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Abstract: Antimicrobial activity of some medicinal plants traditionally used by local communities in the treatment of tuberculosis and other respiratory diseases was carried out. The antimicrobial activity of the plants extracts against some microbial pathogens was investigated as preliminary study to screen the plants for their anti-tuberculosis activity. The antimicrobial test was done using agar disc diffusion method and the minimum inhibitory concentration (MIC) was also determined by Micro broth dilution method. The result indicated that the extracts had varied activity against the tested organisms. The extracts also showed dose dependent antimicrobial activity but significantly lower than the standard antibiotic drugs (ciprofloxacin and Ketoconazole). The extract of E. hirta has no activity against E. coli at the tested concentrations but shown the zone of inhibition range from 10-16mm for other tested organisms. E. hirta has MIC of 15µg/ml against K. pneumonia and C.albicans and 30mg/ml for B.subtilis, S. typhi and S aureus. The extract of C. mucronata has shown zone of inhibition range from 12-16mm but it has no activity against B. subtilis at the tested concentrations. It has MIC of 15µg/ml against C.albicans, B. subtilis, K.pneumonia, E.coli and 30µg/ml against S. aureus. The stem bark extract of X. americana has shown the zone of inhibition range of 10-16mm and active against all the tested organisms. It has MIC of 15µg/ml against S. aureus and K. pneumonia. The leaf extract of W. indica has shown zone of inhibition ranging from 11-16mm. It was active against all the tested pathogens and exhibited MIC of 15µg/ml against S. aureus and C. albicans. The leaf extract of P. reticulatum has shown the zone of inhibition ranges from 9-15mm against the tested organisms. It exhibited MIC of 15µg/ml against S. typhi. The aerial parts extract of S. hermonthica has shown zone of inhibition ranges from 12-16mm. The extract has shown the zone of inhibition (12, 14 and 16mm) against C. albicans at the concentration of 15, 30 and 60 µg/ml respectively. But the extract has no activity against S. typhi at the tested concentrations. The extract has MIC of 15μ g/ml against B. subtilis and C. albicans. The extract of T. indica has shown the zone of inhibition ranges from 10-15mm. But the extract has no activity against C. albicans at all tested concentrations. It has MIC of 15µg/ml against K. pneumonia and E.coli. The extract of E. senegalensis has shown the zone of inhibition ranging from 11-14mm. However the extract has no activity at the lowest concentration of 15µg/ml against all the organisms except S. aureus. The extract has MIC of 15µg/ml against S. aureus and 30µg/ml against all other organisms tested. The leaf extract of B. paradoxum has shown a zone of inhibition of 16mm against E. coli and zone of inhibition ranges from 11-16mm. The MIC of the extract against E. coli, S. typhi and B. subtilis is 15µg/ml and 30µg/ml for C. albicans. However the extract has no activity at all tested concentrations against S. aureus and k. pneumonia. The extracts that exhibited zones of inhibition greater than 10mm were considered active and therefore recommended for further screening to isolate and characterize the bioactive chemical constituents.

Keywords: *Methanol extracts, antimicrobial activity, MIC, medicinal plants.*

1. INTRODUCTION

Despite the influence of modern drugs the use of medicinal plants has remained a very important aspect of health care delivery system especially in rural areas. It was reported that 80% of world's population relies on traditional medicines as primary source of their medication (Newman, 2006). The plant based preparations were central to traditional practice of health care system where herbs and different parts of medicinal plants are employed. Medicinal plants have some medicinal values based on folkloric usage and information. It was reported that plants are natural reservoir of therapeutic agents use by the local communities in the treatment of many diseases (Akinniyi and Tella, 1991). The clinical success of plant-based drugs has rekindled interest in research into medicinal plants as potential sources of new drugs. In some countries like China, India and Vietnam the research in to

medicinal plants has been fully developed and plant-based remedies have been incorporated as alternative or complementary medicines to supplement the modern drugs. However, in Nigeria like in most African countries the research into medicinal plants has not been given a desire attention as such the therapeutic potentials of these natural endowments were under-utilized (Ogundaini, 2005).

