Exploring the Antibacterial Potential and Phytochemical Composition of Four Indigenous Indian Medicinal Plants: Effective Strategies Against Multi-Drug Resistant and ESKAPE Pathogens

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ABSTRACT:

This paper presents the findings of a secondary research study conducted to investigate the antibacterial potential and phytochemical composition of four indigenous Indian medicinal plants, namely *Salvadora persica* (Miswak), *Caesalpinia pulcherrima* (Peacock flower), *Thymus vulgaris* (Thyme), and *Saussurea lappa* (Kuth). The study aimed to explore the efficacy of these plants against multi-drug resistant and ESKAPE pathogens, which pose a significant threat to public health. A comprehensive review of scientific literature was conducted to gather relevant information on the antimicrobial properties and phytochemical constituents of the selected plants. The antibacterial activity of the plant extracts was evaluated against a panel of multi-drug resistant bacteria and ESKAPE pathogens, including *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* species. The results revealed that all four medicinal plants exhibited notable antibacterial activity against the tested pathogens. *Salvadora persica* demonstrated potent activity against *Enterococcus faecium* and *Staphylococcus aureus*, while *Caesalpinia pulcherrima* displayed significant activity against *Klebsiella pneumoniae* and *Acinetobacter baumannii*. *Thymus vulgaris* exhibited broad-spectrum activity against multiple pathogens, including *Pseudomonas aeruginosa* and *Enterobacter* species. *Saussurea lappa* also demonstrated promising antibacterial effects against various ESKAPE pathogens. Furthermore, the phytochemical analysis of the plant extracts revealed the presence of several bioactive compounds, such as alkaloids, flavonoids, phenolic compounds, and terpenoids, which are known for their antimicrobial properties. These compounds likely contribute to the observed antibacterial activity of medicinal plants.

Keywords: *ESKAPE, medicinal plants, antimicrobial activity, antibiotic resistance, bioactive compounds, alternative medicines, multidrug-resistant*

I. INTRODUCTION

The discovery of the first antibiotic - penicillin - was a revolutionary step in medical sciences, providing a dramatically new approach to infection control and healthcare (Bud, 2007). However, the misuse of these wonder drugs has borne another monster - antibiotic resistance. Antibiotic resistance occurs when bacteria change in response to the use of these medications, a challenge that is now associated with high morbidity and mortality from diseases that were otherwise easily treated (Frieri et al., 2017). Antibiotic resistance has been rearing its head wherever these medications are used for humans and animals without prescription. Further, with the lack of standard treatment guidelines in some countries, resistance is becoming increasingly

emergent due to overprescription by doctors and veterinarians as well as misuse by patients (Bud, 2007).

Today, the rapid development of MDR pathogens poses a significant challenge to the treatment of infectious diseases, which could grow as threatening as during the pre-antibiotic era (Brown & Wright, 2016). The ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp.*) are characterized by resistance to multiple first-line and last-resort antibiotics (Denissen et al., 2022) and recognized by the World Health Organisation as a significant danger to human health (Oliveira et al., 2020). Responsible for nosocomial infections, these bacteria place a significant burden on the health care system and economies. They are associated with high morbidity and mortality, increased treatment costs, diagnostic uncertainties, and distrust in traditional medicines (Santajit & Indrawattana, 2016). The known antibiotic resistance mechanisms of these bacteria include drug inactivation or alteration, modification of drug binding sites, reduced intracellular drug accumulation, and biofilm formation (Santajit & Indrawattana, 2016).

Historically, plants have played a role in the development of cosmetics, food, clothing, shelter, and most importantly - drugs. For centuries, Ayurveda has been utilized in India as a treatment for various ailments and illnesses and has popularised the therapeutic uses of plants, initially in the form of crude extracts or powders and further as purified forms (Anand et al., 2019; Khare et al., 2021). As these substances have been proven to inhibit the growth of pathogens and have no or least toxicity to host cells, the discovery of novel drugs in the face of ever-prevalent antibiotic resistance can be achieved with the use of plant extracts (Ahmad & Beg, 2001; Bhatia et al., 2021). The secondary metabolites produced by these plants are of particular interest against MDR due to their antibacterial properties, both in complex mixtures and as a single active component in their purified forms (Khare et al., 2021).

For example, ongoing research at AIIMS Bhopal, India hopes to develop an ayurvedic drug that could prove as a suitable substitute for allopathic medicine without any side effects. The availability of such alternative medicine and its potential as a solution in a post-antibiotic era necessitates further research and reviews regarding various ayurvedic plants and their interactions. However, there is no present compiled review of the efficacy of common Indian medicinal plants against ESKAPE pathogens, despite the prevalence of nosocomial infections and the use of Ayurveda in the country. To remedy the same, secondary research was conducted to explore the antimicrobial and phytochemical properties of *Salvadora persica* (Miswak), *Caesalpinia pulcherrima* (Peacock flower), *Thymus vulgaris* (Thyme), and *Saussurea lappa* (Kuth).

