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Research Paper

Antibacterial activity of three indigenous plants against pathogenic bacteria

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Abstract

In recent years, there has been a growing interest in researching and developing new antimicrobial agents from various sources to combat microbial resistance. Therefore, a greater attention has been paid to antimicrobial activity screening and evaluating methods. Microbial resistance is a major issue now days and is still a challenging situation for the whole globe. Scientists and researchers everywhere in the world are trying to cope this situation. Microbial resistance against drugs makes the microbes more active and more protective. To breakdown this protective zone WHO is still monitoring the drugs and modifying their formulas according to the needs. As well as new drugs from different sources have been discovered. Plants were the major source of drugs in past and also in present century. In present study three plants extracts (i.e., *Carissa opaca*, *Ferula asafoetida* and *Cannabis sativa*) were used against five bacterial strains named *Escherichia coli, Staphylococcus aureus, Salmonella enterica, Pseudomonas aeruginosa, Streptococcus pyogenes*. All the drugs were applied in the form of aqueous extract via well diffusion method and zone of inhibition were measured and photographed after 24 hours. The antibacterial effect of floral drugs in this study showed less or more activity. In conclusion, these antibacterial drugs shown killing of bacteria, on different concentrations zones of inhibition measured were of different size. All these selected plants shown a direct relation with concentration of extract, higher the concentration of extract gives larger zone of inhibition, hence these plants extracts are beneficial with respect to their accessibility as well as economically favorable and can proved a better approach in future.

Introduction

Medicinal plants are the backbone of traditional medicine, as on regular basis more than 3.3 billion people in underdeveloped countries utilize medicinal plants (Davidson-Hunt, 2000). Medicinal plants are a rich source of antimicrobial agents (Mahesh and Satish, 2008). The World Health Organization (WHO) encourages the inclusion of herbal medicine in health care because medicinal plants are source of bioactive compounds that have therapeutic potential (Amos *et al*., 2001).

Human body accept natural medicines more than the synthetic drugs. Medicinal plants contain bioactive compounds such as alkaloids, flavonoids, tannins and terpenoids and have prevailing antimicrobial activity, could be a key source in discovering new drugs because of their key and potential role to inhibit or kill both spoilage and pathogenic microorganisms (Cowan, 1999).

Drugs from these plants are effortlessly available, economical, safe, efficient, and hardly ever accompanied by side effects. Plants which have been preferred for medical use over thousands of years represent the most apparent starting point for new therapeutically efficient drugs for example anticancer drugs (Dewick, 1996) and antimicrobial drugs (Phillipson, *et al.,* 1996). In recent times, practice of medicinal plants has increased despite the advances made in the ground of chemotherapy.

Microbial resistance to conventional antibiotics and their rapid progression has raised severe concerns in treating infectious diseases. A significant increase in the pathogenic resistant strains has been reported, which steered the novel multi-resistant organisms against the current synthetic drugs. The selective pressure on current antimicrobial agents is due to intense and indiscriminate use of drugs and it makes the bacteria less susceptible (Farjana *et al*., 2014). In addition, conventional therapy frequently fails to respond on diseases caused by resistant microorganisms that causes prolonged illness and amplify the chances of death. This severe issue spurs a greater need for more potent plant-mediated antimicrobial agents, alongside uncovering the active ingredients that can act as a template to generate new antimicrobial drugs (Biswas and Das, 2011).

In the past few years, the indiscriminately use of commercial antimicrobial drugs for the treatment of infectious diseases, human pathogenic microorganisms have developed multiple drug resistance (Janovska *et al*., 2003). In addition, the fight against bacterial infections becomes complicated because many bacteria have developed resistance to most antibiotics, which has been a major world health challenge (Benbrinis, 2018). In present study three plants extracts were used against five bacterial strains named *Escherichia coli, Staphylococcus aureus, Salmonella enterica, Pseudomonas aeruginosa, Streptococcus pyogenes*. These plants shown a direct relation with concentration of extract, higher the concentration of extract gives larger zone of inhibition hence these plants extracts would be beneficial with respect to their accessibility as well as economically favorable and can proved a better approach in future.

