



A Study to Check the Antibacterial Activity of Herbal Powder Extracts Against *Klebsiella Pneumonia*

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Abstract

Aim: This study attempted to discover the newer compounds from medicinal herbs. The goal of the present research was to investigate the bactericide activity of herbal powder extracts.

Method: In this study, I choose the method of agar well diffusion to check the antibacterial of herbal powder extracts of Finger millet (*Eleusinecoracana*) and Sandal (*Santalum album*). Agar well diffusion method was the effects were used to check the antibacterial activity against 6 pathogens of Gram-positive which include *Bacillus*, *Methicillin-resistant Staphylococcus aureus* (MRSA), *Klebsiella pneumonia* (*K. pneumon*), *Pseudomonas aeruginosa*, *Escherichia coli* (*E. coli*).

Result: From this research, it was revealed that both extracts of Finger millet and Sandal show antibacterial activity against the *Klebsiella pneumonia* (*K. pneumon*) bacterial strain.

Conclusion: In conclusion, both herbal extracts found to be effective or have broad-spectrum antimicrobial activity and further research or attempts need to be done in this area.

Keywords: *Eleusine coracana*, *Santalum album*, Infectious diseases, Antibacterial activity, Herbal Extracts, Zone of inhibition

INTRODUCTION

Good food or nutrition may be a primary right. So, to possess a healthy or fit population that will encourage development, the connection between nutrition, health, and food, ought to be strengthened [1]. Since the ages, the use of botanical gardens has been globally used to cure and manage medical infirmity. Because of the emergence of synthetic antibiotics and drugs usage of botanical plants and herbs was got decline over the period. According to the WHO survey report, around 80% of people depend on medicinal herbs for their medicinal herbs. The herbal medicines are considered to be generating billions of dollars in trade and also have recorded an impact on the economy globally.

Ayurveda, Unani and homeopathic medicines are highly using botanical medicines. The herbal medicine includes anti-inflammatory compounds, antitumor activity, tannins act as antibacterial substances, phenolic compounds act as antioxidants, and alkaloids act as stimulants. Flavonoid acts as anti-allergic, antidiarrheal activities [2].

For new drug development and scientific as well as clinical study plant secondary metabolites are very much an important source for this purpose. Special importance is given to indigenous and traditional herbs. For a long time, India and China have been using herbal medicines [3]. On the consumption of the herbs, societies have their own

beliefs and applications which mostly depend on their sociocultural status. Few species are grown in the Netherlands but valerian and parsley are presently grown.

About 60 to 70 species of herbal medicines are produced in Bulgaria. Lavender is the main plant in France. A total of 10,000 hectares, herbs are grown for healing and spices in Germany today. About 10-15% of total herb species are grown in Greece.

Herbs are used for an outsized vary of functions including repellents, fragrances, cosmetics beverages, nutrition, flavorings, smoking charms, industrial uses, and dyeing. About 40% of drug prescription is still found in herbs nowadays. Culinary herbs are big and used for many years, and that they have become more and more fashionable within the US for his or her ability to increase and supplement the flavors of a good selection of foods. Even

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ranges of the herbs square measure famous to be sources of synthetic resin compounds, its integrative knowledge is scarce. Numerous herbs at the side of vegetables and fruits carry varied photochemical besides synthetic resin compounds, like atomic number 7 compounds, carotenoids, and vitamin C [4].

A report by information showed that out of the 104 compounds that square measure used globally as medicine over thirty-seven years, 60 of them originate from ancient Chinese healthful plants. More modern reports reveal that a number of the plant-derived flavonoids square measure extraordinarily active at nanomolar levels against bound bacterium and square measure a lot of action than well-established antibiotics like antibiotic and tetracycline.

Hence, screening of healthful plants for their bioactivity is extraordinarily vital to spot promising candidates that square measure source of potential therapeutic agents [5].

Medicinal herbs have a long time ago utilized by completely different ethnic teams everywhere globe for thousands of years. For therapeutic or health-promoting functions, herbs are eaten in different ways. Several of those medicinal herbs could employ their helpful properties, by removing surplus free radicals such as reactive oxygen and chemical element species from the bodies. Current science has supplied a lot of solid proof to hold up this claim [6].