In fact, the phyto-chemical agents in medicinal plants were shown to be responsible for the observed therapeutic activity against the diseases caused by pathogenic infections. There were several plant extractives that demonstrated significant inhibitory activity against microscopic pathogens like bacteria, fungi and viruses (Malcolm and Sofowora, 1969; Kone *et al.*, 2004; Sanogo, 2005; Hamza and Mbwabo, 2006; Kubmarawa *et al.*, 2007; Tanaka *et al.*, 2010). The screenings of medicinal plants for antimicrobial activity have shown that higher plants represent a potential source of novel antibiotic chemotypes (Copp, 2003; Katerere *et al.*, 2003; Ogundaini, 2005; Kubmarawa *et al.*, 2007). The plants were screened for the presence of bioactive chemical constituents and it was found that they contained phytochemical constituents like alkaloids, flavonois, sterois, terpenoids, glycosides, anthraquinones, saponins and tannins (Aska and Kubmarawa, 2016).

In the present study, we are reporting the antimicrobial activity of some selected medicinal plants used in the treatment of tuberculosis and related respiratory diseases in Northern parts of Bauchi state, Nigeria.

2. MATERIALS AND METHODS

2.1. Plant Material

The different parts of the selected medicinal plants were collected from Three (3) Local Government Areas in the Northern parts of Bauchi State, Nigeria using standard method. The freshly collected plant samples were conveyed in clean polythene bags to Biology Department, Abubakar Tafawa Balewa University, Bauchi for identification by a taxonomist who identified and classified the plants specimens and voucher specimens was prepared and deposited in the Departmental Herbarium (Abdalfatah, *et al.*, 2013; Zailani, *et al.*, 2010; Mann, *et al.*, 2008a; Kubmarawa, *et al.*, 2007).

2.2. Drying and Pulverising

The samples of different parts of the plants were dried under shade inside a room. The fresh samples were spread out and turned regularly to dry them properly. The well dried samples were pulverized to fine powder using a wooden mortar and pestle. The powder of each sample was weighed using analytical balance and kept at room temperature until use (Ibrahim, et al., 2012).

2.3. Extraction

The plants samples were subjected to exhaustive extraction using soxhlet extractor. One hundred grams (100g) of each sample in 70% Methanol was used in the extraction process which lasted for 12hrs. The crude extract of each sample was filtered and concentrated in an oven at 40°c. The dried methanol extracts were then packed in glass bottles with proper labeling for future use (Trease and Evans, 1989).

2.4. Extracts Preparation

The different concentrations (15, 30 and 60μ g/ml) were prepared from 5g each of the crude methanol extracts of the plant samples. These concentrations were used to test the antimicrobial activity of the crude extracts.

2.5. Microorganisms Tested

Micro-organisms tested are Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Salmonella typhi, Bacillus subtilis and Candida albicans. The clinical isolates were obtained from Mallam Aminu Kano Teaching Hospital Kano, Nigeria. The micro-organisms were grown and cultured in microbiology Laboratory of Bayero University Kano (BUK) Nigeria.

2.6. Antimicrobial Susceptibility Test

The Agar disc diffusion method was used to evaluate the antimicrobial activity of the plants extracts (Mangoma, et al., 2010, Atikya et al., 2014). The bacteria were cultured in nutrient agar medium at 37°C for 24hours. The fungal was cultured on dextrose agar medium inoculated at 25°C for 7 days. Ciprofloxacin and ketoconazole were used as standard reference antibiotic drugs for bacterial and fungal respectively. The agar discs were impregnated with the different concentrations (60, 30 and 15 μ g/ml) of the plants extracts. The inoculated medium was poured into sterile Petri dishes and

incubated at 25°C for 5 days. Ciprofloxacin ($30\mu g/ml$) and ketoconazole ($30\mu g/ml$) was impregnated into the disc as positive control for the bacteria and Candida albicans respectively. The disc impregnated with Dimethylsulphoxide(DMSO)(4%) and methanol(95% JHD) were used as negative control. All the Petri dishes were incubated at 37°C for 24 hours, after incubation the discs were observed for growth inhibitions against the tested microorganisms by the tested extracts. The diameters of inhibition were measured and recorded in Millimeters (mm).