Materials and Methods

Plants Sample Collection

The plant parts used in this study were collected from different areas of Azad Jammu and Kashmir (AJ&K). Leaves of *Carissa opaca* were collected from the valley areas of Kotli AJ&K in month of March. Resinous material of *Ferula asafoetida* was brought from Khuiratta District Kotli AJ&K. *Cannabis sativa,* was collected from Mughal Pura District Mirpur AJ&K.

Bacterial isolates

The bacterial strains *Escherichia coli, Staphylococcus aureus, Salmonella enterica, Pseudomonas aeruginosa, Streptococcus pyogenes* used in this study were collected from Combined Military Hospital (CMH) Rawalpindi and stored in refrigerator at -4°C. During experiment cultures were grown on agar media. Each bacterial strain was regularly sub cultured and freshly grown cultures were used in each experiment.

Sterilization

For this experiment, all the equipment e.g. Petri plates, test tubes, tips and prepared agar media for bacterial growth and saline solution for preparation of bacterial inoculums were autoclaved at 121°C temperature and 15 psi of pressure. After sterilization in autoclave, whole material was transferred in laminar flow to avoid contamination, poured agar in Petri plates and keep them in laminar flow for 20-25 minutes until agar solidify then transfer the Petri plates in refrigerator at -4°C for 24 hours.

Preparation of agar media and saline solution

We dissolved 28 grams of agar powder in 100ml distilled water and adjusted the amount of water and agar as our demand. To prepare saline solution 0.9g of NaCl was added in 100ml distilled water. Colonies from fresh culture were picked and added in saline solution to mix. The saline solution was swabbed on agar plates to get bacterial growth for experiments.

Preparation of inoculum

We dissolved 0.9 mg of sodium hydroxide in 1000 ml distilled water in a conical flask to get saline solution. A volume of about 10 ml of saline solution was added in every sterilized test tube. Colonies from every freshly cultured bacterium after 24 hours were separated via a sterilized wire loop and then added in saline solution, store it for 24 hours and used in experiments.

Plants collection and extraction

Plant materials were collected from different areas of Azad Jammu and Kashmir. After collection samples were washed and then placed in the place where direct sunlight cannot reach to

destroy their phytochemical compounds. Completely dried plant materials were grinded in grinder and powders saved in labelled airtight containers. To get extract about 15 grams of plant material was dipped in 100 ml distilled water for 24 hours then filtered via filter paper and saved in refrigerator.

Test of antimicrobial activity

To determine the antimicrobial activity, agar well diffusion method was used. In this method first cotton swabbing from saline solution on agar plates was done. When whole plate swabbed with seeded saline solution then wells were formed with sterilized cork borer. Four wells were formed on plate, every well was 6 mm in diameter. Extract of plant was poured in every well with the help of micropipette with sterilized tips and separate tip was used to pour extract in every well. Antimicrobial activity of plant extract was checked by adding 100 µl, 80 µl, 60 µl and 40 µl in respective wells.

After adding the extract, plates were remain kept in the laminar flow for some time so that extract was absorbed completely in agar then transfer the plates in incubator to keep them at 37°C for 24 hours. After 24 hours clear zones of inhibition were seen and measured in millimeters (mm).

Results

Antibacterial activity of aqueous extracts of *C. opaca*, *F. asafoetida* and *C. sativa* was determined by well diffusion method against five bacterial strains named as *E. coli, S. aureus, S. pyogenes, P. aeruginosa* and *S. enterica.* Antibacterial activity of *C. opaca* against selected pathogens were different at different concentrations of extract. The minimum concentration which is used in this study was 40 µl and highest concentration was 100 µl. At 40µl the zones were 13, 12, 2 and 14 mm against *E. coli, S. enterica, S. pyogenes* and *P. aeruginosa* respectively. However, no zone of inhibition was measured against *S. aureus* at 40µl. At 60µl the zones were 15, 14, 14, 13 and 14 mm against *E. coli, S. enterica, S. pyogenes, S. aureus* and *P. aeruginosa* respectively. At 80µl the zones of inhibition were 16, 14, 14, 14 and 15mm respectively. The highest concentration (i.e., 100µl) gave 17, 15, 15, 18 and 15 mm against *E. coli, S. enterica, S. pyogenes, S. aureus,* and *P. aeruginosa*. The *C. opaca* showed the highest activity at 100µl which was 18 mm against *S. aureus* as shown in figures.

