rauvolfia serpentina* belonging to the family of Apocynaceae which is native to the Indian subcontinent and East Asia [11]; *Tagetes erecta* belonging to the family of Asteraceae is

native to the America, it is often called African marigold.; *Brassica nigra* belonging to the family of Brassicaceae is native to the southern mediterranean region of Europe and possibly South Asia where it has been cultivated for thousands of years; *Ocimum tenuiflorum* also known as holy basil, or tulasi, is an aromatic plant in the family Lamiaceae which is native to South Asia and widespread as a cultivated plant throughout the Eastern World tropics [12]. These plants are medicinally important and few of these are used for different purpose which is already established. For example- *Rauvolfia serpentina* extract has been used for the treatment of fever, anxiety, epilepsy, snake bite, rheumatism, insanity, eczema, intestinal disorders, psychiatric disorders, nervous disorders, cardiovascular disorder, bacterial infections and in the management of hypertension schizophrenia [13-15]. The objective of this research was to evaluate the potentiality of plant extracts on standard microorganism strains as well as on the multi-drug resistant bacteria.

II. MATERIALS AND METHODS

2.1 Selection of medicinal plants for the study

Four medicinal plants *Rauvolfia serpentina, Tagetes erecta, Brassica nigra, Ocimum tenuiflorum* were selected based on ethanomedical importance. Healthy, disease free leaves of the selected plants were collected according to the leave's age differences (5-15 days, 15-30 day, > 30 days) from Noakhali district of Bangladesh and later identified by Professor Dr. Md. Jasim Uddin, Taxonomist, Department of Botany, University of Dhaka, Bangladesh.

2.2 Cold extraction

The collected leaves were washed with distilled water and separated from undesirable materials. The washed leaves were categorized according to their species and age differences and dried at room temperature for seven days under shade. After drying the materials were powdered separately by using electric grinder. 50 grams of each category dried powder was filled in the thimble and extracted with 150 ml of methanol (80%) successively up to 48h accompanying occasional shaking and stirring [16]. The mixtures were then undergone a coarse filtration through Whatman filter paper separately. The filtrate obtained were evaporated by rotary evaporator at 5 to 6 rpm and 65ºC temperature. After complete solvent evaporation, one gram of each concentrated solvent extracts were dissolved in 9ml of methanol and stored at refrigerator (4^0 C) for further use.

2.3 Test Microorganisms

Authentic pure cultures of human *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa* were obtained from Department Microbiology, Noakhali Science and Technology university, Bangladesh.

2.4 Inoculum preparation

Ten ml of distilled water was taken into the screw cap tube and pure colony of freshly cultured bacteria of the experimental species were added into the tube and vortex was done. The OD (optical density) was measured with the colorimeter and microbial population was confirmed to be within $10^7 ml^{-1}$ to $10^8 ml^{-1}$ and then plated out as inoculums [17].

2.5 Test solution preparation

One gram of each types of selected plant leaf extracts were dissolved in 9ml of methanol. The sterile nutrient agar medium in petridishes was uniformly smeared with test culture. The test solution is then stored in refrigerator for next use.

2.6 Kirby-Bauer's Disc diffusion method

Antibacterial activities of the selected crude extracts were determined according to Kirby-Bauer's Disc diffusion method (2006) with slight modifications. The Petri dishes were flooded with Mueller Hinton Agar and after solidification of agar 0.1 ml of diluted inoculums were spread over Mueller Hinton Agar in the dishes using sterile L spreader to achieve confluent growth of test organisms and allowed to dry for 10 minutes. The sterile readymade discs loaded with each extract individually (15 µl/disc, 30 µl/disc, 50 µl/disc, 70 µl/disc and 100 µl/disc) were imposed on the inoculated plates. The plates were then incubated at 37^0C for 36 hours. The plates were observed for the zone of inhibition. Zone of inhibition was measured using antibiotic zone scale. Sterile disc with respective solvent (methanol) of 25 µl was used as negative control and Norfloxacin used as positive control.