The earliest literature mentions herbal medicines for age-linked diseases namely diabetic wounds, immune and liver disorders, cognitive state, and pathology. These medicines are made from renewable resources of the raw materials through eco-friendly methods and can initiate economic prosperity to plenty of growing raw materials [7].

Finger millet (*Eleusinecoracana*) 'Ragi' is one of the foremost standard millet in India. Ragi is one of the most important because it contains a high source of minerals, proteins, dietary fiber, and low in fats [8]. Finger millet could be a fast-multiplying cereal crop that comes to maturity within 3-6 months and typically in forty-five days. Finger millet is mostly present in distributed areas, banks, or on the roadside.

It is normally present between one thousand and 2000 m altitude in Southern Africa or Japanese up to 2500 or 3000 m altitude within the Himalayas. Annual downfall ranging from 500 to 1000 mm, area unit appropriate, provided it is evenly distributed throughout the season. It grows better at concerning 23°C temperature however will face up to some cooler and warmer conditions [1].

Finger millet is a lesser widely known cereal of Indian or African origin and it is associate degree underutilized. The grain is high in supermolecule, fat, and minerals like (calcium, iron, and phosphorous), relative to rice, corn, and sorghum. It's a superb dietary supply of essential amino acid, an important organic compound [9].

Ragi or (*Eleusinecoracana*) is a vital food staple for the standard customers and therefore individual's happiness to the lower socio-economic strata within Indian landmass or conjointly in a large number of African countries.

It's a little seeded (1.2 or 1.8-millimeter diameter) minor cereal containing, brown to burnt sienna colored testa with a minutely undulated surface. Their covering is membranous, thin superimposed, or are loosely hooked up tissue covering complete seed or customarily detaches throughout harvest and through easy abrasion [10].

In the Asian countries, it is sixth in production after maize, sorghum, wheat, and rice. It is abare seed with a brick red-colored testa or it is utilized in the shape of total meal preparation of the ancient foods, like muddy and ambali. Epidemiological studies have incontestable that the regular intake of whole-grain cereals or its products will defend in opposition to the chance of various diseases, sort II polygenic disease, epithelial duct cancers, and a range of various disorders.

Since millets are commonly ready from the complete meal, vitamins, wheat, rice, and other millets targeted in the outer layer of the grain or testa kind the half of food or supply its nutritionary and health advantages [11].

Tannins or the flavonoids present in millet reproductive structure square measure multifunctional or vigorous that they behave as reducing agents (free radical terminators), metal chelators. Ragi is a potent supply of antioxidants and this has rich radical-scavenging activity above that of rice, wheat, or some other millets.

The Finger millet not has gluten and thence sweet for the patients plagued by abdomen disease. It has been currently used as composite flour creating biscuit thanks to its rich calcium content. It has the definite benefit of being a blighter resistant crop or drought-free crop, needs very low irrigation or substitutes input, and yet undergoes the best yield [12].

The sandal tree, additionally called Chandana in the Republic of India, is botanically genus *Santalum album L.* and they belong to *Santalaceae*. The plant, *S. album* is indigenous to the highlands of the southern Republic of India. The tree attains a peak of 60-65 feet. It normally happens at altitudes of the 2000-3000 feet. Sandalwood plant is major used as agent, or additionally astringent activity and sedative effect, made useful as a disinfectant in diuretic, medicament and stimulant, reproductive organ and bronchial tracts.

The good strong and lasting fragrance made wood oil helpful in the cologne trade. A similar is additionally used Asanti-poison, fever, memory improvement, as a blood purifier, tonic for heart, and abdomen liver [13].

Folks have used shoe stem (*Santalum spp.*) medicinally to cure many ailments, especially in India. The *S. album* in the main accustomed treat inflammation, facial skin condition,

headaches, and skin itching, the Santalbicacid, which is derived from the oil of *S. acuminatum* R. BR., restricted the growing of *Staphylococcusepidermidis*, *cocci aureus*, and certain infective fungi. Early Hawaiians used the leaves and bark of shoe stem in every remedy to get rid of dandruff and destroy lice, sexually transmitted diseases, and controls eczema [14].