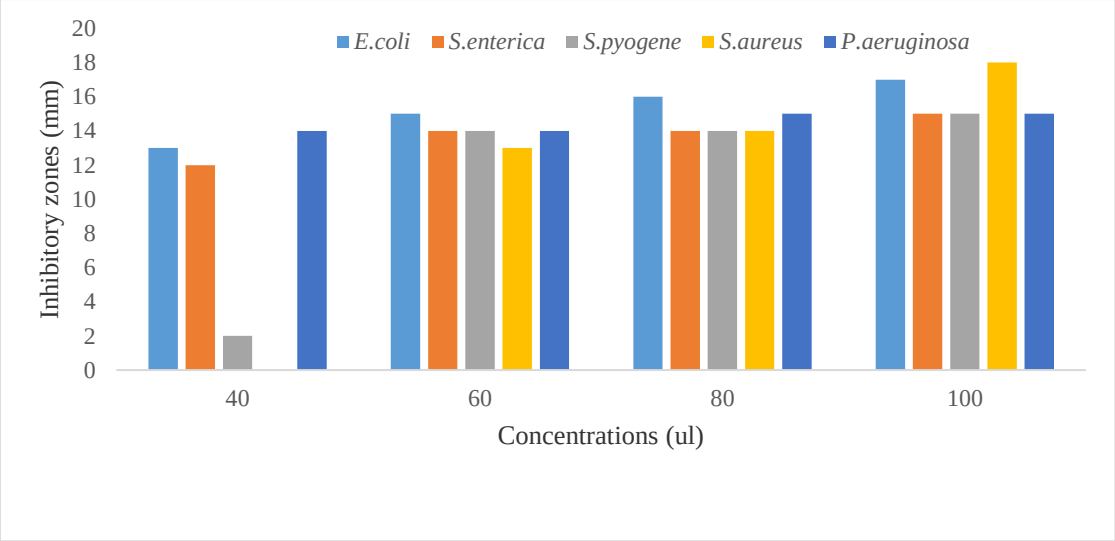

Figure 1: Antibacterial activity of *C. opaca* against pathogens and their zones of inhibition at different concentrations.

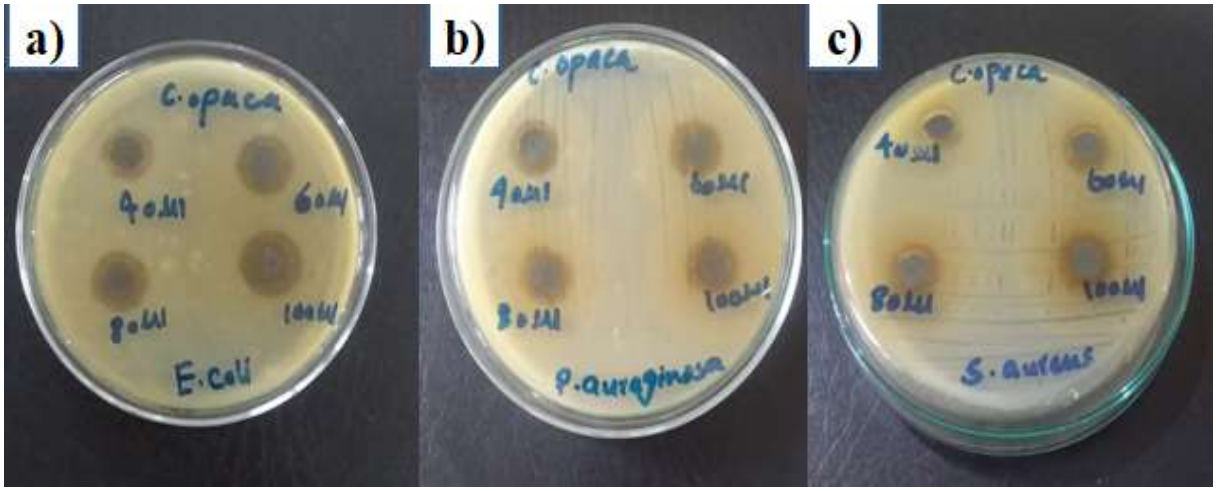

Figure 2: Antibacterial activity of *C. opaca* (a) against *E. coli, (b)* against *P. aeruginosa* (c) against *S. aureus*

