Acta Scientific Microbiology (ISSN: 2581-3226)

Volume 8 Issue 7 July 2025

Research Article

Antibacterial Activity of *Ephedra* alata Extracts: *In Vitro* Evaluation Against Standard and Clinical Human Pathogenic Bacteria

Asma A Elamari[1]*, Nawal M Buzgaia[2], Tahani M Awin[2], Wafa S Zubi[1], Fawzia Mufthah Aljazwia[1], Aida MM Elakeili[3] and Ghazala A Ashour[2]

[1]*Botany Department, Science Faculty, University of Benghazi, Benghazi, Libya*

[2]*Chemistry Department, Science Faculty, University of Benghazi, Benghazi, Libya*

[3]*Biology Department Education Faculty, University of Benghazi, Benghazi, Libya*

***Corresponding Author:** Asma A Elamari, Botany Department, Science Faculty, University of Benghazi, Benghazi, Libya.

Received: June 02, 2025

Published: June 20, 2025

Abstract

The discovery of antimicrobial agents from natural products remains a primary focus for many pharmaceutical scientists seeking to overcome antibiotic resistance. *Ephedra* is one of the commonly used plants in traditional medicine. This study aimed to evaluate the antibacterial activity of leave extracts from Ephedra alata against standard laboratory strains and clinical bacterial isolates; including *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC8739), Staphylococcus aureus, *Escherichia coli*, and *Klebsella pneumonia,* using well diffusion method. 50 µL of *Ephedra alata* leave extracts prepared in methanol and chloroform solvents and were applied at different concentrations (100, 50, 25, and 12.5 mg/ml), to evaluate their efficacy as antibacterial agent against bacterial growth. The results showed that methanol extracts exhibited high inhibitory activity against both standard and clinical strains of Staphylococcus aureus at all tested concentrations. Methanol extracts also was effective against the growth of *Enterococcus faecalis* (ATCC 29212) but only at the highest concentration (100 mg/ml). Chloroform leave extracts had inhibitory effect against a clinical isolates of *Escherichia coli* at high concentrations (50 and 25mg/ml) and *Klebsella pneumonia* at all applied concentrations. Both methanol and chloroform plant extracts did not exhibit any effect on standard *Escherichia coli.* This study confirmed the efficacy of *Ephedra alata l*eave extracts as natural antibacterial products and recommended that they may be used as a therapy.

Keywords: Ephedra; Extract; Antibacterial; Activity

Introduction

The World Health Organization (WHO) reported that 80 % of the world population use herbal medicine for some form of primary health care [30,41]. As of 2018, the global utilization rate of herbal medicine rose to approximately 88%. Its prevalence varies across cultures and is likely influenced by people's understanding, which is shaped by traditional knowledge [2,17,22]. Using plants in folk medicine is a part of our history, and people in both the developed and developing countries considered this therapy as the first line of treating the endemic infectious diseases. About 70,000 different plant species worldwide have been recorded as medicinal plants and commonly used in traditional therapy [7]. The eastern Mediterranean coastal region of Libya, including Benghazi, Albaida, Sahahat, Derna, and Tobruk are consided as arich areaes of traditional herbal medicines [14].

Ephedra is one of the important herbal medicines found in the east part of Libya, and it is well known that it has been used in the treatment of various diseases for over 5000 years [22,38]. Ephedra genus is belonging to the *Ephedraceae* family and consists of 40 species; such as *Ephedra Lristanica*, *Ephedra strobiliacea*, *Ephedra sarcocarp*, *Ephedra procera*, *Ephedra pachyclada*, and *Ephedra alata*, that grow in arid environments [12]. They have been used in folk medicine to deal with some symptoms associated with asthma,

nasal congestion, cough asthma, liver disease, skin conditions, and lately it has shown significant effectiveness in treating COVID-19 as well [4,11,27,39].

Ephedra spp. contain wide varity of bioactive compounds such as glycosides, flavonoids, phenolic compounds, alkaloids, flavonoids, tannins, saponins, and polysaccharides which have unique pharmacological effects [2,3, 9,19]. Previous pharmacological studies have indicated that Ephedra extracts exhibit antimicrobial [43], antifungal [8], antiviral [28], anti-inflammatory [24], antibacterial [15], and antitumor [33] effects. Many reports have demonstrated the antibacterial activity of the *Ephedra spp.* against various bacterial species such as *Staphylococcus aureus*, *Bacillus anthracis*, *Bacillus diphtheriae*, *Bacillus dysenteriae*, *Bacillus typhosus*, *Lactobacillus acidophilus*, and *Pseudomonas aeruginosa* [12,15,25,29,36]. In addition, extensive research has been conducted on this plant, revealing that the leaves, stems, roots, flowers, seeds, and fruits of the Ephedra herb possess antimicrobial activity and various other pharmacological properties [9,25,21,40,43].