Phytochemical estimation of sandalwood extracts discovered that tree is more in tannies, glucoside, phenolics additionally to the terpenoids. The trees are extremely scented and are the costliest style of the wood within the universe, after genus *Dalbergia melanoxylon*, African Blackwood. Sandalwood enlarges in Australia, Pacific Islands, Hawaii, and tropical Asia. In preserving the dead and in the ceremony of burning it is used in Egypt to honor God.

Products of wood are widely used for wood carving, observance pyres; within the food business as a flavor ingredient, and perfumes, soaps, detergents, cosmetics, in insect repellent. Major constituent in wood oil is a santol, a mixture of 2 isomers, α , and β -santalol. Two molecules chiefly linked with sandalwood's fragrance, whereas is principally reported for its antitumor properties. The antimicrobial activity of leaf and stem liquid extracts of true sandalwood were determined against genus *Pseudomonas*, and *E.coli*, *Staphylococcus aureus* and within which leaves extract proclaimed considerably maximum inhibition once comparison to the stem extract [15].

Numerous studies are printed on antimicrobial activities of extracts against differing varieties of microbes, including foodborne pathogens. still, the observations reportable for completely distinct studies square measure hard to check personally, actually due to low variety of plant samples were tested, completely different check strategies or numerous strains of bacteria and sources of antimicrobial samples utilized [16].

The goal is to inspect a different plant extract of different herbs against multiple kinds of organisms that are composed of Gram-negative or positive bacteria [17].

The purpose of the study was intended to research the medication activity of (*Eleusinecoracana*) and sandalwood (*Santalum album*) extracts. The medication activity is to be determined against the pathogens whereas exploitation Well Diffusion technique with seeded cultures.

MATERIALS AND METHODS

Sample material

Sample of *Eleusinecoracana* and *Santalum album* was collected from the commercial source. Sample collection was conducted in February 2020. They after the sample collection first their extracts were prepared.

Plant Extracts Preparation

Finger millet extracts preparation: 25% solution of Finger millet (*Eleusinecoracana*) extract was prepared by using 4ml of water in which 1g of Finger millet powder were added in falcon tube and then centrifuge it well.

Sandal extracts preparation

25% solution of Sandal (*santalum album*) extract was prepared by using 4ml of water in which 1g of sandal powder were added in falcon tube and then centrifuge it well.

ASSAYS FOR ANTIBACTERIAL ACTIVITY

Test Microorganisms: Antimicrobial activity was evaluated by using microorganisms. Six bacterial isolates were used like *Klebsiella pneumonia*.

Sub-Culturing and storage of Microorganisms: Bacterial isolate was maintained by first sub-culturing it on LB agar media under their favorable conditions of growth for 24 h in dark at 37°C. whenever used again on LB agar they were refreshed.

Antibacterial/Antimicrobial Activity: The antibacterial action of the extracts and probiotics was determined through well diffusion assay, Pour plate, and Face to Face method. The basis of these assays is the spreading of antimicrobial agents in a solid medium.

Pour plate method

In the pour plate method, 20 μ l extracts of Finger millet were pipette into the sterile Petri dish. Utilizing a loop or pipette, serial dilution of mixed cultures is required for the pour plate method. LB agar media in the molten state is cooled to 45°C if agar is too hot, then the bacteria may be killed and if agar is too cool, then after adding sample to the Petri dish then mix the 2% bacterial culture (1×10^{-5} CFU/ml) in the liquid agar medium in a separate tube. Put the LB media along with bacterial culture into the same Petri dish containing the specified amount of the diluted sample. Swirl the plate in the clockwise or anticlockwise direction to mix them well. Cover the plates carefully with left hand and plates were wrapped with parafilm and then placed it into an incubator at 37°C for about 24 h. After 24 h, there will be a lawn of inhibition of bacteria on the Petri plate which indicates the presence of activity of extracts (**Figure 1**). But if there will be no zone of inhibition, and then there will be no activity of extracts against selected bacterial strains.