Antibacterial activity of *F. asafoetida*

The resin material of *F. asafoetida* was used in this study showed a very remarkable antibacterial activity against bacterial strains, but *E. coli* showed resistant against *F. asafoetida.* The zones of inhibition measured in case of *S. aureus* were 8mm, 16mm, 16mm and 20mm at 40µl, 60µl, 80µl and 100µl respectively. Zones in case of *S. enterica* were 12mm at both 40 and 60µl and 16mm at 80 and 100µl. It means the slight difference in concentration of extract showed no enhancement of zones. *F. asafoetida* showed 8mm, 12mm, 12mm and 16mm zones against *S. aureus* at these predenoted concentrations. While *P. aeruginosa* shows 8mm, 16mm, 16mm and 20mm zones at these selected concentrations.

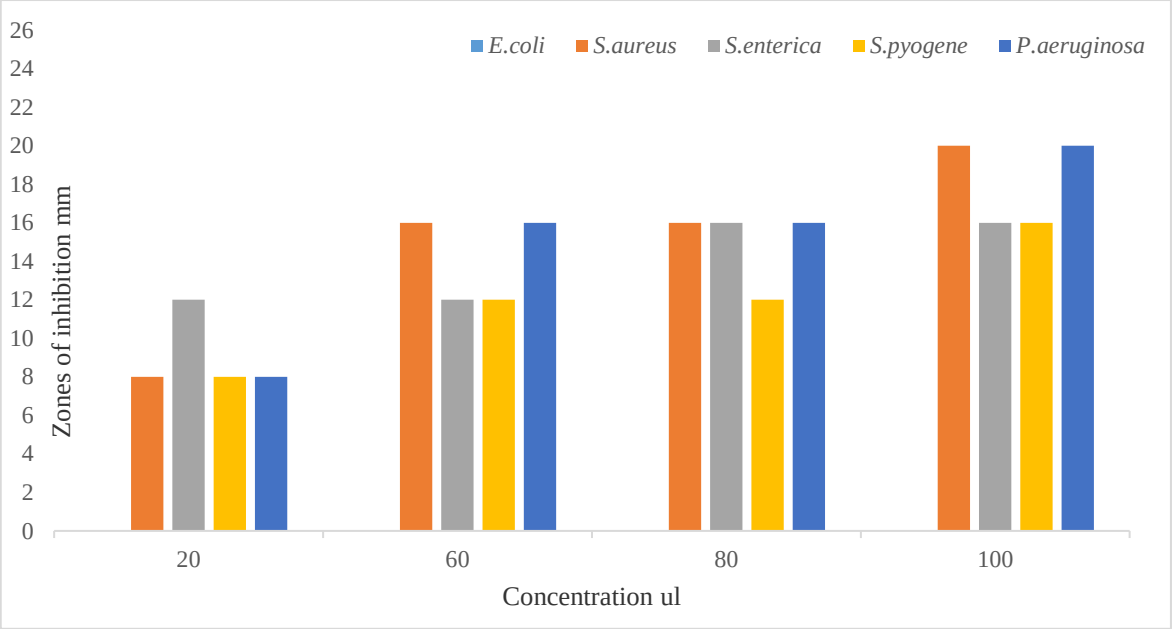

Figure 3: Antibacterial activity of *F. asafoetida* against pathogens and their zones of inhibition at different concentrations

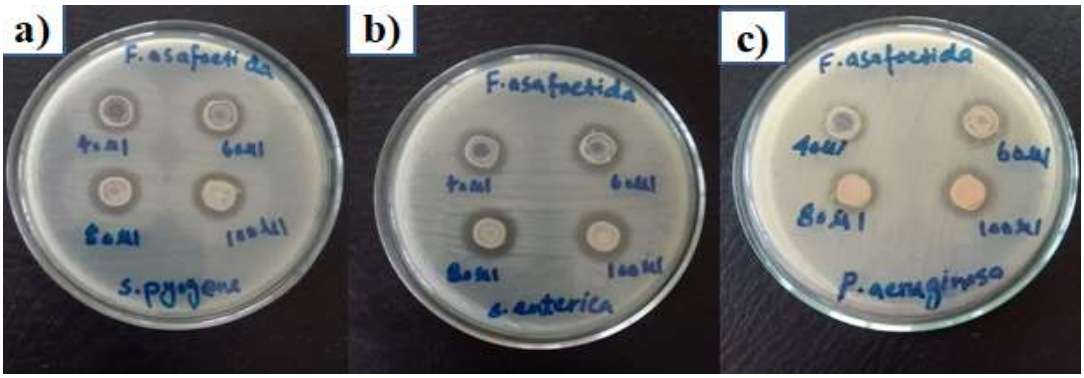

Figure 4: Antibacterial activity of *F. asafoetida* (a) against *S. pyogenes* (b) against *S. enterica* (c) against *P. aeruginosa.*