III. RESULTS AND DISCUSSION

Table 1. In vitro antimicrobial activity of different extracts of experimental plants leaves on the growth of different micro-organisms according to disc diffusion method test.

	R. serpentina,			***T. erecta,***			***B. nigra,***			***O. tenuiflorum***			**Control (Norfloxacin)**
	Leaves aged (5-15 days)	**Leaves aged (15-30 days)**	**Leaves aged > 30 days**	**Leaves aged (5-15 days)**	**Leaves aged (15-30 days)**	**Leaves aged> 30 days**	**Leaves aged (5-15 days)**	**Leaves aged (15-30 days)**	**Leaves aged > 30 days**	**Leaves aged (5-15 days)**	**Leaves aged (15-30 days)**	**Leaves aged> 30 days**	
	Zone of Inhibition (mm) for the species of *Staphylococcus aureus*												
15 µl/disc	9.78 ±0.32	8.55 ±0.75	9.56 ±0.64	7.80 ±0.45	8.50 ±0.78	9.20 ±0.50	-	-	-	-	-	-	16.80 ±0.32
30 µl/disc	12.45 ±0.64	11.62 ±0.35	12.75 ±0.34	11.92 ±0.60	10.50 ±0.86	11.65 ±0.25	-	-	-	-	-	-	18.80 ±0.56
50 µl/disc	14.85 ±0.12	13.65 ±0.48	15.60 ±0.30	12.13 ±0.32	10.95 ±0.48	12.80 ±0.75	-	-	-	-	-	-	20.85 ±0.50
70 µl/disc	18.60 ±0.40	15.72 ±0.44	17.55 ±0.58	13.13 ±0.30	12.65 ±0.64	13.92 ±0.78	-	-	-	-	-	-	25.50 ±0.27
100 µl/disc	20.30 ±0.15	19.50 ±0.58	21.60 ±0.40	16.00 ±0.22	18.00 ±0.55	18. 90 ±0.84	-	-	-	-	-	-	28.12 ±0.40
	Zone of Inhibition (mm) for the species of *Salmonella typhi*												
15 µl/disc	-	-	-	-	-	-	-	-	-	-	-	-	18.70 ±0.50
30 µl/disc	-	-	-	-	-	-	-	-	-	-	-	-	19.12 ±0.24
50 µl/disc	-	-	-	-	-	-	-	-	-	-	-	-	20.64 ±0.72
70 µl/disc	-	-	-	-	-	-	-	-	-	-	-	-	24.45 ±0.70
100 µl/disc	-	-	-	-	-	-	-	-	-	-	-	-	26.46 ±0.35
	Zone of Inhibition (mm) for the species of *Escherichia coli*												
15 µl/disc	8.60 ±0.48	8.14 ±0.30	9.66 ±0.34	-	-	-	-	-	-	-	-	-	17.40 ±0.35
30 µl/disc	10.52 ±0.55	11.55 ±0.52	10.95 ±0.25	-	-	-	-	-	-	-	-	-	18.76 ±0.45
50 µl/disc	12.64 ±0.12	13.40 ±0.48	14.78 ±0.32	-	-	-	-	-	-	-	-	-	23.75 ±0.80
70 µl/disc	15.40 ±0.38	16.85 ±0.35	17.48 ±0.50	-	-	-	-	-	-	-	-	-	27.36 ±0.22
100 µl/disc	19.52 ±0.70	20.38 ±0.25	22.70 ±0.60	-	-	-	-	-	-	-	-	-	29.60 ±0.64
	Zone of Inhibition (mm) for the species of *Pseudomonas aeruginosa*												
15 µl/disc	-	-	-	-	-	-	-	-	-	7.16 ±0.56	8.50 ±0.92	8.76 ±0.25	20.20 ±0.64
30 µl/disc	-	-	-	-	-	-	-	-	-	12.52 ±0.30	14.12 ±0.54	12.80 ±0.42	21.80 ±0.26
50 µl/disc	-	-	-	-	-	-	-	-	-	14.13 ±0.58	15.25 ±0.40	16.80 ±0.20	24.68 ±0.65
70 µl/disc	-	-	-	-	-	-	-	-	-	18.15 ±0.20	19.30 ±0.30	21.36 ±0.72	27.90 ±0.70
100 µl/disc	-	-	-	-	-	-	-	-	-	20.50 ±0.32	22.50 ±0.48	23.75 ±0.86	30.50 ±0.45

Values are the mean of three replicates ± SD (standard error). $P<0.05$; - indicates not to found the zone of inhibition.