II. Exploring Indigenous Medicinal Plants

A. Salvadora persica

Section 1: **Morphology and Common Uses**

Salvadora persica, known as miswak or kharijal, is commonly found in the arid regions of India and often in saline soils. It is an evergreen small tree or large shrub, rarely reaching more than 1 ft in diameter and 3m in height ref. It has a soft, white-yellow wood with barks of old stem rugose and numerous drooping branches. It possesses a pleasant small, of cress or mustard, and a warm and pungent taste. The tree produces flowers greenish-yellow in colour and clusters of small, red edible fruits that are juicy but pungent (Khatak et al., 2010).

Beyond these uses, research has reported multiple biological effects of *S. persica* including antibacterial, antiviral, antifungal, antibiofilm, antioxidant, and even anti-ulcer activities (Aljarbou et al., 2022). With the increasing prevalence of its therapeutic uses, literature reviews regarding its dental uses are ubiquitous. However, there is limited and scattered information as to its action against antibiotic-resistant bacteria. With this and its commonality in India in mind, proven information regarding its efficacy against the ESKAPE pathogens was compiled.

Section 2: Antibacterial Actions of *S. persica*

In line with the aim of this research, another study endeavoured to demonstrate the antimicrobial effects of this medicinal against the clinical isolates of 10 MDR pathogens, amongst which were four ESKAPE pathogens: methicillin-resistant *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa,* and A*ctineobacter baumannii*. The study first showcased that the methanol extracts of this medicinal plant had greater efficacy than its aqueous extract against tested MDR pathogens, with the 400 mg/L methanol extract being the most effective overall. As for specific antimicrobial activities against ESKAPE bacteria, the study recorded the second-highest growth inhibition against *Klebsiella pneumoniae*. However, the highest MIC (Minimum Inhibitory Concentration) values were recorded for MRSA (methicillin-resistant *Staphylococcus aureus*) and *Actineobacter baumannii* along with *Stenotrophomonas maltophilia.* Overall, the authors concluded that *S. persica* is more useful against Gram-negative rather than Gram-positive pathogens. Also supported by other studies, this finding is suspected to be due to the different lipo-polysaccharides in their cell membranes and is crucial knowledge for the use of *S. persica* in the development of anti-bacterial medication (Al-Ayed et al., 2016; Alireza et al., 2014).

Alireza et al., 2014 similarly demonstrated the strong antibacterial action of methanol extracts of *S. persica* against the Gram-negative ESKAPE pathogens *Actineobacter baumannii, Pseudomonas aeruginosa, and Enterobacter cloacae* through procurement and evaluation of relatively low MIC values: 0.02, 0.04 and 0.08 mg/L, respectively. In this study, *S. persica* was also found to be more effective than the antibiotics tested against (Kanamycin, Cefotaxim, Fosfomycin, Colistine, Chloramphenicol, and Ticarcillin), widening the avenues for its use in the potential post-antibiotic era.

S. persica methanolic extract was tested and its efficacy was found highest against *P. aeruginosa* and *S. aureus* and relatively lower with *K. pneumoniae*. Not only did this study demonstrate the antimicrobial properties of this medicinal plant against these pathogens but it also did so specifically for wound infections. Additionally, Ahmed et al. found that the greatest incidence of postoperative wound infections in a hospital in Sudan was the result of poor antibiotic selection and increased levels of contamination within the hospital. These studies

collectively demonstrate that in the case of ESKAPE pathogen-borne infections, could prove to be extremely useful along with, after failure of, or instead of antibiotics (Tatke, 2017).

Section 3: Phytochemical Analysis of *S. persica*

Most studies conducted with *S. persica* attribute its antibacterial properties to its phytoconstituents. Phytochemical analysis of aqueous and methanolic extracts from *S. persica* revealed the presence of flavonoids, sterols, saponins, tannins, basic alkaloids and reducing components in alcoholic extracts and saponins, tannins and reducing components in aqueous extract. The methanolic extract had a wider variety of secondary metabolites that could be responsible for its greater antimicrobial activity in some studies. However, the superiority of solvent for *S. persica* extraction is still debatable due to contradictory results from another research.

Al-Ayed et al., 2016 identified a volatile compound, benzyl isothiocyanate (BITC), in extracts of *S. persica* that showed a strong bactericidal effect against Gram-negative bacteria but only lowered the growth of Gram-positive bacteria. The authors speculated that this Phyto compound may penetrate through the outermost membrane of a bacterium, interfere with its redox systems, and hamper its ability to maintain its membrane potential (Al-Ayed et al., 2016).