Face to face method

In Face-to-face methods, LB agar in a molten state (at nearly 45°C) was poured in a sterile Petri plate and after pouring the LB agar allowed them to solidify. Divide the Petri plate into two portions with the help of the marker on its downside. Pick a colony of bacteria with the help of the autoclaved toothpick and streak one portion of this plate with selected bacteria. On the second half portion, place the 20 μ l diluted sample of an extract with the help of the pipette. Seal the

plate with the help of the parafilm and for 24 h incubate it at 37°C.

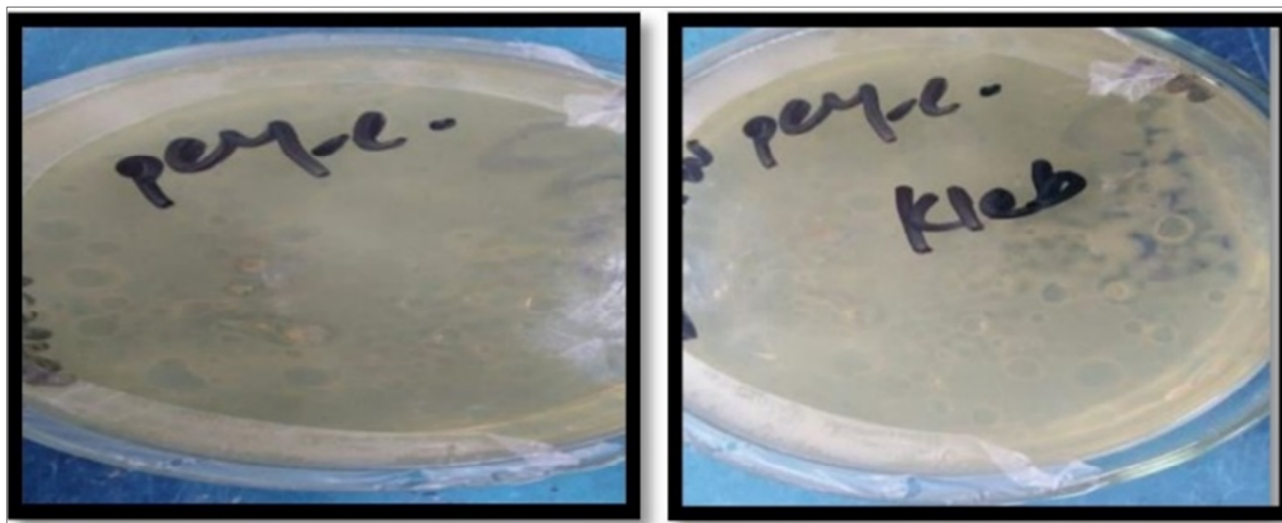


Figure 1. No antibacterial activity of plant extracts against *Klebsiella pneumonia* (*K. pneumon*) by pour plate method.

If bacteria spread on the whole plate after 24 h means that there will be no antibacterial activity. If the bacteria are not spread on the whole plate or it shows its growth only on its

section means that there will show some antimicrobial activity (**Figure 2**).

