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Five Samburu Community (Kenya) Medicinal Plants Show *In Vitro* Antibacterial Activity Against Selected Human Pathogenic Bacteria

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Abstract: ***With the rise in antimicrobial resistances globally, the urge of getting new lead compounds that can inhibit microbial growth and virulence factors production is becoming a reality. In this study Samburu (Kenya) anti-diarrhoeal medicinal plants were bio-assayed against selected bacterial strains (S. aureus - ATCC 20591, B. subtillis - (Local isolate), S. typhi-ATCC 2202, E. coli- STD. 25922 and P.aeruginosa - ATCC 25852). Both disc diffusion and microdilutionmethods were applied to ascertain the antibacterialabilityof 80% methanol extracts of 5 anti-diarhorreal plants in vitro.Results showed that both isolates were moderately susceptible with P. aeruginosa being the most susceptible. Acacia horrida was also found to be the most active extract whose activity was comparable to the positive control (Cefrodoxima) against most isolates. The MICs of the most active plants ranged from 9.375mg ml^{-1} to 37.5mg ml^{-1} while the MBCs ranged between9.375mg ml^{-1} to75mg ml^{-1}. These extractswere found to be rich in various phytochemicals with tannins being the most abundant. These findings therefore demonstrate that some of the bioassayed Samburu anti-diarrhoeal plants have antibacterial properties and compounds. Most of the Samburu bioassayed medicinal plants can offer alternative medicare to conditions caused by the test isolates.***

Keywords: Phytochemicals, Diarrhoea, Resistance, Medicinal plants, Susceptibility

1. Introduction

The World Health Organization (WHO) defines traditional medicine as the health practices, knowledge, beliefs and approaches that do incorporate plants, animals and mineral-based medicine, manual techniques, exercises and spiritual therapies that are applied singularly or in combination to diagnose, treat and prevent illnesses with the sole purpose of maintaining well-being [1]. The use of natural products for treatment of various ailments is the age-old practice that has existed even up to the Precambrian period [2].Currently, WHO is encouraging and supporting programmes that are geared towards documenting medicinal plants use by various communities globally. This has been attributed to the fact that such information has been found to be getting extinct as such information is only passed orally from one generation to another in most populations across the universe [3],[4].

WHO has further estimated that about 80% of the human population globally in one way or another rely or have used medical plants for purposes of managing various ailments. These has been attributed to easy accessibility of the medicinal plants, their affordability as compared to synthetic drugs and the rich history on their usage amongst communities for decades [5], [6]. Therefore, it is clearly indicative of the value and potential of the natural plant derived natural products towards new pharmaceutical discovery and development that will assist humankind to deal with the current monster of antimicrobial resistances and easy management of common ailments [1].

The burden of management of diseases due to the rise in antimicrobial resistances and multidrug resistance cases to commonly used antibiotics is real. As such, antimicrobial resistance is now a devastating global problem, whose magnitude is currently accepted. Over the last 45 years, no new classes of antibiotics have been discovered and resistance has evolved against virtually every antibiotic that has been deployed [7].Estimates foresee that in the year 2050, 10 million lives per year and cumulative 100 trillion USD of economic output will be at risk due to the rise of resistant infections if no proactive measures are taken today to slow down the present rising trend in drug resistance [8], [9]. Among the suggested measures, to tackle infections is the search for alternative products as antibiotics. Even though vaccination is good as a major preventive strategy, other alternatives to replace antibiotics as treatment, or make them more effective, have been recognised and are in various stages of development[10]. Such options include probiotics, antibodies, wild type and engineered bacteriophages, lysins, immune stimulation, and peptides (namely, host defence, antimicrobial, biofilm formation inhibitory peptides and innate defence)or preventing the adhesion of pathogenic bacteria to their host (e.g., *H. pylori*)[11]. Attacking some of the virulence traits(e.g., biofilm formation) via disruption of quorum sensing is advocated as a potentially promising new approachto deal with bacterial infections [12].