Antimicrobial activity of *C. sativa*

The aqueous extracts of *S. sativa* showed antimicrobial activity. The aqueous extracts of *C. sativa* were effective against three pathogenic bacteria while two microbes showed resistance against *C. sativa*. The maximum activity was shown against *E. coli,* while lowest activity was shown against *S. enterica*. The zone of inhibition of *S. aureus* were 12, 13, 14 and 18mm at 20, 40, 60 and 80 µl concentration. The zone of *S. enterica* were in the range of 9, 11, 13 and 16 mm. The resistance shown by two bacteria were *S. pyogenes* and *P. aeruginosa*. The results of pathogens against *C. sativa* for all concentration are shown in Figure 5.

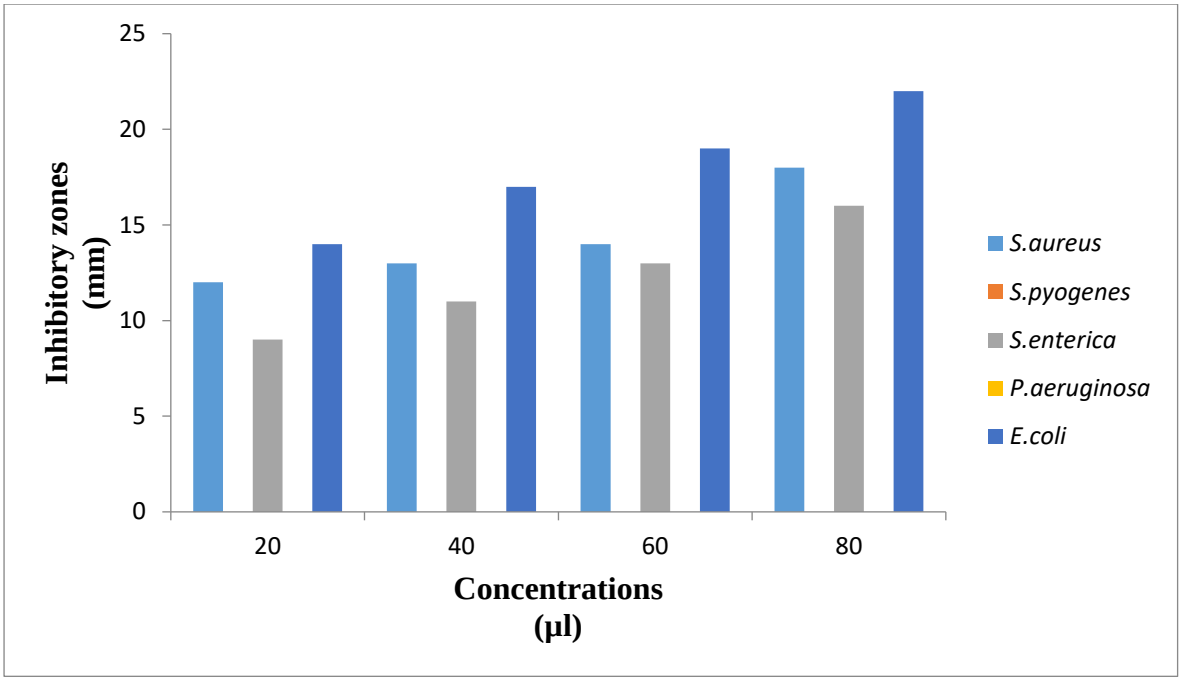

Figure 5: Antimicrobial activity of *C. sativa* against different pathogenic bacteria.

