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In vitro study of the antibacterial activity of *Taraxacum officinale* root extracts against methicillin resistant *Staphylococcus aureus* bacteria

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Abstract
The current study set out to determine whether plant extracts from *Taraxacum officinale* roots exhibited antibacterial activity against a strain of methicillin-resistant *Staphylococcus aureus* (MRSA). The research was carried out at the Kampala International University Uganda Western Campus, Microbiology Laboratory. There were four extracts used: methanolic, ethanolic, chloroform, and distilled water (D. H_2O). The diffusion method in an agar well was applied. The tested bacterial pathogen, MRSA, was found to be susceptible to the effects of *Taraxacum officinale* extracts, both methanolic and ethanolic root extracts being the most effective; chloroform extract exhibited lower potency, and queous extracts exhibited no activity against MRSA. Minimum inhibitory concentration (MIC), of the ethanolic and methanolic extracts against this bacterial strain was around 3 mg/ml and 6 mg/ml for chloroform extract and the average MBA was 25 mg/ml for ethanolic, methanolic chloroform extract. The results of various phytochemical analyses showed the existence of secondary metabolites that may be involved in the antibacterial assay, including flavonoids, saponins, alkaloids, tannins, terpenoids, cardiac glycosides, and phenol. Based on the findings, it can be said that *Taraxacum officinale* extracts may be the next antibacterial agent to be created since they have potential efficacy against the tested bacterial pathogenic strain, MRSA.

Keywords: Agar well diffusion method, *Taraxacum officinale*, Antibacterial activity, methicillin resistant *Staphylococcus aureus*

1. Introduction
The gram-positive, non-motile bacteria *Staphylococcus aureus*, or *S. aureus*, has a spherical form [1]. This widespread human pathogen proliferates on the skin and mucous membranes, where it invades the body and causes severe illnesses in both humans and animals, including as blood infections, suppurative skin infections, acne, osteomyelitis, endocarditis, and respiratory tract infections [2]. Food-borne infections are brought on by the enterotoxins produced by *S. aureus*, which is also present in food and food-related areas like kitchens [3]. Over the years, *Staphylococcus aureus* has attracted clinical attention due to its ability to quickly adjust to antibiotic pressure and acquire antibiotic resistance [4]. The majority of *S. aureus* strains about 94 percent have a noticeable resistance to penicillin and its derivatives because of the lactamase enzyme that is released. The mecA gene's production, which codes for the MRSA Methicillin-resistant *Staphylococcus aureus* is caused by penicillin-binding protein (MRSA) [5]. All WHO regions have reported methicillin resistance in *Staphylococcus aureus*, with rates reaching 80% in 2018 [6]. MRSA prevalence was shown to vary within and between countries of Africa in a study conducted MRSA prevalence was shown to vary within and between African countries in a study conducted by Wangai *et al*. (2019), with national statistics from 9 African countries revealing MRSA rates ranging from 12 to 80 percent, with some countries reaching 80 percent. Uganda has high prevalence rates of 325 to 42 percent among patients and healthcare workers, Rwanda has a prevalence rate of 31 to 82 percent MRSA, and Tanzania has a prevalence rate of 10 to 50 percent MRSA [7]. In addition to penicillin, MRSA has shown multidrug resistance to a range of antibiotic families, including aminoglycosides, fluoroquinolones, macrolides, lincosamides and tetracyclines [8]. In the World Health Organization's (WHO) report of antimicrobial resistance, Methicillin-resistant *Staphylococcus aureus* (MRSA) was identified as a significant cause of increased death, post-infection morbidity, ICU length of stay, and treatment costs [5]. In people and animals, MRSA can infect the mammary glands, mucous membranes, serous membranes, skin, and internal