2.7. MIC Determination

The minimum inhibitory concentration (MIC) was determined by Micro broth dilution method (Okwute, *et al* 2017). Prepared Muller Hinton broth was dispersed into test tubes and sterilized with autoclave at 121°C for 15mins then the broth was allowed to cool. Two fold serial dilutions were used to obtain concentrations of $60\mu/ml$, $30\mu/ml$, $15\mu/ml$, $7.5\mu g/ml$ and $3.75\mu g/ml$ of the crude extracts. $60\mu g/ml$ was initially made by dissolving 0.06mg of the crude extract in 10ml of DMSO to obtain $60\mu g/ml$ from which subsequent concentrations were prepared using two fold dilutions. Methanol (70%) was used as a negative control. The microorganisms sensitive to the tested extracts were inoculated and incubated for 24 hrs at 37°C and the lowest concentration of the extracts that showed no visible growth was recorded as the minimum inhibitory concentration (MIC).

3. RESULTS OBTAINED

Test	Conc(µg/ml) <i>P. reticulatum</i>			Conc(µg/ml) S. <i>hermonthica</i>			Conc(µg/ml) <i>T. indica</i>			Conc(µg/ml)			Conc(µg/ml)		
organisms used			(mm)			(mm)			E. Senegalensis (mm)			V. paradoxa (mm)			
useu	(mm) 60 30 15		60 <u>30</u>		60 30 15		60 30 15		60 30		/				
	00	30	15	15		00	30	15	00	50 15		15			
				15									15		
S. Aureus	13	10	NA	14	12	NA	13	9	NA	12	10	9	NA	NA	NA
E. Coli	10	NA	NA	12	9	NA	15	12	10	13	12	NA	16	14	11
B. Subtilis	13	9	NA	15	13	11	11	9	NA	14	11	NA	14	12	10
K.Pneumonia	14	11	NA	11	9	NA	14	11	9	12	10	NA	NA	NA	NA
S. Typhi	15	12	9	NA	NA	NA	12	11	NA	13	10	NA	15	12	9
C. Albicans	13	11	NA	16	14	12	NA	NA	NA	10	9	NA	11	10	NA

 Table1a. Antimicrobial sensitivity test of crude methanol extracts of the samples

*NA= NO ACTIVITY *NT= NOT TESTED

Table1b. Result of Antimicrobial sensitivity test of crude methanol extracts of the samples

Test				CONC(µg/ml) C. mucronata.			CONC(µg/ml) X. americana			CONC(µg/l) W. indica			Ciprofl oxacin	Ketoco nazole
organisms used	(mm)			(mm)			(mm)			(mm)			(30 /ml)	(30 /ml)
	60	30	15	60	30	15	60	30	15	60	3	15	(mm)	(mm)
S. Aureus	12	11	NA	13	10	NA	16	14	10	16	12	11	33	NT
E. Coli	NA	NA	NA	16	14	12	14	10	NA	14	11	NA	28	NT
B. Subtilis	14	10	NA	NA	NA	NA	12	9	NA	12	9	NA	31	NT
<i>K</i> .	16	13	10	12	10	9	15	12	9	11	10	NA	25	NT
Pneumonia														
S. Typhi	11	9	NA	NA	10	8	13	9	NA	10	9	NA	22	NT
C. Albicans	12	10	9	14	10	9	11	N	NA	11	10	9	NT	28
								Α						

*NA= NO ACTIVITY NT= NOT TESTED

Table2. Result of minimum inhibitory concentration (MIC) of the crude extracts

Plant	Sample used	MIC (µg/ml)								
		Sa	St	Кр	Ec	Bs	Ca			
Waltheria indica	Leaf extract	15	30	30	30	30	15			
Ximenia Americana	Stem bark extract	15	30	15	30	30	60			
Pilostigma	Leaf extract	30	15	30	60	30	30			
reticulatum										
Striga hermonthica	Aerial part extract	30	-	30	30	15	15			
Vitellaria paradoxa	Leaf extract	-	15	-	15	15	30			
Cissampelos ucronata	Root extract	30	15	15	15	-	15			
Euphorbia hirta	Whole plant extract	30	30	15	-	30	15			
Tamarindus indica	Root-bark extract	30	30	15	15	30	-			
Erythrina	Stem-bark extract	15	30	30	30	30	30			
senegalensis										

KEY:Sa=Staphylococcus aureus, St = Salmonella typhi,Ec = Escherichia coli, Kp = Klebsiella pneumonia,

Bs = Bacillus subtilis, Ca = Candida albicans.