Medicinal plants have been documented to possess a rich library of secondary metabolites/compounds that could be potential sources of the next generation of antibiotics but they are not fully explored[13], [14]. Such metabolites have been found to possess various modes of action, which potentiates them to be lead compounds that can be used to manage various conditions causing harm to humankind. For instance, alkaloids have been documented to possess cytotoxic effects while Cardiac glycosides have been found to inhibit the pumping of sodium/ potassium ion[15]. Other

phytochemicals like baicalein and quercetin (flavonoids) have also been found to inhibit bacterial quorum sensing which is a main pathway bacterial pathogen use to produce various virulence traits like biofilm formation, toxin production etc.[16]. These amongst many more mechanisms can be harnessed and be used in management of conditions caused by pathogenic bacteria. This study therefore aimed at validating the antibacterial profiles of five Samburu anti-diarrhoeal medicinal plants.

2. Materials and Methods

Ethnobotanical survey

By use of questionnaires which mainly targeted the herbalists, a survey was carried out in Wamba sub-county, Samburu County, Kenya on the commonly used anti-diarrhoeal medicinal plants.

Collection of plant material

Fresh plant/plant parts of the commonly used medical plants by the Samburu community were harvested from the various Samburu-WambaConservancies as shown in figure 1 below. Voucher specimens were also collected, pressed and deposited at Kenyatta University herbarium where they aided in identification of the botanical names by a taxonomist. The specimens obtained from the field were air dried under the shade before they were ground by the laboratory-grinding mill (IKA M20) and then used for bioassays.

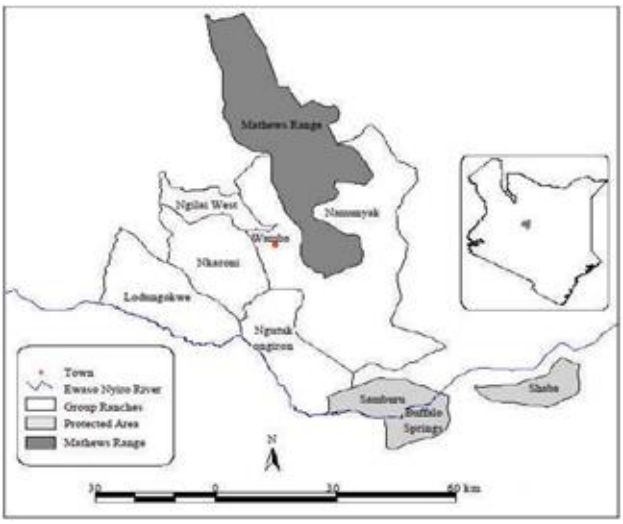


Figure 1: Map of Kenya showing the location of Wamba Division and its conservancies

Extraction

Fifty grams (50g) of the dried, ground plant materialswere soaked in300 ml of 80% methanol (MW-32.04) for 12-48hwith intermittent shaking to enable the dissolvingof the active phytochemicals in the solvent. Themethanol-soaked plant extracts were filtered byuse of Whatman No. 1 filter paper. Then the solvent in the supernatant was evaporated by use of the Rotar evaporator (BuchiRotavapor R-210) around 5-10mls. The paste was deep-frozen with liquid nitrogen and then placed in the freeze drier (Christ Alpha 1-4 LD plus) overnight to completely get rid of the solvent.The resulting powder was packed and used for the bioassays.