Ephedra alata, also known as *Alanda* in Arabic [21], is commonly used in folk medicine and is a part of Libyan heritage—especially in the eastern region of the country, where a significant portion of the population considers medicinal plants a first-line treatment for endemic infectious diseases.

A study conducted in Libya indicated the antibacterial efficacy of methanolic extracts from the aerial parts of *Ephedra sp.* against the growth of both Gram-positive and Gram-negative bacteria [6]. Furthermore, it has been reported that the flower extracts from *E. alata* inhibit the growth of a wide range of bacteria such as *Bacillus subtilis* ATCC 6051, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* ATCC 11778 [10].

It was recorded that *E. alata* is considered environmentally friendly, less toxic, and safe for use in traditional medicine [20]. For a long time, the pharmaceutical industries have developed numerous new antibiotics, but they are still in fighting with the progressive of the bacterial resistance of these antibiotics and consequently fail in therapy. Although awareness of the random use of antibiotics has increased, their widespread use in therapy continues and contributes to the growing resistance of bacteria to antibiotics, which is still not fully under control. Because of that, there is a strong need of replacing the antibiotics with safe alternative natural products such as herds with antimicrobial properties in human therapy. Therefore, the aim of this research was to assess the antibacterial activities of methanolic and chloroformic extracts from *Ephedra alata* leaves against the growth of standard and clinical bacteria in vitro.

Materials and Methods

Plant collection and preparation extract

The herb of *Ephedra alata* was collected from Wadi Alkouf in East mountain of Libya in March, 2023. The plant was identified and classified at Department of Botany, Faculty of Science, University of Benghazi. The *Ephedra alata* herbs were washed several times with deionized water and left to dry in open air for a week. The dried leaves were crushed into a fine powder using an electric blender. According to [18] with modifications, 50g of the ground plant were used to extract the bioactive compounds from the leaves of the plant with methanol (99 %) and chloroform (99%) solvents through ultrasonic extraction (i.e., WiseD; Daihan Scientific, Ltd Co, South-Korea) under controlled temperature (45 °C) for an hour. The procedure was repeated three times with adding fresh solvent. The leave extracts then were filleted using Whatman filter paper (Whatman NO. 1 USA), and the filtrates were evaporated in a rotary evaporator under reduced pressure at 45°C. Finally, the solid residue was stored at - 4°C until further use.

The crude dissolved in the 1% (*v*/v) of Dimethyl Sulfoxide (DMSO) and four concentrations of the each extract (100, 50, 25, 12.5 mg/ml) were prepared.

Collection of bacteria

In this research, three standard bacterial strains were used: *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* (ATCC 29212), and *Escherichia coli* (ATCC 8739). Additionally, three clinically isolated bacteria were included: *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*. The source of the standard bacterial strains was Microbiology Department at the University Of Tunis El Manar (UTM), Tunis while the clinical isolates were obtained from microbiology laboratory at Saleem Medical Analysis Laboratory in Benghazi City, Libya. The clinical isolates were identified using the VITEK® 2 COMPACT system (bioMérieux, France), with VITEK 2 GP Card (bioMérieux) for gram-positive bacteria and VITEK 2 GN Card (bioMérieux) for gram-negative bacteria, which offers accurate and efficient identification.

Preparation of the bacterial suspension

Bacterial stock cultures were sub-cultured onto Nutrient Agar (NA) plates and incubated overnight at 37 °C. Three to four discrete bacterial colonies were then transferred into 10 ml of sterile Mueller-Hinton Broth (MHB) and incubated overnight at 37°C. The resulting bacterial suspensions were adjusted to a 0.5 McFarland standard using sterile MHB, corresponding to approximately 1.5 × 10^6 CFU/ml.