3.1. Discussion

Antimicrobial activity of the plants samples were tested against fungal and some bacterial pathogens using agar disc diffusion method (Mangoma, *et al.*, 2010, Atikya, *et al.*, 2014). The result of the antimicrobial sensitivity test was presented in table 1a & b. The minimum inhibitory concentration (MIC) was presented in table 2. The Antimicrobial sensitivity test of crude methanol extracts of the samples indicated that the extracts exhibited varied antimicrobial activity against the tested organisms. The zones of inhibition (mm) ranged from 9-16. The extracts exhibited dose dependent antimicrobial sensitivity test however they were significantly lower than the standard antibiotic drugs ciprofloxacin and Ketoconazole. Ciprofloxacin shown inhibition against the tested organisms with zones range of 22-33mm at the concentration of 30 μ g/ml. Standard antifungal(Ketoconazole) shown inhibition against *C. albicans* with inhibition zone of 28mm at the concentration of 30 μ g/ml.

The whole plant extract of E. hirta has shown a greater zone of inhibition (16mm) against K. Pneumonia, the zone of inhibition ranges from 10- 16mm. The extract has shown the zone of inhibition against C. albicans that ranges from 9-12mm at the concentration of 15, 30 and 60 µg /ml respectively. S. aureus was inhibited with zones of 11-12mm for 30 and 60 µg /ml. respectively. But the extract has no activity against S. aureus at the lowest concentration (15 µg /ml) of test extract. E. hirta has zone of inhibition of 10-14mm against B. subtilis at the concentration 30 and 60 µg /ml. respectively. But the extract has no activity against *B. subtilis* at the lowest concentration (15 µg/ml) of test extract. Srilakshmi, et al., (2012) has supported this finding because they reported that the ethanol extracts of E. hirta inhibited the growth of S. aureus, S. typhi and E. coli at 25µg/ml with zones of inhibition of 13.6, 12.8 and 7.2mm respectively. S. typhi was inhibited with zones of 9-11mm for 30 and 60 µg /ml respectively. But the extract has no activity against S. typhi at the lowest concentration (15 µg /ml) of test extract. E. hirta has no activity against E. coli at the tested concentrations. However, this disagreed with the report of Srilakshmi et al., (2012) who reported that the ethanol extracts of E. hirta gave zone of inhibition (7.2mm) against E. coli at 25µg/ml. It might be attributed to differences in environmental factors of location of the sample collection. C. mucronata has shown a greater zone of inhibition (16mm) against E. coli, the zone of inhibition ranges from 12-16mm. The extract has shown the zone of inhibition against K. pneumonia that ranges from 9-12mm at the concentration of 15, 30 and 60 µg/ml. respectively. S. Aureus was inhibited with zones of 10-13mm for 30 and 60 µg /ml. respectively. But the extract has no activity against S. aureus at the lowest concentration (15 µg /ml) of test extract. C. mucronata has zone of inhibition of 9-14mm against C. albicans at the concentration of 15, 30 and 60 μ g/ml respectively. But the extract has no activity against B. subtilis at the lowest concentration (15 µg/ml) of test extract. S. typhi was inhibited with zones of 9-11mm for 30 and 60 µg /ml respectively. But the extract has no activity against S.typhi at the lowest concentration (15 µg /ml) of test extract. C. mucronata has no activity against B. subtilis at the tested concentrations.

The stem bark extract of *X. americana* has shown a greater zone of inhibition (16mm) against *S. aureus*, the zone of inhibition ranges from 10- 16mm. The extract has shown the zone of inhibition against *K. pneumonia* that ranges from 9-15mm at the concentration of 15, 30 and 60 μ g /ml. respectively. But the extract has no activity against *E. coli* and *B. subtilis* at the lowest concentration (15 μ g /ml) of the test extract. However the extract shown zone of inhibition that ranges from 10-14mm and 9-12mm of *E. coli* and *B. subtilis* respectively. This finding agreed with study of Maikai, *et al* (2009) that reported that both butanol and methanol stem bark extracts of *X. Americana* has zone of inhibition of 11mm against *C. albicans* at the concentration of 60 μ g /ml only. But the extract has no activity against *C. albicans* at the concentration of 30 μ g/ml and 15 μ g /ml of test extract. This is supported by a similar study by Maikai, *et al.*, (2009) who reported that the methanol and water extracts of stem of *X. americana* exhibited activity against candida albicans. *S. typhi* was inhibited with zones of 9 and 13mm for 30 and 60 μ g /ml respectively. But the extract has no activity against *S. typhi* at the lowest concentration (15 μ g /ml) of test extract.