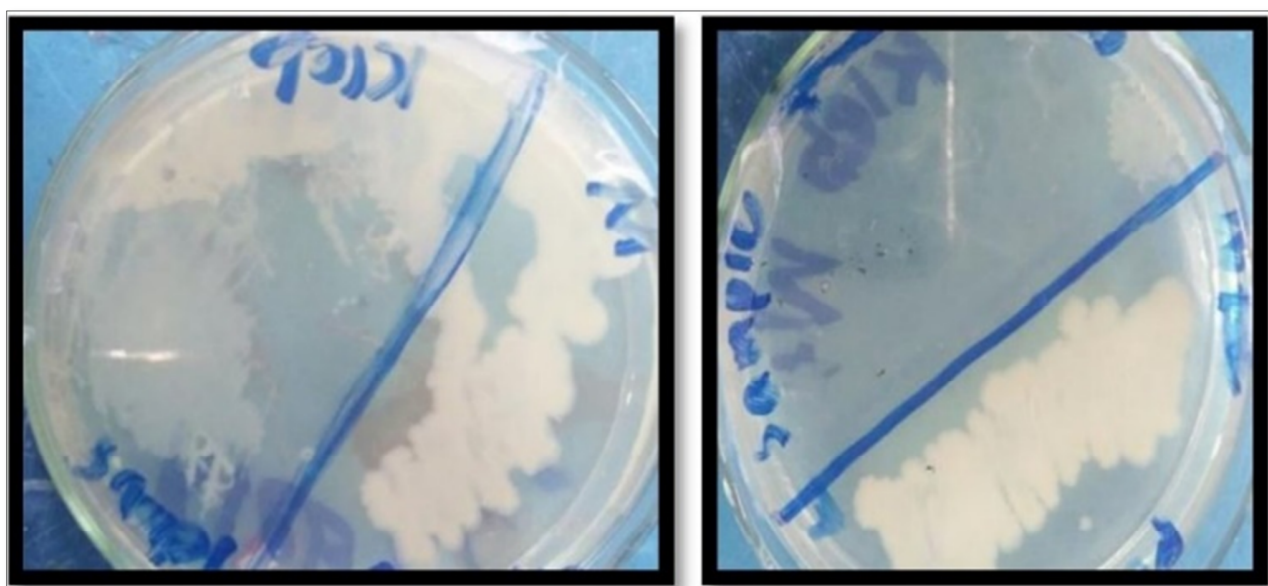


Figure 2. No antibacterial activity of plant extracts against *Klebsiella pneumonia* (*K. pneumon*) by Face-to-face method.

Well Diffusion Method

LB in a molten state (at nearly 45°C) was seeded with 2% bacterial inoculum (1×10^5 CFU/ml) and allowed to solidify. Made wells of 6mm or height were bored in the inoculated agar plate using a sterile plastic tip and with the help of micropipette 20µl of plant extracts was added into the wells.

Carefully wrapped the plates with the parafilm and incubate them. If there will be inhibition or a zone is formed then it indicates the activity of plant extracts against selected bacterial strains (**Figures 3 & 4**). But if no zone of inhibition will be formed, then no activity of plant extracts against that bacterial strain were showed.



Figure 3. Zone of inhibition of (*Eleusinecoracana*) extracts against *Klebsiella pneumonia* (*K. pneumon*) by well diffusion method.

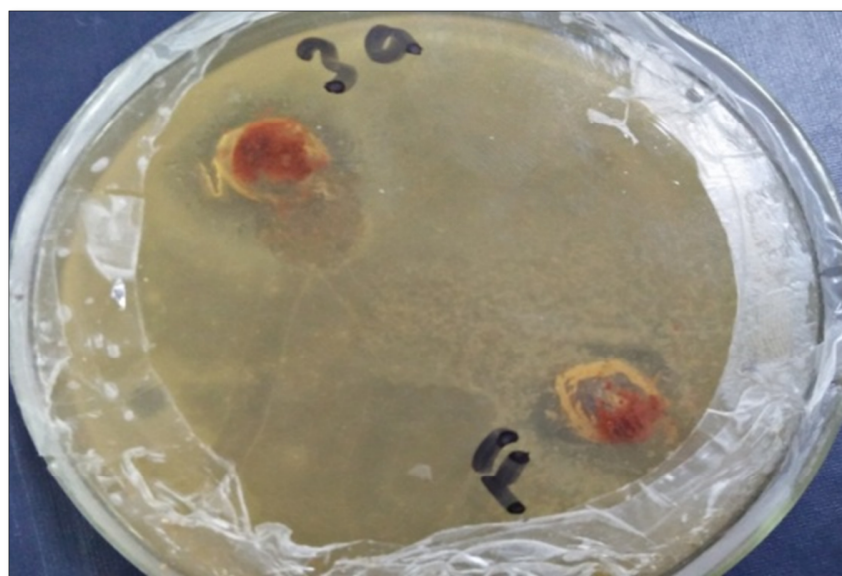


Figure 4. Zone of inhibition of santalum album extracts against *Klebsiella pneumonia* (*K.pneumon*) by well diffusion method.