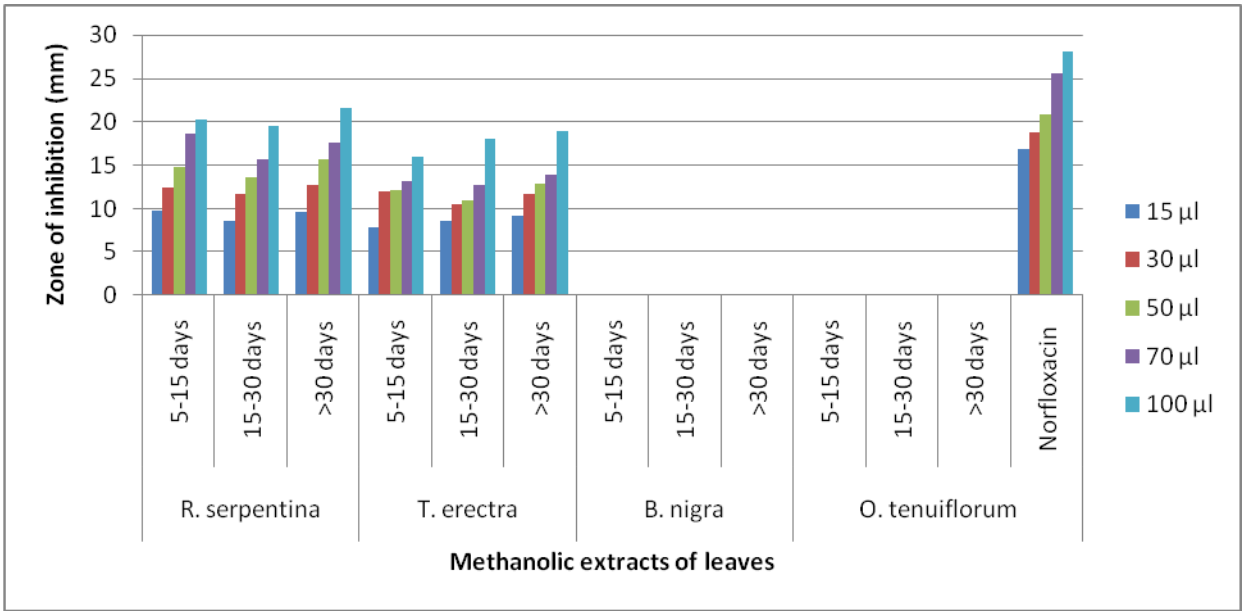

Figure 1. Antibacterial activity of methanolic leaves extracts of selected plants against *S. aureus.*

In vitro antibacterial activities of the methanolic leaves extract of four medicinal plants were screened individually by the presence or absence of zone of inhibition. Fig 1 represents the antibacterial activity of selected plants leaves of different age categories against gram-positive bacteria *S. aureus.* The figure showed that *R. serpentine* and *T. erectra* showed antibacterial activities against *S. aureus*. Although, *B. nigra* and *O. tenuiflorum* did not show antimicrobial activity against *S. aureus*. Maximum of 21.60 ±0.40 mm zone of inhibition was recorded for the leaves aged >30 days (100µl) of *R. serpentina*. This result revealed that due to the increases of ages, antimicrobial activities of leaves also increases. *R. serpentina* contains good amount of reserpine and exhibited strong antibacterial activity against most of the tested human pathogenic bacteria [18]. Perhaps, due to the maturation of the leaves, amount of reserpine also increases. Again, maximum of 18.90 ±0.84 mm was recorded for the leaves of *T. erectra* aged >30 days (100µg), whereas the least inhibitory zone was found 7.80± 0.45 mm for the leaves aged 5-15 days (15 µl). Maximun inhibition 28.12±0.40 mm was found for the positive control, Norfloxacin.