Antimicrobial Activity of plant extract

According to [35] with modifications, the antibacterial activity of *Ephedra alata* herb extracts (methanol and chloroform) was determined by agar well diffusion assay as the following; Mueller-Hinton Agar (MHA) was applied as basal medium for the antibacterial activity test (MHA was prepared by suspending 38 g in 1000 ml of distilled water and sterilized using autoclaving at 15 lbs pressure and 121°C for 15 minutes).Then the medium allowed to cool at 45-50°C and 15 ml of the sterilized medium was poured into sterile petri plates. Following solidification, each MHA plate was inoculated with 100 µl of bacterial suspension (0.5McFarland) and spread evenly using a sterile swab. Plates then left for at least 3 min to allow inoculum to be absorbed.

Wells of 5 mm in diameter were made using a serial cork-borer in the medium of each plate and 50 µl of Ephedra extracts (100, 50, 25, and 12.5 mg/ml) were injected into the wells. The plates were then left at room temperature for one hour to permit the antibacterial agent to diffuse before the organism was allowed to grow [37]. Gentamycin (10 µg) was applied as a positive control while 1% (*v*/v) DMSO was used as a negative control (DMSO would not affect the growth of bacteria) [34]. Triplets of the experiment were maintained for each bacterial species to ensure reliability. Finally, the plates were incubated at 37°C for overnight, and after incubation time the diameter of the inhibitory zones formed around the well were measured in mm.

Statistical analysis

The experiments were designed according to the complete random design. Statistical analysiswas performed using SPSS program and ANOVA variance analysis tables. The averages were compared using LSD test at $P < 0.05$.

Results

The results of well diffusion assay are shown in tables (1,2) and the figures (1,2). In this research, the leave extracts from *Ephedra alata* was investigated to evaluate its antibacterial activity against the standards strains and clinical isolates. The results indicated that the methanolic extracts of *Ephedra alata* inhibited the growth of both standard and clinical *Staphylococcus aureus* strains at all tested concentrations (Table 1). *S. aureus* was the most sensitive strain to methanol extracts, and there was no significant difference in susceptibility between the standard and clinical isolates ($P > 0.05$). Also, the methanol extract affects the growth of *Enterococcus faecalis* ATCC 29212 but only at high concentrations (100 and 50 mg/ml) with inhibition zone 6 and 5.66 mm in diameter; respectively.

On the other hand, methanol extract of *Ephedra alata* was ineffective against the standard *Escherichia coli* (ATCC8739) and all gram negative clinical isolates.

In addition, the chloroform extracts of *Ephedra alata* were active against gram negative isolates *Escherichia coli,* at the highest concertation with inhibition zone 8 mm in diameter, and *Klebsella pneumonia,* at all concentrations applied with inhibition zone 10, 9.66, 9, 8 mm in diameter; respectively (Table 2). In contrast, chloroform extracts did not exhibit inhibitory effect against standard and clinical *Staphylococcus aureus*. Moreover, this extract was not effective for all the standard bacteria used in this study. Finally, the results showed that all the tested bacteria were sensitive to gentamicin.

Discussion

The Ephedra genus is widely distributed globally and it's bioactive compounds composition upon on its species. In addition, the growth stage, plant organs, and geographical area factors can reflect the antibacterial efficiency of the plant extract [16]. It is well known that *Ephedra* species contain bioactive compounds including; the alkaloids and phenolic compounds, such as trans-cinnamic acid, catechin, epicatechin, symplocoside, flavonoids (leucodelph-

Name of cultured bacteria	Plant extract concentrations (mg/ml) 100% 50% 25% 12.50				Gentamycin 10 µg	DMSO 1%
Staphylococcus aureus (ATCC 6538)	11.5 ± 0.86	9.7 ± 1.15	7 ± 0	5 ± 0	10 ± 0	-
Enterococcus faecalis (ATCC 29212)	6 ± 1.73	5.66 ± 0.57	0.00	0.00	9 ± 0	-
Escherichia coli (ATCC8739)	0.00	0.00	0.00	0.00	9 ± 0	-
Staphylococcus aureus	11 ± 0	9.66 ± 0.57	8.66 ± 0.57	6.3 ± 0.57	10 ± 0	-
Escherichia coli	0.00	0.00	0.00	0.00	8.66 ± 0.57	-
Klebsella pneumonia	0.00	0.00	0.00	0.00	9.33 ± 0.57	-

Table 1: Antibacterial activity of methanolic extract from *Ephedra alata* leaves against bacterial strains.

(-): no inhibition zone.