Antimicrobial screening/ bioassay

i. Test cultures

The type culture isolates were obtained from Kenyatta National Hospital in Nairobi, which included *Staphylococcus aureus* (Gram-positive cocci) - ATCC 20591, *Bacillus subtillis* (Gram-positive spore forming *bacilli*) - Local isolate, *Salmonella typhi* (Gram-negative rod) - ATCC 2202, *Escherichia coli* (Gram-negative rod) - STD. 25922 and *Pseudomonas aeruginosa* (Gram-negative rod) - ATCC 25852. They were selected based on their cell wall properties and their ability to cause gastrointestinal infections, which are commonly characterised by diarrhoea.*B. subtilis* and *P. aeruginosa* were however chosen purely on their typical cell wall characteristics. These test strains were kept refrigerated on Muller-Hinton (Merck, Germany) agarslants during the experimental period, were sub -cultured, and incubated for 24h at 37°C thentested biochemically for purity before use.

ii. Disc diffusion assay

The antimicrobial activity of the 80% methanol extracts of the five medicinal plants were measured by agar well diffusion method[16], [17]. Briefly, a few colonies were taken from the sub-cultured plate of test isolatesand mixed with 1ml of the broth. This was adjusted to match a turbidity of 0.5 McFarland standards. 100µL of the adjusted bacteria in broth was then cultivated unto the agar via spread plate technique. Then a sterile dry disc (6 mm) that had been saturated with 100µl of the plant extract (made by dissolving 300mg of the extract in 1ml of nutrient broth), were introduced on the upper layer of the seeded agar plate. The plates were incubated overnight at 37°C. Microbial growth inhibition was determined by measuring the diameter from the end of growth to disc at one end to the beginning of growth at the other end of inhibition. Amoxicillin (250 mg) was used as positive control for each bacterial strain and methanol as the negative control after they had been soaked on sterile discs (6mm) and allowed to dry prior to their usage. Any disc (6 mm) showing no zone of Inhibition was considered as having no antimicrobial activity. All experiments were done in triplicate and the average values were calculated.

iii. Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

A micro-dilution technique using 96 well microplates, [18] wasused to obtain MIC values of the crude extractsagainst all the test bacteria. Each plant extractwas serially diluted to obtain concentrations of 150mg ml^{-1}, 75mg ml^{-1}, 37.5mg ml^{-1}, 18.75mg ml^{-1}, and 9.375mg ml^{-1}. Similar serial dilutions wereperformed for Cefrodoxima (250mg) as positive control and methanol as negative control. Equal volumes of 50µl fresh bacterial cultures wereadded to each of the wells. Micro titre-plates werecovered and incubated at 37°C overnight for the bacterial strains.The MIC values were determined as thelowest concentrations of the extract showing nogrowth. All the wells where no growth (not turbid)was observed were sub-cultured, and the lowestconcentration of the plant extracts that did notyield any colony growth on the solid nutrient mediumfor the bacterial cultures after sub-culturing and incubating

for12-24h for the bacterial cultures were taken as the MBC.All experiments were done in triplicate and the average values were calculated.

Preliminary phytochemical screening

The analysis for the presence of various phytochemicals of the crude powder of the five plants collected was determined byestablished methods [19], [20], [21]. Various phytochemicals like cardiac glycosides (Keller-Killani method), flavonoids, saponins, alkaloids (Wagner's method), terpenoids (Salkowski test) and tannins were screened for their presence in extracts. Based on the colour changes that occurred during this screening a (+) was used to represent low compound concentration, (++) for moderate, and (+++) for high/dense concentration after the reaction with the various reagents used.

Statistical analysis

All experiments were done in triplicates that are independent of each other to validate reproducibility. The data obtained was analysed by using Graph Pad Prism software (version 6.01; Graph Pad Software, Inc., La Jolla, CA, USA). All values of diameter zones of inhibition, MIC and MBC of extracts were reported as mean ± standard error. One-way analysis of variance was performed to compare differences between groups with significance difference determined at $P \leq 0.05$. Tables and graphs were used to represent data.