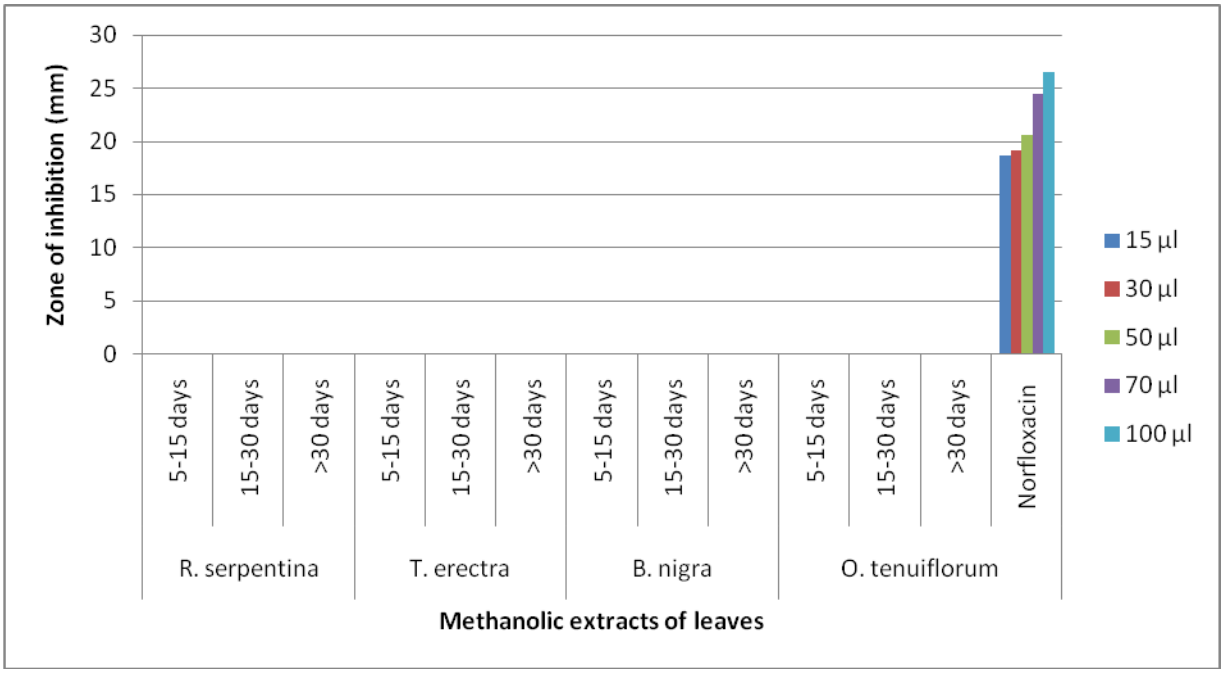

Figure 2. Antibacterial activity of methanolic leaves extracts of selected plants against *S. typhi.*

Fig 2 represents the antibacterial activity of selected plants leaves of different age categories against gram negative bacteria *S. typhi.* The figure showed that none of the leaves extracts of these plants showed antibacterial activities against *S. typhi.*