organs. This can lead to serious infections such as septic arthritis, otitis media, pneumonia, osteomyelitis, pyogenic endocarditis, and infections of the skin and soft tissues. Methicillin-resistant *Staphylococcus aureus* (MRSA) infections have surged or increased worldwide, along with related morbidity and discharge to long-term care. As a result, additional resources are being used to manage and treat MRSA infections in both acute and long-term settings. The burden on healthcare resources is well-known worldwide, as more than 60% of *Staphylococcus aureus* isolates causing nosocomial infection in intensive care units have been verified to be MRSA [7]. The emergence of multidrug-resistant MRSA strains has affected the management of MRSA infections as a result of widespread antibiotic use in humans and animals [6]. The extensive and indiscriminate use of these antimicrobials, according to [9], has led to a loss of efficacy of the clinical use of first-line antimicrobials, driving a shift in therapy to newer, more expensive drugs. As a result, multidrug-resistant virulent MRSA strains have become tough to treat pathogens, posing a serious public health risk and leading to treatment and control failures [10]. MRSA causes nosocomial infections and is a major source of community-acquired infections (CA-MRSA), whose morbidity is on the rise all over the world [11]. MRSA strains have been treated using a variety of strategies, including the use of newer antimicrobial medicines like telavancin and tedizolid in combination with vancomycin. Austroeupatorium inulaefolium (H.B.K.) essential oil and Leoheo domatiophorus Chaowasku leaves-extracted essential oil are used in alternative herbal therapy regimens [8]. Worldwide, medicinal products derived from plants have been used for thousands of years to treat microbial infections. Interest in employing plants to treat microbial diseases, especially those that are resistant to antibiotic therapy, is expanding [12]. *Taraxacum officinale*, often known as the dandelion, is a common perennial herbaceous plant that blooms in Eurasia, North America, Africa, and other parts of the world [13]. In temperate climes, it grows in a range of locations, such as wayside vegetation, orchards, vegetable gardens, horticultural crops, good soil, dispersed banks, and coasts along waterways. They live in garbage dumps, abandoned fields, and lawns at elevations between 500 and 11,000 feet [14]. Medicinal herbs and plant extracts have been used by people to treat MRSA infections. According to published research, the plants may be used to treat microbial diseases, such as skin conditions with staphylococcal origins [15], but there is little to no evidence to support their use in the management of MRSA infections [16, 17]. The leave extract of the *taraxicum officinale* plant has been said to possess antimicrobial properties [13]. Therefore, this study explored the anti-bacterial activity of *Taraxicum officinale* root extracts against methicillin resistant *Staphylococcus aureus* clinical isolates.

2. Materials and Methods

2.1 Collection of Plant Material

Fresh and strong roots of *Taraxacum officinale*, a disease-free plant, were gathered. Three (3) to four (4) times under running water and once with sterile distilled water, the leaves were cleansed. They were then allowed to air dry in a sterile blotter in the shade and heated to 40 ^{0}C in a hot air oven for 4 to 5 days, or until their weight stabilized. Every day, plant material was checked for bacterial or fungal decay. A clean grinder was used to turn the dried plant material into a powder [13].

2.2 Preparation of plant extracts

In a beaker (1L), 100 grams of powdered roots sample were put in 500ml of 70% ethanol, methamol, chloroform and water separately and allowed to mix for 72 hours with vigorous shaking. After 72 hours, the mixture was filtered using a Whatman No. 1 filter paper grade 1 of 0.5L and a clean white cotton cloth. Drying the filtrate in an oven set to 40°C (Binder, Model E28) concentrated it and the % yield of extracts determined [13].

2.3 Phytochemical Analysis

Several phytochemical substances that may have antibacterial activity were tested in this investigation. Among the substances included in *Taraxicum officinale* (dandelion root), saponins, terpenoids, Tannins, Flavonoids, Alkaloids, cardiac glycosides, and phenol are anticipated to have antibacterial properties [13, 18, 19].

2.3.1 Test for flavonoids

0.2g of extract was added to 5 mL of weak ammonia solution. After that, 2 mL of concentrated sulphuric acid was slowly added. Flavonoids were identified by a yellow solution that turned colourless [20].

2.3.2 Tests for alkaloids

(a) The Dragendorff test: When 1 ml of Dragendorff's reagent was added to 2 ml of extract, an orange-red precipitate was produced, indicating the presence of alkaloids.

(b) Mayer's examination: 1 ml of extract was mixed with a few drops of Mayer's reagent. Alkaloids were present because a white or yellowish precipitate developed.

(c) The Hager test: A few drops of Hager's reagent were added to 2 ml of extract. There was a yellow precipitate that showed alkaloids were present [20].