3. Results

Ethnobotany survey

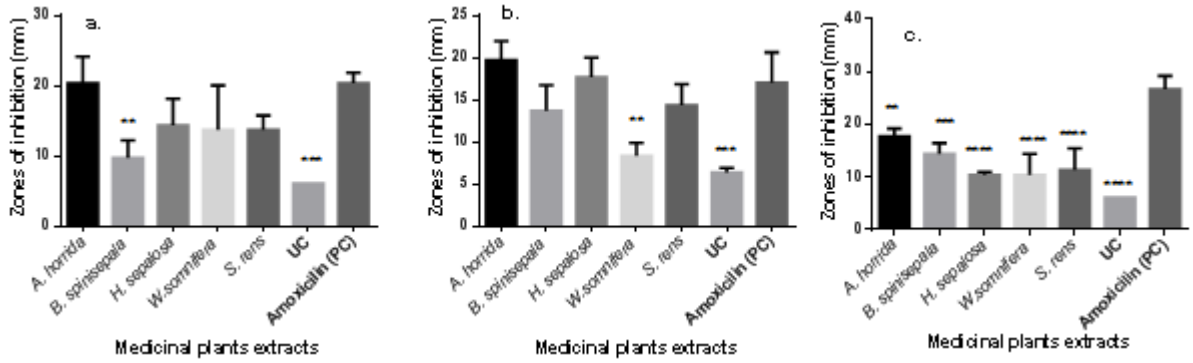
Five commonly used medicinal plants used to manage gastrointestinal tract ailments by the Samburu community were collected from the various conservancies of Wamba (Namunyak, Ngilai west and NgutukOngiron). The medicinal plant were from different families as represented in Table 1 below. Roots were the most predominant part harvested amongst the plant species identified and this was followed by the stem barks and then whole plant. It was also deduced that out of the five medicinal plants collected no species had leaves as the harvested part.

Table 1: Ethno botanical information of some medicinal plant species traditionally used by the Samburu people of Kenya

Botanical name	Local (Samburu) name	Family	Part Used	medicinal use	Area collected
Hildebrantia sepalosa	Nyirman	Convolvulaceae	Roots	Diarrhoea, stomach ache	Ngutuk Ongiron
Acacia horrida (L.) Willd	Lmarti	Mimosaceae	Roots	Diarrhea	Ngutuk Ongiron
Barleria spinisepala E.A. Bruce	Sucha	Acanthaceae	Whole plant	Diarrhea	Ngilai west
Withania somnifera (L.) Dunal	Lekuru	Solanaceae	Roots/bark	Diarrhea	Namunyak
Solanum rens L.	Ltururai/ Ntulelei	Solanaceae	Bark	Diarrhoea	Ngilai west

Disc diffusion

Antimicrobial activity of the 5 medicinal plants collected was deduced by subjecting 30mg ml^{-1} of each extract to disc diffusion assay. Various pathogens were used as model microorganisms for determining the ability of the extracts to inhibit their growth as presented in figure 2 below. *A. horrida* seemed to be the most active extract as it produced significant inhibitory results in all microorganisms studied e.g.*S. aureus*ATCC 20591(20.33mm); *B. subtillis* - Local isolate(19.67mm); *S. typhi* - ATCC 2202(17.67mm); *E. coli* - STD. 25922 (20.33mm) and *P. aeruginosa* - ATCC 25852(24mm). In addition,*H. sepalosa* did have significant inhibitory effects against *Bacillus subtilis* isolate. All medicinal plants did show some substantive inhibitory effects as presented in figure one below against the isolates used as models for bio assaying. In most isolates there was some significance difference (**P*<0.05, ***P*<0.01, ***P<0.001 and ****P<0.0001) when the findings were compared to the positive control (Amoxicillin) as presented in the Figure 2. However, it should be noted that the Gram positive isolates were more susceptible as compared to the Gram negative isolates.