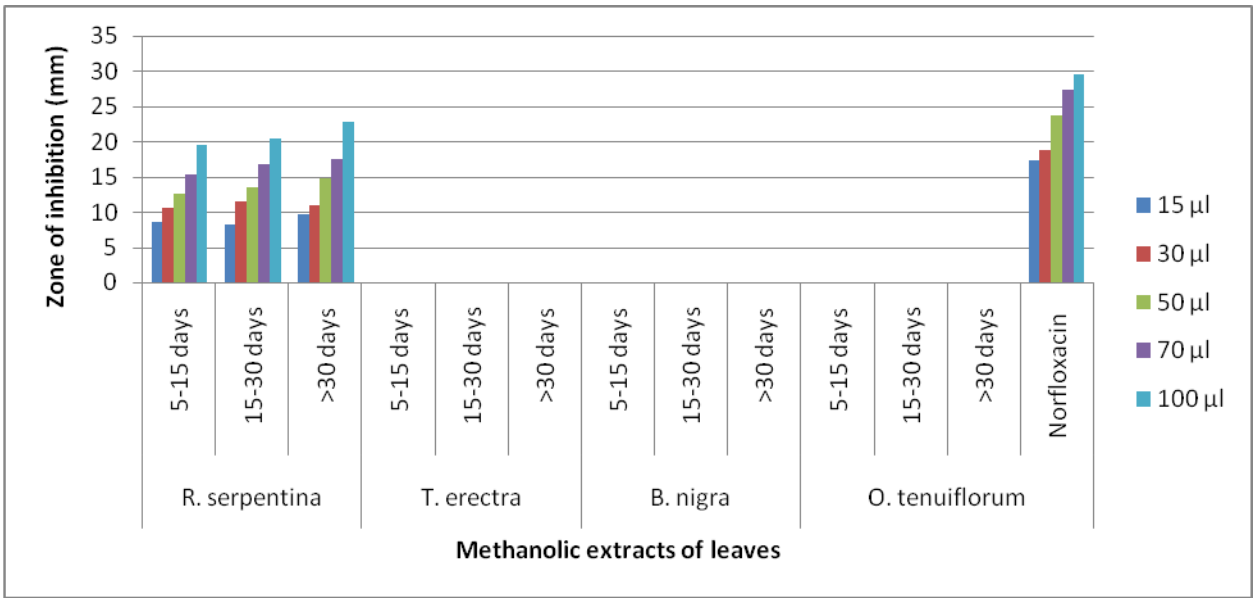

Figure 3. Antibacterial activity of methanolic leaves extracts of selected plants against *E. coli.*

Fig 3 represents the antibacterial activity of selected plants leaves of different age categories against the gram negative bacteria *E. coli.*This figure showed that *R. serpentine* and *O. tenuiflorum* showed antibacterial activities against *E. coli.* Although, *T. erectra* and *B. nigra* didn't show antimicrobial activity against *E. coli.* Maximum of 22.70±0.60 mm(100µl) zone of inhibition was recorded for the leaves aged >30 days of *R. serpentine;* whereas the least inhibitory zone was found 8.14± 0.30 mm for the leaves aged 15-30 days (15 µl). Maximun inhibition 29.60±0.64 mm (100µl) and least inhibition 17.40±0.35 (15µl) were found for the positive control, Norfloxacin.