RESULTS

A sample of Finger millet (*Eleusinecoracana*) was purchased from the local market and a sample of sandal (*santalum album*) was also collected from the same source. The antimicrobial activities of both samples were estimated against specific pathogen bacterial strains.

Antibacterial Activity

Pour Plate Method: The extracts of Finger millet (*Eleusinecoracana*) and sandal (*santalum album*) were screened against the strain of Bacteria. e.g., *Bacillus*,

Klebsiella pneumonia (*K.pneumon*) with the pour plate method (**Figure 1**). This method shows no antibacterial activity of selected bacteria against the extract.

Face to face method: The extracts of Finger millet (*Eleusinecoracana*) and sandal (*santalum album*) were screened against six strains of Bacteria and microorganisms used in the assay. e.g., *K.pneumon*, through the pour plate method (**Figure 2**). This method also shows no antibacterial activity of selected bacteria against the extract.

Well diffusion Method: The antibacterial activity of Finger millet (*Eleusinecoracana*) and sandal (*santalum album*)

extracts were investigated by using a well diffusion method against a bacterial strain (Figures 3 & 4). *Klebsiella pneumonia* (*K.pneumon*) showed susceptibility with a zone

of inhibition 1.5cm (Figure 5). *Klebsiella pneumonia* (*K. pneumon*) showed susceptibility to sandal extract with inhibition of 1.5cm (Table 1).

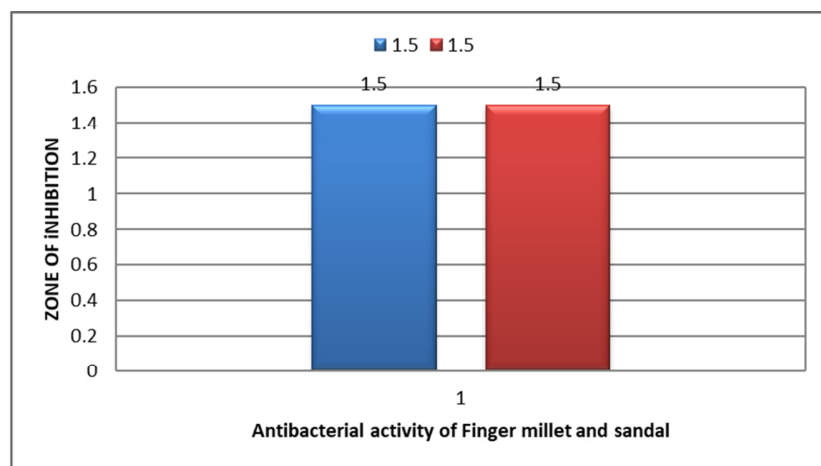


Figure 5. Graph showing the antibacterial activity of Finger millet and Sandal.

Table 1. The antibacterial activity of Finger millet (*Eleusinecoracana*) and sandal (*santalum album*) extracts were investigated by using a well diffusion method against a specific bacterial strain.

Bacterial Strain	Extract of Finger millet	Extract of Sandal
<i>Klebsiella pneumonia</i>	<i>Eleusinecoracana</i>	<i>Santalum album</i>
Zone of Inhibition(cm)	1.5cm	1.5cm

DISCUSSION

The antimicrobial activities of various pathogens that are accountable to infectious diseases were progressively investigated by using healthful plants or herbs as the alternative to artificial medicines.

Plant-derived antimicrobials are present in different plants or herbs [18]. The antimicrobial efficiency of plants is believed to result in tannins, saponins, phenolic resin compounds, necessary oils, and flavonoids. It's fascinating to see that even crude extracts of those plants show smart activity against multidrug-resistant strains wherever recent antibiotic therapy has unsuccessful [19]. Different photochemical with biological properties present in herbs and herbal extracts stimulate human health and facilitates the threat of chronic disease. Phenolic acids and flavonoids referred to as bioactive agents, often take place in seasoning or herbaceous plants [20]. In some studies, it is reportable that there's an extremely positive linear relationship between antioxidant activity, antibacterial activity, and total phenolic content in a few herbs [21].

According to the world health organization (WHO), over the world 21,000 plants were used for medicinal purposes. Because India is the largest producer of medicinal plants such as Finger millet and Sandal they are called as botanical

garden of the world. Out of the 21,000 plant species 2500 species were used in India and 150 species were used locally on large scale [22].