2.3.3 Saponins tests

5 mL of extract was put in a test tube together with a drop of Na_2CO_3 solution. Then it was shake and then allowed to rest for five minutes. The presence of foam indicated the presence of saponins [20].

2.3.4 Test for terpenoids

Horizon examination. One milliliter of extract was mixed with two milliliters of trichloroacetic acid. The development of a crimson precipitate indicated the presence of terpenoids [20].

2.3.5 Test for glycosides

Killiani Keller test. Two milliliters of extract were combined with a 0.5 milliliter solution of glacial acetic acid (glacial CH_3CO_2H) and two to three drops of ferric chloride ($FeCl_3$). Afterwards, 1 mL of concentrated H_2SO_4 was added to the test tube's walls. The presence of cardiac glycosides was suggested by the formation of a brown ring [20, 21].

2.3.6 Test for phenolic compounds

5 ml of distilled water was used to dissolve the 0.5 g of extract. A small amount of a neutral 5% ferric chloride ($FeCl_3$) solution was added to this. Phenolic chemicals were shows dark green color [21].

2.3.7 Tannins

In a test tube, 0.5g of powdered plant material is boiled in 20ml of distilled water, then filtered. 0.1% $FeCl_3$ is then

added to the filtered samples, and the presence of tannins is determined by looking for a brownish green or blue-black coloration. [21].

2.4 The Test Microorganism Culture and the Maintenance for Antibacterial Study

Certain staphylococci that do not manufacture coagulase create little pink or red colonies without changing the color of the medium, while *Staphylococcus aureus* develops yellow colonies with yellow zones. The Methicillin Resistant *Staphylococcus aureus* was placed in broth culture at 37 °C using the medium Brain Heart Infusion (BHI) broth, which allowed it to develop to a high concentration overnight. Subcultures were then made daily to a single selective medium, mannitol salt agar, which showed golden yellow, allowing the isolate to be recognized as illustrated in the diagram below [13].

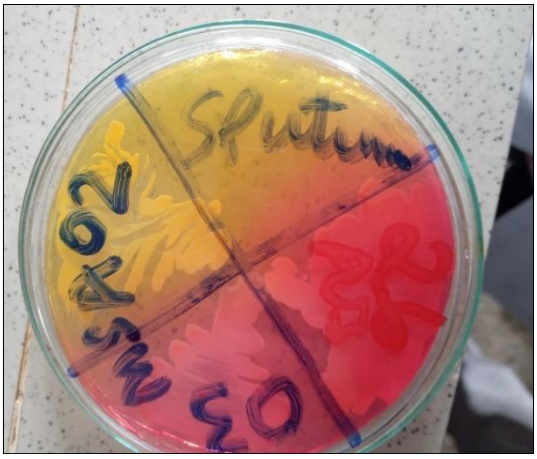

Fig 1: Cell cultured in Manitol Salt Agar

2.5 Antimicrobial Activity Screening

For the antibacterial research of methanol, chloroform, and distill water, agar-well diffusion methods were used. To combat the test bacteria, 50 µl (microlitres) of each root extracts of, methanolic, ethanolic, distil water and chloroform were used [22].

2.5.1 Antibacterial screening by agar well diffusion method

Petriplates were filled with 25 ml of sterile Muller-Hinton Agar (MHA) and left to set. A sterile glass rod was used to spread 0.10 ml of 24-hour-cultured bacteria into the petriplates before the plates were left to dry. On the plates, sterile hole punctures (10 mm in diameter) were used to create wells. A micropipette was used to fill six wells with around 50 µl of each concentration of the plant, 50 mg/ml, 100mg/ ml, 200mg/ ml, and 400mg /ml in each well and a vancomycin disk was placed in another well as positive control and 50 µl blank water was used as negative control and this was done for all the different respective solvent extracts. After that, plates were incubated at 37 °C for a whole day. We looked at the plates after a day (24h). An inhibition zone encircling the well served as a gauge for the plant root extract's antibacterial activity, and the zone of inhibition was quantified and expressed in millimeters [13, 22].

2.6 Measurement of Minimum Inhibitory Concentration

The lowest concentration necessary to prevent the development of methicillin-resistant *Staphylococcus aureus* is the MIC of *taraxicum officinale* roots crude ethanolic, methanolic, chloroform extract. Broth tube modified dilution method was used. Using sterile conical centrifuge tubes with 1 ml of Brain Heart Infusion broth (BHI), a two-fold serial dilution was performed [13, 19].