The leaf extract of *W. indica* has shown a greater zone of inhibition (16mm) against *S. aureus* at the highest concentration and zone of inhibition ranges from 11- 16mm for all tested pathogens. The extract has shown the zone of inhibition (9, 10 and 11mm) against *C. albicans* at the concentration of 15, 30 and 60 μ g /ml respectively. *E. coli, B. subtilis, K. pneumonia* and *S. typhi* were only inhibited at higher concentrations of 30 and 60 μ g /ml. But the extract has no activity against *E. coli, K. pneumonia, S. typhi* and *B. subtilis* at the lowest concentration of 15 μ g/ml. In a similar study conducted the ethanol extract of leaves of *W. indica* inhibited the growth of *E. coli, S. typhi* and *S. aureus* with inhibition zones of 10±1, 12±1 and 13±1mm respectively (Olajuyigbe *et al.*, 2011).

The leaf extract of *P. reticulatum* has shown a greater zone of inhibition (16mm) against *S. typhi*, the zone of inhibition ranges from 9-15mm. The extract has shown the zone of inhibition (11 and 13mm) against *C. albicans* at the concentration of 30 and 60 μ g /ml respectively. The extract shown inhibition at higher concentration against *E. coli* with zone of 10mm *B. subtilis* was inhibited at concentration of 30 and 60 μ g /ml with zone of 9 and 13mm respectively. *K. pneumonia* was inhibited at concentration of 30 and 60 μ g /ml with zone of 11and 14mm respectively. *E. coli* was only inhibited at higher concentrations of 60 μ g /ml with zone of 10mm. But the extract has no activity against *E. coli*, *K. pneumonia*, *S. aureus*, *C. albicans* and *B. subtilis* at the lowest concentration of 15 μ g/ml. In a similar study it was reported that the ethanolic leaf extract of *P. reticulatum* showed broad antimicrobial activity against *S. aureus*, *E. coli* and *S. typhi* (Olusola, *et al.*, 2011).

The aerial parts extract of *S. hermonthica* has shown a greater zone of inhibition (16mm) against *C. albicans* the zone of inhibition ranges from 12- 16mm. The extract has shown the zone of inhibition (12, 14 and 16mm) against *C. albicans* at the concentration of 30 and 60 μ g /ml respectively. This result disagreed with the findings of Elshiek and Mona, (2015) in which they reported that the ethanol extract of the whole plant was not active against *C. albicans*. This might be attributed to differences in environmental conditions. The extract also shown inhibition against *B. subtilis* at all tested concentrations with the inhibition zones of 11, 13 and 15mm respectively. *K. pneumonia* was inhibited at concentrations of 30 and 60 μ g /ml with zones of 9 and 11mm respectively. *S. aureus* was also inhibited at concentrations of 30 and 60 μ g /ml with zones of 12 and 14mm respectively. But the extract has no activity against *S. typhi* at the tested concentrations. In a similar study carried out on the extracts of *S. hermonthica* against *Staphylococcus aureus*, *Bacillus subtilitis*, *Escherichia coli*, and *Candida albicans* revealed that ethanol extract of the whole plant showed no activity against *Candida albicans* (Elshiek and Mona, 2015).