Various bacterial agents like pathogenic *E. coli*, *Vibrio cholera*, *Shigella spp*, *Salmonella spp*, *Pseudomonas spp*, *Klebsiella spp*, and *Staphylococcus aureus* are the microorganisms that most commonly causing the diseases [23].

Plants primarily based on antimicrobials represent an enormous trapped supply for medicines and additional exploration of plant microorganisms would like to occur. Compared to artificial antibiotics, antimicrobials of plant origin aren't related to many aspects or side effects and have a vast therapeutic potential to heal several infectious diseases [24].

Jirovetz [25] reported that phenolics and terpenoids are the major categories of an *S. album* for their antibacterial property and the constituents of *S. album* seeds oil constituents and their synthetic analogs have strong antimicrobial and antibacterial agents. Chourasia [26] evaluated that the *S. album* essential oil shows most potent against *E. coli* and *Bacillus mycoides*.

Jones [27] reported that in *S. album* the major component is Santalbic acid and in charge of the antimicrobial activity of the sandalwood. In *S. album* seeds, the non-polar component Santalbic acid is very effective against some pathogenic fungi and Gram-positive bacteria. Jones [27] reported that in *S. album* the major component is Santalbic acid and accountable for the bactericidal activity of the sandalwood. The seeds of *S. album* have non-polar components like Santalbic acid that is very effective against some pathogenic fungi and Gram-positive bacteria.

Herbal plant Ragi (Finger millet) has many useful properties such as methionine is important in ragi as it is a small or less expensive millet and it contains a large amount of calcium, iron, and fiber. For children of 6 months, Ragi (Finger millet) is the most suggested food. Ragi is the most consumed in villages but now people in cities started using the ragi is an important part of their diet reported by VidyaLaxme [28].

Cesar [29] investigated that around 90% of people living in Africa and Asia and about 3.5 billion were at a danger of Ca deficiency in 2011 and reported that the Finger millet is more superior or high nutritional property as compared to rice or wheat for these deficiencies. Few researches described the antimicrobial activity of *Eleusinecoracana* and sandal herbal powder extract.

Hammer [17] evaluated that those plants which are used for treatment or traditional medicines have a large-scale potential of antimicrobial activity. Hence, the purpose of this study was to reveal, in-vitro antimicrobial activity of herbal powder extracts such as Finger millet and sandal against the bacterial strains. The intend of this short study was to focus on herbal extracts against various strains of bacteria and to compare their antimicrobial activity against bacterial strains, which are responsible for infectious diseases.

The Antibacterial activity of plant extracts was performed through the agar well diffusion method against one bacterial strain including *Klebsiella pneumonia* (*K. pneumon*) and which shows the inhibitory activity. The bacterial strain showed a zone of inhibition or susceptibility against these plant extracts (**Figures 6**). The bactericidal activity of herbaceous plant extracts was performed with pour plate or face-to-face method but it shows no inhibition against the selected bacteria (**Figures 4 & 5**).

CONCLUSION

As there was less research on the antimicrobial activity of Finger millet so, this study was to check the susceptibility against 6 bacterial strains like *Bacillus*, *Methicillin-resistant Staphylococcus aureus* (MRSA), *Klebsiella pneumonia* (*K. pneumon*), *Pseudomonas aeruginosa*, *Escherichia coli* (*E. coli*) of these plants alone or with combination by using different methods. But only against *Klebsiella pneumonia* (*K. pneumon*) was checked by pour plate, face to face, or agar well diffusion. It is concluded that both herbal extracts

were found to be effective or have broad-spectrum antimicrobial activity and further research or attempts need to be done in this area.

AUTHOR'S CONTRIBUTION

Sample collection and culturing of bacterial strains have been done by Aqsa Qurban and all other works like well diffusion method, face-to-face has been done by Huda Ishfaq.

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DISCLOSURE STATEMENT

The authors declare no conflict of interest.

ETHICAL COMPLIANCE

This article does not contain any studies involving human participants or animals performed by any of the authors.

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