400mg extracts was dissolve in 1ml distilled water to form 400 mg/ml and added to 1ml of BHI broth form 400 mg/ml extract concentration, which was carefully mixed. 1 ml of this dilution was then transferred to tubes in a two-fold dilution of the original extract concentration until the last tube. Where 1 ml of the broth and extract mixture was discarded. This result to 400 mg/ml extract, yielding 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml and 1.563 mg/ml final dilution extracts concentration [23] and this was done for all the extract.

To get 1.0 x 10^6 cfu/ml, a further dilution of the 0.5 McFarland standard of a 24-hour clinical culture of methicillin-resistant *Staphylococcus aureus* was done. Then, in each of the tubes containing serially diluted extract, 0.5 ml of this concentration of bacterial organism was added. To determine whether the media supports the growth of methicillin resistant *Staphylococcus aureus* and to check the viability of the organism, two controls were created as follows: (1) control one (1) had bacteria and broth but no extract. (2) Control two (2) had only broth and but there is no bacterium inoculation added, which helped determine whether the broth was contaminated with other species. The experiment was repeated for each of the extracts [13, 19].

After that, the tubes were incubated for 24 hours at 37 °C in 5% CO_2. The minimum inhibitory concentration (MIC) of the dandelion root extracts against the test methicillin resistant *Staphylococcus aureus* clinical isolate was taken at the lowest concentration of the crude extract at which there was no physical growth and when the solution clearer as compared to the next tube dilution and the controls [19, 23, 24]. The experiment was repeated for each of the extracts.

2.7 Measurement of Minimum Bactericidal Concentration

The test tubes that had no turbidity for 400 mg/ml extract, test tubes one and two with 200 mg/ml and 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml respectively were swabbed and aseptically inoculated on Mueller Hinton Agar and incubated at 37 °C for another 24 hours. The Minimum Bactericidal Concentration was defined as the lowest concentration that prevented microorganism development. The goal of this experiment was to find the lowest dose of extracts that would kill MRSA [19, 25].

2.8 Statistical Analysis

All the experiments were performed in triplicates. The measured zones of inhibition, MIC and MBC. The data was analyzed as mean and Standard deviation (S.D), presented in tables and graphs.

3. Result and Discussion

3.1 Extracts

The grinded root was mixed with each of the solvents separately. Extraction was performed for different solvent at 100 g per 500 ml of each solvent and the % yield for, Ethanolic, Methanol, Chloroform and Aqueous extracts was (50.4%),(48.3%) (41.6%) and (41.5%) respectively.

3.2 Phytochemical Analysis

All of the phytochemicals that were examined were present in the roots of *Tataxicum officinale*, according to the preliminary phytochemical analysis performed on the ethanolic and

methanolic extract of the plants under study. The chloroform and the aqueous extract also show presence of some phytochemicals but not all, as indicated by the data presented in Table 1. Ethanolic and methanolic phytochemical of *Tataxicum officinale* roots extract are richer in phytochemicals that are tested such as Alkaloids, cardiac glycosides, phenols, flavonoids, terpenoids, tannins, and saponin. Whereas chloroform and aquous extract shows absence of phenols. Based on crude extracts of *Tataxicum officinale* root extracts, we can hypothesize that these bioactive components are what cause the antibacterial activity.

Table 1: Phytochemical test for *Taraxicum officinale* root extracts

	Constituents	Tests	Inference			
			Ethanolics	Methanolic	Chloroform	Water
1.	Alkaloids	Dragendorff's	+	+	+	+
		Meyer's test	+	+	-	-
		Hager's	+	+	+	-
2.	Flavonoids	Alkaline reagent	+	+	-	+
		Shinod's test	+	+	+	-
3.	Saponins	Na2CO3 solution	+	+	+	+
4.	Terpenoids	Horizon test	+	+	+	+
5.	Tannins	Ferric chloride test (FeCl3 solution)	+	+	+	+
6.	Cardiac Glycosides	Keller Killiani test	+	+	+	+
7.	Phenol	Ferric chloride test (FeCl3 solution)	+	+	-	-