Discussion

Now a days microbial resistant is a major problem and a challenging situation which make the microbes more potent to promote the discovery of novel drugs. Researchers are trying to switch towards a safe and natural solution. To have floral remedies were very common in past and have a great importance in present (Holmes *et al*., 2016).

Hundreds of medicinal plants in every region of world are being used as medicinal plants, some of their types are region specific but some species found in various regions. In this study, three types of plants named as *Carissa opaca, Ferula asafoetida* and *Cannabis sativa,* were used to check their antibacterial activity against selected bacterial strains. The primary phytochemical selection of methanolic mine of *C. opaca* leaves showed the incidence of tannis, flavonoids, phlobatannins, alkaloids, coumarins, terpenoids and anthraquinones (Shareen *et al*., 2011).

In a relative study carried out by Ahmed and Hammad (2013) of *C. opaca* fruits, leaves and seeds, leaves were proved to show greater antioxidant value. In a different revision which is completed by Izhar and Ahmed (2016) Ethyl acetate mine exhibited killing action counter to *E. coli, S. aureus, K. pneumonia* and *P. aeruginosa* while acetone abstract displayed the bustle against *P. aeruginosa, E. coli, B. cereus, Proteus vulgaris* and *Salmonella typhi* correspondingly. The concentration of ethanolic leaves extract of 3 mg/ml gave 18.5 mm zone against *P. aeruginosa,* against *S. aureus* 23 mm and against *E. coli* 15.1 mm (Ahmed *et al*., 2011) while in this study 40µl of aqueous extract of leaves gives 13mm zone of inhibition against *E. coli*, 15 mm against *S. enterica*, 2mm against *S. pyogenes* and 11 mm

against *P. aeruginosa* while against *S. aureus* it showed no activity at 40µl but 13 mm zone of inhibition at 60µl.

Rendering to Niazmand and Razavizadeh (2021), *Ferula* is the bush that has extraordinary Sulphur, which is related with its antimicrobial possessions. Essential oils (EO) that can have recognized in the *Ferula asafoetida* generally as (Z) 1-Propenyl sec-butyl disulfide (10.20%) 10-epi-γ-Eudesmol (19.21%) and (E)-1-Propenyl sec-butyl disulfide (21.65%) (Dissanayake and Perera, 2020). The supreme corporate sulfur complex in the EOs of *Ferula* species, which shows a vital part in the aroma and flavor of these floras (Iranshahy and Iranshahy, 2011).

The EO has showed antiseptic action contrary to methicillin-sensitive and resistant *S. aureus* in studies of this research. 0.5-4µl / ml concentration range, and at a concentration of 2µl / ml, EO of this plant successfully inhibited the growth of both normal and clinical isolates of *S. mutans* and *S. sanguinis* (Divya *et al.*, 2014). In this study, the minimum inhibitory concentration of *F. asafoetida* is 40µl which showed antibacterial effect against all selected strains and zone of inhibition in case of *E. coli* was 8mm, 12mm of *S. aureus*, 8mm of *S. pyogenes* and of *P. aeruginosa* zone of inhibition was 12mm.

Antibacterial activity of *Cannabis sativa* leaf extracts was checked against some selective bacterial strains. The activity of this plant leaf extract is due the presence of phenyl moiety of cannabinoids which act as a good antimicrobial agent extract of *Cannabis sativa* leaf showed good activity against both Gram-positive and Gram-negative bacteria. These reports and presence of Cannabinoid in different extract of *Cannabis sativa* confirm its potential against all selected pathogenic bacterial strains. The study suggests that the Ethanol and aqueous leaf extract of *Cannabis sativa* have a broad range of antimicrobial activity, although the degree of susceptibility could be different between different microorganisms (Appendino *et al.,* 2008).

The study concluded that antibacterial effect of floral drugs used in this study showed less or more activity with respect to their different concentrations. All these selected plants shown a direct relation with concentration of extract, higher the concentration of extract gives larger zone of inhibition. Therefore, these plants extracts can be beneficial with respect to their accessibility as well as economically favorable and can proved a better approach in future.

References

Ahmed, D., & Hammad, M. (2013). Comparative Study of Antioxidant, Antimicrobial and a-Amylase Inhibitory Activities of Leaves, Fruits and Seeds of Carissa opaca. *Asian Journal of Chemistry*, *25*(7), 3913.