(DMSO): Dimethyl Sulfoxide.

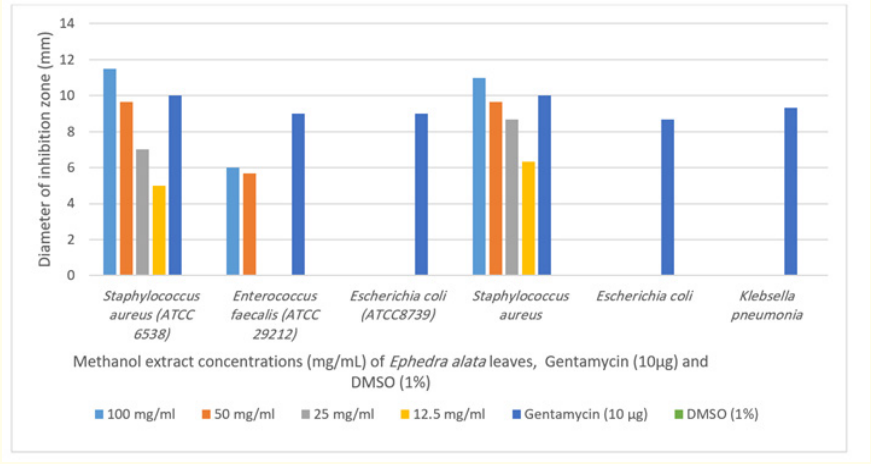

Figure 1: Effects of methanol leave extracts against the tested bacteria.

Name of cultured bacteria	Plant extract concentrations (mg/ml) 100 50 25 12.50				Gentamycin 10 µg	DMSO 1%
Staphylococcus aureus (ATCC 6538)	0.00	0.00	0.00	0.00	10 ± 0	-
Enterococcus faecalis (ATCC 29212)	0.00	0.00	0.00	0.00	9 ± 0	-
Escherichia coli (ATCC8739)	0.00	0.00	0.00	0.00	9 ± 0	-
Staphylococcus aureus	0.00	0.00	0.00	0.00	10 ± 0	-
Escherichia coli	8 ± 0	0.00	0.00	0.00	10 ± 0	-
Klebsella pneumonia	10 ± 0	9.66 ± 0.57	9 ± 0	8 ± 0	11 ± 0	-

Table 2: Antibacterial activity of chloroformic extract from *Ephedra alata* leaves against bacterial strains.

(-): no inhibition zone

(DMSO): Dimethyl Sulfoxide

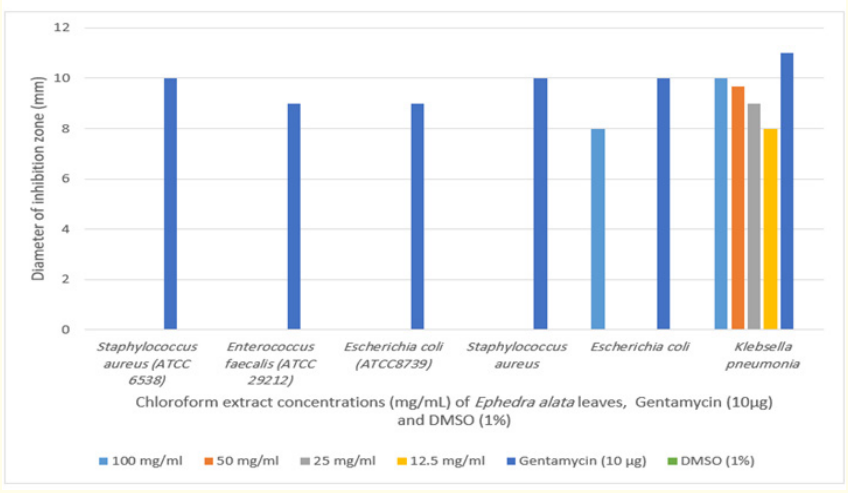

Figure 2: Effects of methanol leave extracts against the tested bacteria.

inidin, leucopelargonine, leucoanthocyanidin, lucenine, vicenin-1, and vicenin-2); which are aromatic compounds with anti-oxidative properties, and tannins [5,16,26]. Ephedras' pharmacological characteristics are mainly attributed to alkaloids [42]. However, combinations of all of the previous mentioned bioactive compounds attributed to its antibacterial activities.