3.3. Determination of Antimicrobial activity of *Taraxacum officinale* root extracts

The current study examines *Taraxacum officinale*'s antibacterial activity in various solvents. Extracts in four solvents - ethanol, methanol, chloroform, and distilled water extract, are used for this purpose. The extract demonstrated efficacy against the studied bacterial pathogen in methanol, ethanol and chloroform, but no action was observed in plane D.H2O. The highest activity zone of inhibitions was noted against methicillin resistant *S. aureus* (MRSA) at concentration of 400 mg/ml at 20.54±0.07 mm in ethanolic extract followed by methanolic extract 400 mg/ml at 19.80±0.6 mm, followed by Chloroform extract at 16.04±0.7 mm, and water extract at 400 mg/ml at 10.80±0.8 mm and no sign of inhibition on plain distilled water. Moreso *S. aureus* at concentration of 200 mg/ml at 10.99±0.06 mm in ethanolic extract followed by methanolic extract 200 mg/ml at 9.80±0.8 mm, followed by Chloroform extract at 6.04±0.9 mm, and water extract at 200 mg/ml at 0 4.80±0.5 mm and no sign of inhibition on plain distilled water.

At 400 mg/ml concentration, the result in ethanol, methanol and chloroform was good, water extract shows least inhibiting zoon while no activity was shown in Distilled.H2O zoon. At 200 mg/ml concentration, the result in ethanol, methanol and chloroform has low inhibiting zoon, water extract shows least inhibiting zoon while no activity was shown in Distilled. H2O zoon as shown in Table 2 and Figure 2.

Table 2: Activity of *Taraxicum officinale* root extracts against bacterial strains

Treatment Type	Concetration of Extract	Volume used	Zone of Inhibition of MRSA (mm) mean ± SD			
			Ethanolic	Methanolic	Chloroform	Water
Roots Plant Extract	50	120	0.00	0.00	0.00	0.00
	100	120	0.00	0.00	0.00	0.00
	200	120	16.10±0.14	12.99+0.4	8.30±0.1	0.00
	400	120	20.54±0.07	19.8±0.6	12.08±0.2	0.00
Control Positive Vancomucin		19.00±0.00	19.00±0.00	19.00±0.00	19.00±0.00	19.00±0.00
Control Negative Distilled water		0.00	0.00	0.00	0.00	0.00

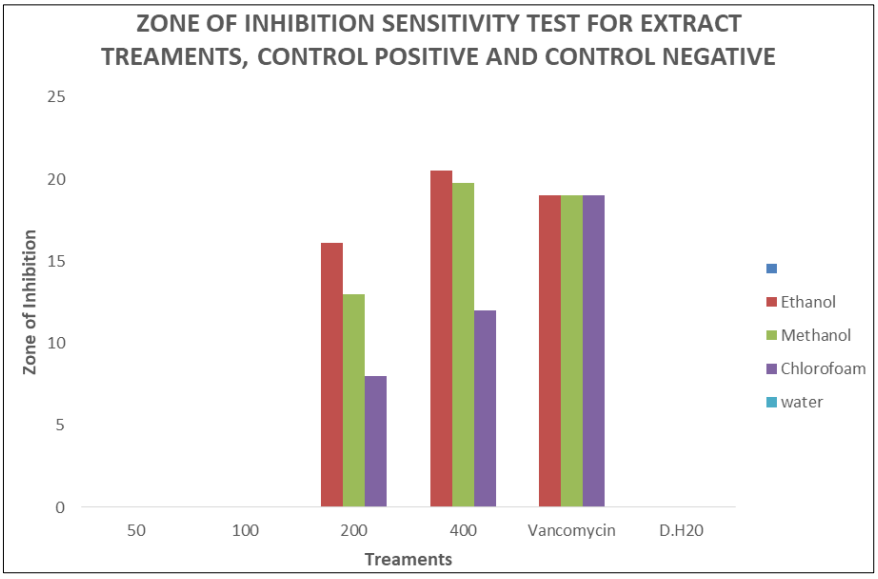

Fig 2: Antibacterial screening by agar well diffusion method

Table 3: MIC and MBC of *Taraxicum Officinale* root extracts MRSA

Solvent extracts	Minimum Inhibitory Concentration (MIC)	Minimum Bacterialcidal Concentration (MBC)
Ethanolic and Methanolic Extracts	˜ 3 mg/ml	˜ 25 mg/ml
Chloroform Extracts	˜ 6 mg/ml	˜ 25 mg/ml