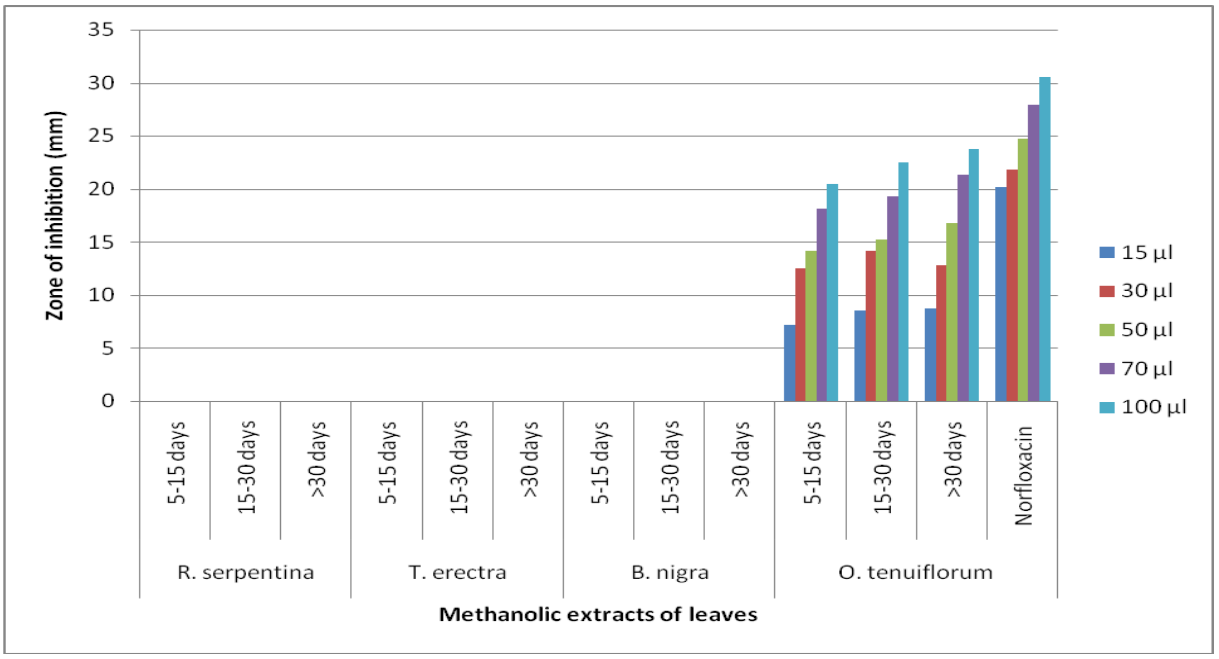

Figure 4. Antibacterial activity of methanolic leaves extracts of selected plants against *P. aeruginosa.*

Fig 4 represents the antibacterial activity of selected plants leaves of different age categories against the gram negative bacteria *P. aeruginosa.* This figure showed that only the leaves extracts of *O.tenuiflorum* showed antibacterial activities against *P. aeruginosa*. Although *R. serpentine, T. erectra* and *B. nigra* didn't show antibacterial activity against *P. aeruginosa.* Maximum of 23.75±0.86mm (100µl) zone of inhibition was recorded for the leaves aged >30 days of *O.tenuiflorum;* whereas the least inhibitory zone was found 7.16±0.56 mm for the leaves aged 5-15days (15 µl). Maximun inhibition 30.50±0.45 mm (100µl) and least inhibition 20.20±0.64 (15µl) were found for the positive control, Norfloxacin. The antibacterial activity of methanol extracts of selected plants leaves against human pathogenic bacteria both Gram-positive and Gram-negative bacteria are presented in Table 1. After analyzing the above results, it is clear that gram positive bacteria *S. aureus* is more susceptible than other experimental species of gram negative bacteria- *E. coli, S. typhi, P. aeroginosa*. The methanol extracts of *R. serpentina* exhibited excellent antibacterial activity against tested bacterial organisms as compared to the standard norfloxacin. This result supports the previous claim of [19]. *S. typhi* showed no sensitivity for all tested plant leaves extracts, whereas other Gram-negative bacteria showed some degree of susceptible for other plants leaves extracts. *E. coli* showed excellent susceptibility against *R. serpentina. P. aeruginosa* showed susceptibility against *O. tenuiflorum*. Although, it is resistant against the other leaves extracts.This implied that the gram-positive bacteria were more susceptible to the extract than the gram-negative bacteria. Possibly because of the presence of outer membrane that serves as an effective barrier in gram negative species [20,21].

IV. CONCLUSION

From this study it is concluded that among the leaves of four plants, *R. serpentine* possess greater antibacterial activity against tested human pathogenic bacteria than other plant's leaves. *T. erecta, O. tenuiflorum* showed moderate antimicrobial activity; whereas *B. nigra* didn't show any antimicrobial activity. Our results demonstrated that methanol extracts of selected plants leaves have age and concentration dependent antibacterial activity against some of the tested organisms. The results obtained by this study cannot be directly extrapolated to human; further studies should be undertaken to elucidate the exact mechanism of action by which extracts exert their antimicrobial effect to identify the active ingredients which can be used in drug development program for safe health care services.

V. ACKNOWLEDGMENTS

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VI. CONFLICTS OF INTEREST

All authors have none to declare.

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