The results of the current study showed that the high concentrations (100 and 50 mg/ml) of the methanolic and chloroformic leave extracts of *Ephedra alata* exhibited antibacterial activity against most of standard and clinical bacteria. The lowest concentrations of the plant extracts (12.5 mg/ml) were only active against standard and clinical *Staphylococcus aureus* and clinical isolate of *Klebsella pneumonia*. Methanol extract was effective on the growth of standard and clinical *Staphylococcus aureus* at all concentrations used while only the highest concentration of this extract (100 mg/ml) had inhibitory effect on the growth of *Enterococcus faecalis* (ATCC 29212) indicating consistent antibacterial activity across both laboratory and clinical isolates. Our results are in line with the study conducted by [13] where the methanol extracts of the aerial parts of *Ephedra alata* inhibited the growth of five isolates of methicillin- resistant *Staphylococcus aureus* strains and *Enterococcus faecalis* ATCC 29212. Additionally, our findings align with the previous study which reported significant antibacterial activity of the methanol extract from the aerial parts against *Staphylococcus aureus* [6]. However, our results did not correspond with their findings for the Gram-negative bacteria (*Escherichia coli*). Methanolic extract of *Ephedra alata* plant contains alkaloids, carbohydrates, flavonoids, steroids, terpenoids, tannins, and phenolic compounds, and this may attributed to its antibacterial activity [6,13]. On the other hand, methanol leave extracts of the *Ephedra alata* showed no effect on the growth of other tested bacteria.

The lack of activity of both methanol and chloroform leaf extracts of *Ephedra alata* against the standard *Escherichia coli* strain (ATCC 25922) suggests that this strain is either inherently resistant or not susceptible to the bioactive compounds present in the extracts at the concentrations tested. In contrast, the chloroform extract inhibited the growth of the clinical *E. coli* isolate but only at the highest concentration. The limited activity and concentration dependence also suggest that the antimicrobial potential of this extract may be relatively weak or specific, and further fractionation and compound isolation would be needed to identify the active constituents and enhance efficacy. In addition, chloroform extracts had a great effect against the clinical *Klebsella pneumonia* at all used concentrations, which is a positive outcome highlighting the probability of using *Ephedra alata* in treating infection caused by such gram negative bacteria. In general, this result supports the previous research which presented that butanol, ethyl acetate, and dichloromethane extracts from *Ephedra alata* had antibacterial activity against Gram-positive and Gram-negative bacteria [10].

Plant extracts, particularly their phenolic compounds, are hydrophobic and can disrupt bacterial membranes by embedding into the lipid bilayer. This disturbs membrane structure, collapses the proton motive force and membrane potential, causes leakage of cellular contents, denatures proteins, and damages cellular integrity. These compounds can also affect membranes by increasing their permeability and contributing to further structural harm [31].

The lack of antibacterial activity of leave extracts of *Ephedra alata* on some bacteria may be due to the fact that this part of plant may contain level of phenolic compounds, which is not enough for inhibiting bacterial growth. Also, the low level of alkaloids and lack of some secondary metabolites that improve the efficiency of alkaloids, or act synergistically may attribute to their deficiency as antibacterial agent [45]. Therefore, there is a strong need for comprehensive researches in *Ephedra alata* as a natural product in term of it's antimicrobial activity and the safety of it's use must be guaranteed as well.

Conclusion

The results indicated the antibacterial activity of *Ephedra alata* herb extract against standard and clinical bacteria. Methanol herb extract was effective on Gram positive bacteria, but it was not the case of chloroform extracts. Chloroformed herb extract did not exhibit any effect on the growth of standard bacteria but had a significant effect on the gram negative clinical isolates. Further research is needed to fully elucidate the mechanisms of action of *Ephedra alata* extracts as antibacterial agents, in order to enhance their potential therapeutic applications.

Bibliography

1. H Ali and S Dixit. "*In vitro* antimicrobial activity of flavanoids of *Ocimum sanctum* with synergistic effect of their combined form". *Asian Pacific Journal of Tropical Disease* (2012): 396-S398.

2. OM Akhagba. "Cultural influence in the consumption of herbal medicine among Nigerian women : a theoretical exploration". Miscellanea Anthropologica et Sociologica 18.5 (2017): 193-206.