3.4 Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MIC) of the *Taraxicum officinale* root extracts which is the concentration giving the least inhibitory activity and below which there is no further inhibition [13, 19]. The *Taraxicum officinale* root extracts minimum inhibitory concentration of ethanolic and methanolic extract on MRSA, was 3 mg/ ml and 6 mg/ ml for chlorofoam extract as shown in Figure 3. The *Taraxicum officinale* root minimum inhibitory concentration of aqueous extract on MRSA was not done, because it shows little to no activity on the zoon of inhibition as shown in table 2 and Figure 2. The antimicrobial activity of *Taraxicum officinale* root extracts against test MRSA with varying zones of inhibition has revealed the antimicrobial potency of the root of this plant. The results showed that *Taraxicum officinale* root inhibited the growth of organism. This is presumed to be due to the active compound present in this plant as related to previous analysis [18, 22]. The extracts of *Taraxicum officinale* root showed conspicuous degrees of antibacterial activity. The MRSA bacterial species were susceptible to the root extract of *Taraxicum officinale* but variations may occur depending on the type of extraction method used. For instance, ethanolic and methanolic extraction method inhibited the growth of MRSA more than the chloroform extracts. This result conformed to the result of Iqbal, 2014 on similar study. The minimum bactericidal concentration for ethanolic, methanolic, chloroform extract of *Taraxicum officinale* root is around 25 mg/ml. MBC was not performed for water extract. It was observed that the *Taraxicum officinale* root extract activities against MRSA is concentration dependent due to the MIC and MBC inhibition concentration variation as stated in methodology. This result conformed to the result of investigators on similar studies such as [13, 18, 19, 23]. Due to ever increasing microbial antibiotic resistance, it is important to identify natural antimicrobial compounds and the future development of this compound. The results presented above showed that ethanolic, methanolic and chloroform extracts of *Taraxicum officinale* root extracts had appreciable antimicrobial activity against MRSA. Aquous extract had little to no visible activity on MRSA on zoon of inhibition. The minimum inhibitory concentration (MIC) of each ethanolic, methanolic and Choroform extract of the *Taraxicum officinale* root extract revealed the best solvent for extraction. It was determined that *Taraxicum officinale* root extract had inhibitory effects against MRSA bacteria, causing diseases in humans. *Taraxicum officinale* root extract can be alternative to chemicals used in medication, food and cosmectics. It is believed that this research may identify certain chemicals that can be utilized to create new, more effective natural antibacterial medications.

4. Conclusion

The demonstration of *Taraxicum officinale* root extract activity against MRSA strain is an indication that it could be an origin of bioactive substances that could be used for antibacterial activity. Therefore, *Taraxacum officinale's* antibacterial activity may aid in the discovery of novel chemical families of antibiotic compounds that may function as selective agents for the treatment and control of infectious diseases.