They explored the ethnopharmacology of this medicinal plant and discovered that the concentration of BITC is highest (73.5%) in *S. persica* tree bark extract rather than root or tender stem extracts. As this compound has been identified as a potent bacterial inhibitor, further research into the Phyto compounds present in the tree bark extract and their isolation could help create potent drugs to combat bacterial action.

B. Caesalpinia pulcherrima

Section 1: Morphology and Common Uses

Also known as Gulmohar or the peacock flower in India, *Caesalpinia pulcherrima* is a species of flowering plant belonging to the pea family Fabaceae. Usually a shrub or small tree, this plant grows to different heights in different climates. When in climates with few to no frosts, it is only semi-evergreen and can grow up to 3m while in Hawaii, it is evergreen and grows to 5m (Bakshi et al., 1999). It possesses a bi-pinnate foliage with small, oval leaflets and green or greyish-brown stems, depending on how woody they are. After its name, it produces flowers of orange, red, and yellow and is scentless with long stamens and pistils (Bakshi et al., 1999).

Typically, its flowers are harvested for medicinal uses such as to combat intestinal worms and cure sores. West Indians also make decoctions from its leaves and flowers that treat fever. As its leaves have purgative properties, they can be used for abortions and to make decoctions with its flowers to treat fever. Indonesians even pound it to treat children experiencing convulsions (Kumar et al., 2010). A phytochemical and pharmacological review of this plant elucidated its anticancer, antimicrobial, antioxidant, anti-inflammatory, anti-diabetic, immunosuppressive, and vasorelaxation properties (Pankaj et al., 2014). Considering the broad scope of *C. pulcherrima*'s applications worldwide as an ayurvedic plant and its various

therapeutic properties, this review regarding its phytoconstituents and efficacy against ESKAPE pathogens was prompted.

Section 2: Antibacterial Action of *C. pulcherrima*

Parekh & Chanda 2007, made aqueous and methanolic extracts of the aerial parts of *C. pulcherrima* and used agar well and disc diffusion to test their antibacterial activity against six bacterial species: *Bacillus cereus, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli,* and *Enterobacter aerogenes*. While the methanolic *C. pulcherrima* extract inhibited the three ESKAPE pathogens, the aqueous extract was only effective against *S. aureus* and *K. pneumoniae*. Overall, the maximum antibacterial activity was exhibited by *C. pulcherrima* out of the 12 medicinal plants tested. Additionally, it was found to be most effective against Gram-negative *K. pneumoniae* (Parekh & Chanda, 2007). While Exner et al., 2017 similarly reported that Gram-negative bacteria, *K. pneumoniae*, were most susceptible to this medicinal plant, however, other researchers have opposed this result (Exner et al., 2017). Authors of Swami et al. are of the opinion that the flower parts of *C. pulcherrima* were more effective against Gram-positive than Gram-negative bacteria, attributing this to the presence of an outer membrane possessing hydrophilic polysaccharide chains that forms an additional barrier (Swamy et al., 2014). Similarly, Pulipati et al. also reported that Gram-positive bacteria were more susceptible to *C. pulcherrima*'s flower extract than Gram-negative bacteria (Pulipati et al., 2012). This inconsistency could be due to varying experimental conditions or other extraneous variables and necessitates further research to arrive at a conclusion.

In the study conducted by Refahy et al. various extracts of *C. pulcherrima* were tested against *S. aureus* and *P. aeruginosa*: 100%, 95%, 85%, and 70% methanol, diethyl ether, petroleum ether, methylene chloride, ethyl acetate, and n-butanol. The total phenolic content (TPC) and total flavonoid content (TFC) in these extracts were also measured. It was found that the 70% methanol extract was most effective against *S. aureus*. In comparison, the 100% methanolic one inhibited *P. aeruginosa* the most, despite the TPC and TFC being the highest in other concentrations (Refahy et al., 2015).

Urinary tract infections are those caused anywhere in the urinary tract by microorganisms and are amongst the most common nosocomial infections. Of the 6 ESKAPE pathogens, *S. aureus, E. faecalis, P. aeruginosa,* and *K. pneumoniae* have been implicated as causes of UTIs. While antibiotics are commonly used to treat these infections, with the rise of MDR pathogens, their management, and treatment become more difficult. This occurs especially in hospital settings, where UTIs represent 30-40% of all nosocomial infections (Swamy et al., 2014). Swami et al. explored the efficacy of *C. pulcherrima* extracts against the aforementioned bacteria and found that they exhibited the strongest inhibition activity against them as compared to other flower extracts tested (*P. ferrugineum* and *D. regia*) (Swamy et al., 2014).

Multiple establishments of *C. pulcherrima*'s inhibition of *S. aureus*, *K. pneumoniae,* and *P. aeruginosa* across various studies indicates that it is an effective antimicrobial agent for drug-resistant as well as drug-susceptible strains of these pathogens.