3. EA Abourashed., *et al.* "Ephedra in perspective - A current review". *Phytotherapy Research* 17.7 (2003): 703-712.

4. R Andraws., *et al.* "Cardiovascular Effects of Ephedra Alkaloids: A Comprehensive Review". *Progress in Cardiovascular Diseases* 47.4 (2005): 217-225.

5. Al-Rimawi., *et al.* "Analysis of phenolic and flavonoids of wild Ephedra alata plant extracts by LC/PDA and LC/MS and their antioxidant activity". *African Journal of Traditional, Complementary and Alternative Medicines* 14.2 (2017): 130-141.

6. SA Alshalmania., *et al.* "Antimicrobial, antioxidant and anticancer activity of Ephedra alata growing in East of Libya1". *Libyan Journal of Science and Technology* 11.2 (2020): 87-90.

7. AJ Brinckmann., et al. "A New Global Estimation of Medicinal and Aromatic Plant Species in Commercial Cultivation and Their Conservation Status". *Economic Botany* 76.8 (2022).

8. S Bagheri-Gavkosh ., *et al.* "Inhibitory effects of Ephedra major host on Aspergillus parasiticus growth and aflatoxin production". *Mycopathologia* 168 (2009): 249-255.

9. S Caveney., *et al.* "New observations on the secondary chemistry of world Ephedra (Ephedraceae)". *American Journal of Botany* 88.7 (2001): 1199-1208.

10. E Chebouat., *et al.* "Assessment of antimicrobial activity of flavonoids extract from Ephedra alata". *Der Pharmacia Lettre* 6. (2014): 27-30.

11. JK Chen and TT Chen. " Chinese Medicinal Herbology and Pharmacology". *Art of Medicine Press City of Industry, CA, USA* (2004): 31-34.

12. MM Deabes., *et al.* "Impact of silver nanoparticles on gene expression in Aspergillus Flavus producer aflatoxin B1". *Open Access Macedonian Journal of Medical Sciences* 6. (2018): 600-605.

13. A Dbeibia., *et al.* "Control of *Staphylococcus aureus* methicillin resistant isolated from auricular infections using aqueous and methanolic extracts of *Ephedra alata"*. *Saudi Journal of Biological Sciences* 29. 2. (2022)1021-1028.

14. FM El-Mokasabi. "Floristic Composition and Traditional Uses of Plant Species at Wadi Alkuf, Al- Jabal Al-Akhder, Libya". *American-Eurasian Journal of Agricultural and Environmental Science* 14 .8 (2014): 685-697.

15. B Fazeli-Nasab and SR Mousavi. "Antibacterial Activities of Ephedra sinica Herb Extract on Standard and Clinical Strains of Pseudomonas aeruginosa Bacteria". *Journal of Medical Bacteriology* 8.3. 4. (2019): 40-48.

16. H Freitag., *et al*. "In: Tutin TG, editor". F*lora Europaea Cambridge: University Press* 1. (1993).

17. PE Harris., et al. "Prevalence of complementary and alternative medicine (CAM) use by the general population: A systematic review and update". I*nternational Journal of Clinical Practice* 66.10 (2012): 924-939.

18. S Herur., *et al.* "Antibacterial activity by Dombeya wallichii plant extracts obtained by ultrasound-assisted extraction". *Journal of Emerging Investigators* 6. (2023): 100-101.

19. S Ibragic and E Sofi. "Chemical composition of various Ephedra species". *Bosnian Journal of Basic Medical Sciences* 15.3 (2015): 1-7.

20. S Jadhav., *et al.* "Lipid oxidation in biological and food systems". *Food Antioxidants*. CRC Press. USA (1995): 19-78.

21. N Jaradat., *et al.* "Ephedra alata fruit extracts: phytochemical screening, anti-proliferative activity and inhibition of DPPH, α-amylase, α-glucosidase, and lipase enzymes". *BMC Chemistry* 15.41 (2021).

22. C Jon and T Tilburt. "Herbal Medicine Research and Global Health: An Ethical Analysis". (2008).

23. HK Kim., *et al.* "Metabolic fingerprinting of Ephedra species using 1H-NMR spectroscopy and principal component analysis". *Chemical and Pharmaceutical Bulletin* 53.1 (2005): 105-109.

24. IS Kim., *et al.* "Ephedrannin A and B from roots of Ephedra sinica inhibit lipopolysaccharide-induced inflammatory mediators by suppressing nuclear factor-κB activation in RAW 264.7 macrophages". *International Immunopharmacology* 10.12 (2010): 1616-1625.