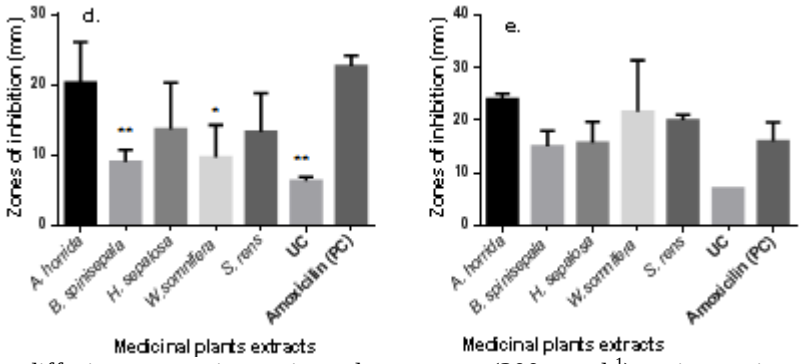


Figure 2: Results for disc diffusion assay using various plant extracts (300mg ml^{-1}) against various bacterial isolates used a. *S. aureus* ATCC 20591(b)*B. subtillis* - Local isolate (c) *S. typhi* - ATCC 2202 (d) *E. coli* - STD. 25922 (e) *P. aeruginosa* - ATCC 25852 (n=3; **P*<0.05, ***P*<0.01, ***P<0.001 and ****P<0.0001 compared with the control group)

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Our findings did indicateaverage MICs and MBCs as produced by the various extracts bioassayed and shown in Table 2. However, *Acacia horrida*extract produced substantive inhibitory effects as it exhibited bactericidal effects on all pathogens screened except for *P. aeruginosa* that showed bacteriostatic activity.The MIC and MBC values for this extract ranged between9.375±00 to18.75±00 mg ml^{-1}which are comparable to thoseconcentrations produced by the positive control. Most extracts were also deduced to be possessing bactericidal effects on the tested isolates. In terms of pathogen susceptibility, *P. aeruginosa* was found to be the most susceptible as low dosages were found to inhibit its growth or even kill the pathogen. A very positive finding as this pathogen is known to cause a lot problems to man. *S. typhi, S. aureus* and *B. subtilis* isolates were inhibited with high dosages of most extracts used.

Table 2: The MICs and MBCs (mg ml^{-1}) produced by the selected Samburu medicinal

	S. aureus		*S. typhi*		*B. subtilis*		*E.coli*		*P. aeruginosa*	
	MIC (mg ml^{-1})	MBC (mg $ml^{-1)}$	MIC (mg ml^{-1})	MBC (mg ml^{-1})	MIC (mg ml^{-1})	MBC (mg ml^{-1})	MIC (mg ml^{-1})	MBC (mg ml^{-1})	MIC (mg ml^{-1})	MBC (mg ml^{-1})
A. horrida	18.75 ± 00	18.75 ± 00	18.75 ± 00	18.75 ± 00	18.75 ± 00	18.75 ± 00	9.375 ± 00	9.375 ± 00	9.375 ± 00	18.75 ± 00
H. sepalosa	37.5± 00	37.5 ± 00	37.5 ± 00	37.5 ± 00	37.5 ± 00	37.5 ± 00	37.5 ± 00	37.5 ± 00	18.75 ± 00	18.75 ± 00
B. spinisepala	37.5 ± 00	75 ± 00	37.5 ± 00	75 ± 00	37.5 ± 00	37.5 ± 00	18.75 ± 00	37.5 ± 00	37.5 ± 00	37.5 ± 00
W. somnifera	37.5 ± 00	75 ± 00	37.5 ± 00	75 ± 00	37.5 ± 00	37.5 ± 00	37.5 ± 00	37.5 ± 00	18.75 ± 00	37.5 ± 00
S. rens	18.75 ± 00	37.5 ± 00	37.5 ± 00	37.5 ± 00	37.5 ± 00	75 ± 00	37.5± 00	37.5 ± 00	37.5 ± 00	37.5 ± 00
Cefrodoxima (PC)	18.75 ± 00	18.75 ± 00	18.75 ± 00	18.75 ± 00	18.75 ± 00	18.75 ± 00	18.75 ± 00	18.75 ± 00	9.375 ± 00	9.375 ± 00