Ahmed, D., Waheed, A., Chaudhary, M. A., Khan, S. R., Hannan, A., & Barkaat, M. (2011). Nutritional and antimicrobial studies on leaves and fruit of Carissa opaca Stapf ex Haines. *Asian Journal of Chemistry*, *23*(5), 2072.

Amos, S. E. Kolawole, P. Akah, C. Wambebe, & Gamaniel, K. (2001). Behavioral effects of the aqueous extract of Guiera senegalensis in mice and rats," Phytomedicine, 8(5), 350-361.

Appendino, G., Gibbons, S., Giana, A., Pagani, A., Grassi, G., Stavri, M., ... & Rahman, M. M. (2008). Antibacterial cannabinoids from Cannabis sativa: a structure− activity study. *Journal of natural products*, *71*(8), 1427-1430.

Benbrinis, S. (2018). *Evaluation des activités antioxydante et antibactérienne des extraits de Santolina chamaecyparissus* (Doctoral dissertation). Universty of Ferhat Abbas-SETIF. Algeria.

Biswas, S. K., Chowdhury, A., Das, J., Hosen, S. Z., Uddin, R., & Rahaman, M. S. (2011). Literature review on pharmacological potentials of Kalanchoe pinnata (Crassulaceae). *African Journal of Pharmacy and Pharmacology*, *5*(10), 1258-1262.

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical microbiology reviews*, *12*(4), 564-582.

Davidson-Hunt, I. (2000). "Ecological ethnobotany: Stumbling toward new practices and paradigms," MASA Journal, *16(1)*, 1-13.

Dewick, P. M. (1996). Tumor inhibitor from plants, in Trease and Evans' Pharmacognosy, Elsevier Health Sciences, Philadelphia, Pa, USA.

Dissanayake, K. G. C., & Perera, W. P. R. T. (2020). Medicinal importance of Ferula asafetida oligo-gum resins against infective diseases. *Journal of Medicinal Plants Studies*, *8*(2), 135-139.

Divya, K., Ramalakshmi, K., Murthy, P.S. & Rao, L.J.M. (2014). Volatile oils from Ferula asafoetida varieties and their antimicrobial activity. LWT Food Sci. Technol. *59*, 774-779.

Farjana, A., Zerin, N., & Kabir, M. S. (2014). Antimicrobial activity of medicinal plant leaf extracts against pathogenic bacteria. *Asian Pacific Journal of Tropical Disease*, *4*, S920-S923.

Holmes, A. H., Moore, L. S., Sundsfjord, A., Steinbakk, M., Regmi, S., Karkey, A., ... & Piddock, L. J. (2016). Understanding the mechanisms and drivers of antimicrobial resistance. *The Lancet*, *387*(10014), 176-187.

Iranshahy, M., & Iranshahi, M. (2011). Traditional uses, phytochemistry and pharmacology of asafoetida (Ferula assa-foetida oleo-gum-resin)—A review. *Journal of ethnopharmacology*, *134*(1), 1-10.

Izhar, S., & Ahmed, D. (2016). Carissa opaca: A plant with great potential for future drugs for degenerative and infectious diseases. *Chemistry Select*, *1*(12), 3005-3011.

Janovska, D., Kubikova, K., & Kokoška, L. (2003). Screening for antimicrobial activity of some medicinal plants species of traditional Chinese medicine. *Czech journal of food sciences*, *21*(3), 107.

Mahesh, B., & Satish, S. (2008). Antimicrobial Activity of Some Important Medicinal Plant Against Plant and Human Pathogens. *World Journal of Agricultural Sciences*, *4*, 839-843.

Niazmand, R., & Razavizadeh, B. M. (2021). Ferula asafoetida: chemical composition, thermal behavior, antioxidant and antimicrobial activities of leaf and gum hydroalcoholic extracts. *Journal of Food Science and Technology*, *58*(6), 2148-2159.

Phillipson, J. D., & Wright, C. W. (1996). "Plants with antiprotozoal activity," in Trease and Evans' Pharmacognosy, W.B. Saunders company, London, UK, 14.

Sahreen, S., Khan, M. R., & Khan, R. A. (2011). Hepatoprotective effects of methanol extract of Carissa opaca leaves on CCl 4-induced damage in rat. BMC complementary and alternative medicine, 11, 1-9.