25. A Khan., et al. "In Vitro Antioxidant and Antimicrobial Activities of Ephedra gerardiana (Root and Stem) Crude Extract and Fractions". *Evidence-Based Complementary and Alternative Medicine* (2017): 4040-4254.

26. D Kustrak and A Ramic. "Ephedra species and their alkaloids". *Farm Glas* 50. 12. (1994) :321-336.

27. S Liang., *et al*. "Polysaccharide from Ephedra sinica Stapf inhibits inflammation expression by regulating Factor-β1/Smad2 signaling". *International Journal of Biological Macromolecules* 106 (2018): 947-954.

28. L Li., *et al*. "Antiviral effects of modified Dingchuan decoction against respiratory syncytial virus infection in vitro and in an immunosuppressive mouse model". *The Journal of Ethnopharmacology* 147.1 (2013): 238-244.

29. CH Lee and HS Lee. "Growth inhibiting activity of quinaldic acid isolated from Ephedra pachyclada against intestinal bacteria". *The Korean Society for Applied Biological Chemistry* 52. (2009): 331-333.

30. H Mbali., *et al.* "Prevalence and correlates of herbal medicine use among Anti-Retroviral Therapy (ART) clients at Queen Elizabeth Central Hospital (QECH), Blantyre Malawi: a cross-sectional study". *Malawi Medical Journal* 33.3 (2021): 153-158.

31. J Mushore and M Matuvhunye. "Antibacterial properties of Mangifera indica on Staphylococcus aureus". *African Journal of Clinical and Experimental Microbiology* 14.2. (2013): 62-74.

32. AS Naidu. "Phytoantimicrobial (PAM) agents as multifunctional food additives. In: Bidlack WR, Omaye ST, Meskin MS and Topham D (Eds.), Phytochemicals as bioactive agents". *Technomic Publishing. Lancaster, United Kingdom* (2000). 106-110.

33. NH Nam., *et al.* "Antiinvasive, antiangiogenic and antitumour activity of Ephedra sinica extract". *Phytotherapy Research* 17.1 (2003): 70-76.

34. NY Naguib., *et al*. "A comparative study on the productivity and chemical constituents of various sources and species of Calendula plants as affected by two foliar fertilizers". *The Journal of Applied Sciences Research* 1.2 (2005): 176-189.

35. SM Pawaskar and KU Kale. "Antibacterial activity of successive extracts of Mimosa pudica". *INDIAN DRUGS-BOMBAY* 43.6. (2006): 476.

36. A Parsaeimehr., et al. "A comparative study of the antibacterial, antifungal and antioxidant activity and total content of phenolic compounds of cell cultures and wild plants of three endemic species of Ephedra". Molecules 15 (2010): 1668-1678.

37. SA Selim. "Chemical composition, antioxidant and antimicrobial activity of the essential oil and methanol of the Egyptian lemongrass *Cymbopogon proximus* Stapf". *Grasas y Aceites* 62.1. (2011): 55-61.

38. MG Soni., *et al*. "Safety of Ephedra: lessons learned". *Toxicology Letters* 150 (2004): 97-110.

39. S Tang., *et al.* "Ephedrae Herba: A Review of Its Phytochemistry, Pharmacology, Clinical Application, and Alkaloid Toxicity". *Molecules* 28.2 (2023): 663.

40. H Tao., *et al.* "Dimeric proanthocyanidins from the roots of Ephedra sinica". *Planta Medica* 74.15 (2008): 1823-1825.

41. "Who Global Report on Traditional and Complementary Medicine" (2004).

42. A Walaa., *et al.* "Clinical uses and toxicity of Ephedra sinica". *Pharmacognosy Journal* 1 (2019): 447-452.

43. X Zang., *et al.* "A-Type proanthocyanidins from the stems of Ephedra sinica (Ephedraceae) and their antimicrobial activities". *Molecules* 18.5 (2013): 5172-5189.

44. JR Zgoda and JR Porter. "A Convenient Microdilution Method for Screening Natural Products against Bacteria and Fungi". *Pharmaceutical Research* 39. 3. (2001): 211-225.

45. S Garba and SO Okeniyi. "Antimicrobial activities of total alkaloids extracted from some Nigerian medicinal plants". *Journal of Microbiology and Antimicrobials* 4.3 (2012): 60-63.