Preliminary phytochemical screening

On carrying out qualitative screening of the phytochemicals, some phytochemicals (flavonoids, tannins, terpenoids, saponins, alkaloids and cardiac glycosides)proved to be present on the five extracts as summarized in table 3 below. All the five extracts proved to be rich with tannins phytochemicals. In addition, saponins and alkaloids were only absent in two medicinal plants (*B. spinispela* and *S. rens*). The cardiac glycosides were only absent in two medicinal plants (*H. sepalosa* and *S. rens*). Flavonoids were present only in *H. sepalosa* and *B. spinisepala.*Generally, amongst the phytochemicals screened most of them were present in *H. sepalosa*in abundance except the cardiac glycosides. *Acacia horrida* had a rich library of most of the phytochemicals screened only that it did not possess the flavonoids and terpenoids. It was also deduced that only tannins were present in *S. rens* extracts amongst the phytochemicals screened.

Table 3: Preliminary results for phytochemical screening

Plant	Tannins	Saponins	Flavonoids	Terpenoids	Cardiac glycosides	Alkaloids (Wagner's test)
Hildebrantia sepalosa	+++	+++	++	+++	-	++
Acacia horrida	+++	+++	-	-	++	+++
Barleria spinisepala E.A. Bruce.	+++	-	+	-	+++	-
Withania somnifera (L.) Dunal.	+++	+	-	+	+	++
Solanum rens L.	+++	-	-	-	-	-

Key: +++ (Most abundant), ++ (Abundant), + (Less abundant) and - (Not present)

4. Discussion

From the survey that was carried out, five anti-diarrhoeal Samburu medicinal plants were harvested and bioassayed for their efficacy against selected bacterial species. These plants were obtained from various conservancies around Wamba Sub County namely Namunyak, Ngilai west and Ngutuk Ongiron. The medicinal plant were from different families with solanaceae being the most abundant. Roots were the most predominant part harvested amongst the plant species identified and this was followed by the stem barks and then whole plant. A clear reason as to why some of the medicinal plants are becoming extinct as harvesting the roots and the bark does not promote plant species survival hence not sustainable. Conservationists have further eluded that any medicinal plant whose bark, bulbs, roots, stems,

tubers or whole plant is harvested face an uphill task of existence [22], [23], [24]. Therefore, their need to promote conservatory measures amongst such species that have been validated to be having antimicrobial properties.

By use of the disc diffusion method the antibacterial activity of the extracts obtained from the five anti-diarrhoeal plants collected was determined. *A. horrida* was found to be the most active extract as it produced significant inhibitory results in all microorganisms studied e.g. *S. aureus* ATCC 20591 (20.33mm); *B. subtillis* - Local isolate (19.67mm); *S. typhi* - ATCC 2202 (17.67mm); *E. coli* - STD. 25922 (20.33mm) and *P. aeruginosa* - ATCC 25852 (24mm). In addition, *H. sepalosa* had significant inhibitory effects against *Bacillus subtilis* isolates. However, the Gram-positive isolates were more susceptible as compared to the Gram-negative isolates and *P. aeruginosa* was most susceptible to most extracts. As such, their modes of action cannot be fully attributed to the cell wall properties of the test microorganisms.

Other medicinal plants could also be having other modes of action (e.g. immunomodulation abilities) other than antimicrobial activity more so the ones that did not produce substantive inhibitory effects against the test isolates like *S. rens* & *B. spinisepala* against *S. typhi; B. spinisepala* & *W. somnifera* against *E.coli* and *W. somnifera* against *B. subtilis*. For instance, [25] reported that *Withania somnifera* up regulated IL-7 cytokine to two times in IEC- 6 (ATCC) mammalian cell line. This group of cytokines have been found to stimulate immune system that ends up producing more of the immune response lymphocytes(CD4+ and CD8+) that are commonly involved in elimination of the invading microbes. Therefore, further studies on this line could be encouraged, as it is evident that some medicinal plants do have such capabilities.