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6. References

1. Liyun Shi, H.W., Zhe Lu. Staphylococcal Infection and Infertility; c2016.
2. Fisher EL, Otto M, Cheung GYC. Basis of Virulence in Enterotoxin-Mediated Staphylococcal Food Poisoning. Front Microbiol. 2018;9:436.
3. Kadariya J, Smith TC, Thapaliya D. *Staphylococcus aureus* and staphylococcal food-borne disease: an ongoing challenge in public health. Biomed Res Int. 2014;2014:827965.
4. Chambers HF, Deleo FR. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nat Rev Microbiol. 2009;7(9):629-41.
5. Gnanamani A, Hariharan P, Paul-Satyaseela M. *Staphylococcus aureus*: Overview of Bacteriology, Clinical Diseases, Epidemiology, Antibiotic Resistance and Therapeutic Approach. Chapter Metrics Overview; c2017.
6. Chakraborty S, *et al*. Antimicrobial activity of Cannabis sativa, Thuja orientalis and Psidium guajava leaf extracts against methicillin-resistant *Staphylococcus aureus*. J Integr Med. 2018;16(5):350-357.
7. Wangai FK, *et al*. Methicillin-resistant *Staphylococcus aureus* (MRSA) in East Africa: Red alert or red herring? BMC Infect Dis. 2019;19(1):596.
8. Algammal AM, *et al*. Methicillin-Resistant *Staphylococcus aureus* (MRSA): One Health Perspective Approach to the Bacterium Epidemiology, Virulence Factors, Antibiotic-Resistance, and Zoonotic Impact. Infect Drug Resist. 2020;13:3255-3265.
9. Fernandes TG, *et al*. *In vitro* synergistic effect of Psidium guineense (Swartz) in combination with antimicrobial agents against methicillin-resistant *Staphylococcus aureus* strains. Scientific World Journal. 2012;2012:158237.
10. Bento EB, *et al*. Association between Food and Drugs: Antimicrobial and Synergistic Activity of *Annona muricata* L. International Journal of Food Properties. 2013;16(4):738-744.
11. Cho JC, *et al*. Treatment of methicillin-sensitive *Staphylococcus aureus* bacteremia secondary to septic phlebitis using dalbavancin. J Clin. Pharm. Ther. 2015;40(5):604-606.
12. Subramani R, Narayanasamy M, Feussner KD. Plant-derived antimicrobials to fight against multi-drug-resistant human pathogens. 3 Biotech. 2017;7(3):172.
13. Iqbal SZ, *et al*. *In vitro* antibacterial study of *Taraxacum officinale* leaves extracts against different bacterial pathogenic strains. Journal of Pharmacognocy and Phytochemistry; c2014. p. 15-17.

14. Stewart-Wade SM, *et al*. The biology of Canadian weeds. 117. *Taraxacum officinale* G. H. Weber ex Wiggers. Canadian Journal of Plant Science. 2002;82(4):825-853.
15. Gadisa E, *et al*. Combined antibacterial effect of essential oils from three most commonly used Ethiopian traditional medicinal plants on multidrug resistant bacteria. BMC Complement Altern. Med. 2019;19(1):24.
16. Okwu MU, *et al*. Methicillin-resistant *Staphylococcus aureus* (MRSA) and anti-MRSA activities of extracts of some medicinal plants: A brief review. AIMS Microbiol. 2019;5(2):117-137.
17. Zouhir A, *et al*. Inhibition of methicillin-resistant *Staphylococcus aureus* (MRSA) by antimicrobial peptides (AMPs) and plant essential oils. Pharm Biol. 2016;54(12):3136-3150.
18. Yebpella GG, *et al*. Phytochemical screening and a comparative study of antibacterial activity of Aloe vera green rind, gel and leaf pulp extracts. International Research Journal of Microbiology (IRJM). 2011;2(10):382-386.
19. Stanley MC, Ifeanyi OE, GodsonEziokwu O. Antimicrobial effects of Aloe vera on some human pathogens. International Journal of current microbiology and applied sciences. 2014(3):1022-1028.
20. Kancherla N, *et al*. Preliminary Analysis of Phytoconstituents and Evaluation of Anthelminthic Property of Cayratia auriculata (*In vitro*). Maedica (Bucur). 2019;14(4):350-356.
21. Amin MM, SS S, MM JM. Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale*. Wudpecker Journal of Pharmacy and Pharmocology. 2013;2(1-5).
22. Ahmad I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J Ethnopharmacol. 2001;74(2):113-23.
23. Ho R, Dn K. *In vitro* Antifungal activity of leaf extracts from Aloe secundiflora, Bulbine frutescens, Vernonia lasiopus and Tagetes minuta against Candida albicans. Medicinal & Aromatic Plants, 2016, 5(02).
24. Kumar RS, Rajkapoor B, Perumal P. Antioxidant activities of Indigofera cassioides Rottl. Ex. DC. Using various *In vitro* assay models. Asian Pac J Trop Biomed. 2012;2(4):256-61.
25. Yusuf B, *et al*. *In vitro* Antibacterial Evaluation of Four Selected Medicinal Plants against *Staphylococcus aureus* Isolated from Bovine Mastitis in Mieso District West Hararghe Zone, Oromia Regional State, Ethiopia. The Open Microbiology Journal; c2022.