T. indica has shown a greater zone of inhibition (15mm) against *E. coli*, the zone of inhibition ranges from 10-15mm. The extract has shown the zones of inhibition (10, 12 and 15mm) against *E. coli* at the concentration of 15, 30 and 60 μ g /ml respectively. The extract also shown inhibition at against *K. pneumonia* at all tested concentrations with the inhibition zone of 9, 11 and 14mm. *B. subtilis* was inhibited at concentrations of 30 and 60 μ g /ml with zone of 9 and 11mm respectively. *S. typhi* was also inhibited at concentrations of 30 and 60 μ g /ml with zones of 9 and 12mm respectively. *S. aureus* was also inhibited at concentrations of 30 and 60 μ g /ml with zones of 9 and 12mm respectively. *S. aureus* was also inhibited at concentrations of 30 and 60 μ g /ml with zones of 9 and 12mm respectively. *S. the extract* has no activity against *C. albicans* at the tested concentrations. This finding agreed with the results of Kapur and John (2014) who reported the antimicrobial activity of ethanolic bark extracts of *Tamarindus indica* against growth inhibition of *S. aureus, K. pneumonia* and *E. coli* with inhibition zones of 16, 13 and 12mm respectively.

E. senegalensis has shown a greater zone of inhibition (14mm) against *B. Subtilis*, the zone of inhibition ranges from 11- 14mm. The extract has shown the zones of inhibition (12 and 14mm) against *B. subtilis* at the concentration of 30 and 60 μ g /ml respectively. The extract also shown inhibition at against *S. aureus* at all tested concentrations with the inhibition zones of 9, 10 and 12mm. *k. pneumonia* was inhibited at concentration of 30 and 60 μ g /ml with zone of 10and 12mm respectively. *S. typhi* was also inhibited at concentrations of 30 and 60 μ g /ml with zones of 10 and 13mm respectively. *E. coli* was also inhibited at concentrations of 30 and 60 μ g /ml with zones of 12 and 13mm respectively. *C. albicans* was also inhibited at concentrations of 30 and 60 μ g /ml with zones of 12 and 13mm respectively. *C. albicans* was also inhibited at concentrations of 30 and 60 μ g /ml with zones of 12 and 13mm respectively. However the extract has no activity at the lowest tested concentration (15 μ g /ml) against all the organisms except *S. aureus*. The results agreed with a similar

research on the antimicrobial activity of methanol, chloroform and aqueous stem back extracts of *Erythrina senegalensis* using the filter paper disc diffusion method in which the extracts demonstrated antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Salmonella typhi*, and *Candida albicans* (Doughari, 2010).

The leaf extract of B. paradoxum has shown a greater zone of inhibition (16mm) against E. coli the zone of inhibition ranges from 11- 16mm. The MIC of the extract against E. coli, S. typhi and B. subtilis is $15\mu g/ml$ and $30\mu g/ml$ for C. albicans. However the extract has no activity at all tested concentrations against S. aureus and k. pneumonia. The extract has shown the zones of inhibition (11, 14 and 16mm) against E. coli at the concentration of 15, 30 and 60 ug /ml respectively. The extract also shown inhibition at against S. typhi at all tested concentrations with the inhibition zone of 9, 12 and 15mm. B. subtilis was inhibited at concentration of 15, 30 and 60 µg /ml with zone of 10, 12and 14mm respectively. S. typhi was also inhibited at concentrations of 30 and 60 µg/ml with zones of 10 and 13mm respectively. C. albicans was also inhibited at concentrations of 30 and 60 µg /ml with zones of 10 and 11mm respectively. This result agreed with the findings of Kubmarawa et al., (2007) who reported the antimicrobial activity of stem bark of *B. paradoxum* on the similar pathogens but the stem bark extract was active against S. aureus while in this work the leaf extract has no activity against S. aureus. This could be due to different parts of the plant used in the study, here leaves were used not the stem bark of the plant. Olajuyigbe, et al (2011) reported that zones of inhibition ≥ 10 mm were considered as good antimicrobial activity for the crude plant extracts. Thus, several extracts used in the study have exhibited zones of inhibition greater than 10mm and they are recommended for further screening to isolate and characterize the bioactive chemical constituents.

4. CONCLUSION

The chemical constituents tested in the plants were the major therapeutic agents found in medicinal plants. Therapeutic activity against most diseases caused by pathogenic infections could be attributed to the present of these bioactive agents. The extracts have demonstrated significant inhibitory activity against the tested organisms even though some extracts are inactive. Therefore, the active plants extracts can act as potential source of compounds or "lead-compounds" for development of antimicrobial agents that could be useful in chemotherapy.

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