MIC and MBC were also determined with *A. horrida* extract producing greater inhibitory effects (9.375 ± 00 to 18.75 ± 00 mg ml^{-1}) and it exhibited bactericidal effects on all pathogens screened except for *P. aeruginosa* that showed bacteriostatic activity. In terms of pathogen susceptibility, *P. aeruginosa* was found to be the most susceptible as low dosages were found to inhibit its growth or even kill the pathogen. A very positive finding as *P. aeruginosa* is one of the notorious opportunistic pathogen that has been found to cause a lot of havoc to humankind especially among the immunocompromised patients and those suffering from cystic fibrosis. Additionally, the pathogen has been found to use quorum sensing to induce various virulence traits amongst them biofilm formation, toxin production and even antimicrobial resistance which is a big issue in the world today [26]. These clearly demonstrates that the medicinal plants collected could be possessing some lead compounds that can be harnessed and be used to manage conditions caused by *P. aeruginosa*.

On the other hand, *S. typhi*, *S. aureus*, *E.coli* and *B. subtilis* isolates were inhibited at higher dosages of most extracts used that ranged from 37.5 ± 00 to 75 ± 00 mg ml^{-1}. These pathogens are among the priority list of WHO that have been grouped to have multiple resistances to commonly used antibiotics[27]. As such therefore, these medicinal plants may not offer lead compounds that can assist in management of conditions related to these pathogens. On the other handthe difference in the activity may not be attributed to the cell wall/membrane properties, but could be related to the inhibition of specific important biological pathway(s) that is/are crucial to a given species of bacteria that could be absent on the other species. The different rates of inhibition with these extracts might also be attributed to the the phytochemical compounds quantities present in the extracts. Such phytochemicals have also been documented to be possessing antagonistic activity and as such it may not be ruled out especially to those extracts that did not have more pronounced activities against the test pathogens [28].

The presence of various phytochemicals (flavonoids, tannins, terpenoids, saponins, alkaloids and cardiac glycosides) in the five medicinal extracts clearly demonstrates the reason why they exhibited some antimicrobial activities together with the unscreened ones. Tannins were the most abundant phytochemicals that were present in all the medicinal plants screened. In addition, saponins and alkaloids were only absent in two medicinal plants (*B. spinispela* and *S. rens*). The cardiac glycosides were absent in two medicinal plants (*H. sepalosa* and *S. rens*). Generally, amongst the phytochemicals screened most of them were present in *H. sepalosa* in abundance except the cardiac glycosides. *Acacia horrida* had a rich library of most of the phytochemicals screened only that it did not possess the flavonoids and terpenoids. This could clearly demonstrate why this medicinal plant was the best concerning its antimicrobial activity. It was also deduced that only tannins were present in *S. rens* extracts amongst the phytochemicals screened. However, the quantity and quality of the phytochemicals could be affected by the geographical region and climatic conditions and partly could have contributed to the diversity that was observed in this study[29]. Some of these phytochemicals like tannins have been documented to be having antimicrobial activity through their ability to inactivate specific key enzymes, microbial adhesions, mineral uptake and even cell envelope transport proteins [30], [31], [32], [33].

Additionally, the inhibitory effects of terpenoids have also been documented in both bacteria and viruses [34], [35],[38].

On the other hand, the rich library of phytochemicals in these medicinal plants screened clearly demonstrates why the Samburu community use more than one medicinal plant (concoction) to treat a given condition. Scientifically it could be related to the additive or synergistic activity. The phytochemicals could target various sites within the bacterial cell hence easily overcoming their defence mechanisms [28], [29].

5. Conclusion

The five medicinal plants could offer medicare to conditions caused by the test isolates and as such, this study supports their use by the Samburu community. They possess a rich library of phytochemicals, which could offer diverse modes of action hence easy management of the infections.

6. Future Scope

More research work needs to be done on these medicinal plants especially on *Acacia horrida* as it did have good inhibitory effects on the test microbes. Such work should focus on mining and structural elucidation of lead antimicrobial compounds and deducing their mode of action. Further work needs to be done in usage of modern techniques to enhance delivery of the active phytochemicals into the bacterial cell.

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