Section 3: Phytochemical Analysis of *C. pulcherrima*

For the purpose of identification of major phytochemical constituents in a methanolic extract of *C. pulcherrima* flowers, a phytochemical analysis was carried out. Overall, it revealed the presence of secondary metabolites such as glycosides, flavonoids, phenolics, and tannins (Refahy et al., 2015). A similar analysis of another methanol extract of this medicinal extract indicated the presence of saponins and terpenoids along with the aforementioned phytoconstituents. Additionally, this study once again demonstrated how secondary metabolites found in extracts of this flower differ from solvent to solvent. The methanolic extract consisted of the largest number of phytochemicals, followed by petroleum ether, acetone, and finally ethanol (JM & Sreeja, 2018). As the antibacterial properties of medicinal plants are speculated to be the result of their phytoconstituents, this serves to highlight how crucial the choice of solvent for their extraction is, especially for drug development.

JM & Sreeja 2018, isolated and tested some phenolic compounds from *C. pulcherrima* flower extracts against clinical isolates of some ESKAPE pathogens. Methyl gallate was found to be a potent bactericidal agent against methicillin-resistant *S. aureus* and *K. pneumoniae*. A time-kill kinetics assay of MRSA (methicillin-resistant *Staphylococcus aureus*) using methyl gallate was carried out, showing a reduction in CFU (colony forming units) in two hours and complete bactericidal activity at 24 hours. Methyl gallate was also synergistically tested with Amoxicillin against MRSA and a remarkable fall in MICs (minimum inhibitory concentrations) of both - from 256 µg/ml to 0.0625 µg/ml and 500 µg/ml to 31.25 µg/ml, respectively - was discovered. Along with this compound, gallic acid, and ethyl gallate also exhibited inhibitive properties against the MDR isolates (Khan et al., 2021).

C. Thymus vulgaris

Section 1: Morphology and Common Uses

Thymus vulgaris, or common garden thyme, is an aromatic and perennial flowering plant from the Lamiaceae family (Patil et al., 2021). While it originated from Southern Europe, it is now widely cultivated in Indian regions such as the Nilgiris and the foothills of the Himalayas (Hosseinzadeh et al., 2015). It can grow to 6-12 feet tall and its numerous, somewhat woody stems from a foliage mound and are covered in highly aromatic grey-green leaves. Tiny, tubular, and lilac flowers appear at the end of the stems during late spring and early summer (Stahl & Venskutonis, 2012). The plant flourishes well in arid environments and unshaded areas in coarse, rough, and well-drained soils that normally prove hostile to other plants (Javed et al., 2013).

A commonly used plant, *T. vulgaris* possesses a variety of ethnobotanical and culinary applications. It has been used as a treatment for wounds due to its healing and antiseptic properties. The plant has also been reported to possess antiseptic, astringent, carminative, tonic, and anthelmintic properties. Overall, its wide array of properties, especially bactericidal ones, necessitates a thorough review of its potential use against MDR pathogens (Patil et al., 2021).

Section 2: Antibacterial Action of *Thymus vulgaris*

Alibi et al., 2020 investigated the antimicrobial, antibiofilm, and antiphase activity of *Thymus vulgaris* extracts against four multidrug-resistant bacteria - *E. coli, S. aureus, C. albicans,* and *P.*

aeruginosa. The bioactivity of thyme oil and thyme aqueous extract was investigated, with both showing bactericidal action against these ESKAPE pathogen isolates. Thyme oil was reported to have greater antimicrobial action than aqueous thyme extracts due to its lower MIC values across the board. The authors speculated that this may be due to its higher phenolic content as the aqueous extract loses its active components during grinding and boiling. However, both thyme oil and its aqueous extracts were still found to be most effective against *S. aureus*, with MIC values of 0.2 mg/L and 40 mg/L, respectively. Notably, even though *P. aeruginosa* was found to have the greatest antibiotic resistance out of all isolates, thyme oil showed strong inhibitory action against it even at lower concentrations (0.8 mg/L). Using scanning electron microscopy, the authors researched the effect of thyme on biofilm formation by MDR strains, a previously reported mechanism of antibiotic resistance. Biofilms are microbial communities that adhere to biotic and abiotic surfaces with the cells inside them sheathed in a self-produced matrix (Alibi et al., 2020). The bacterial cells exposed to thyme were observed to be irregularly shaped, with holes and cracks. Accordingly, it was suggested that thyme treatment induces damage to the cell membrane, eventually causing lysis. These findings are concordant with the results of (Helander et al., 1998), which reported the mechanisms of action of thymol (a major active component of thyme) in attacking the outer membranes, resulting in the disruption of the cell wall and a subsequent release of intracellular components and depletion of ATP (Qureshi et al., 2022).

Similar effects were also reported by Alibi et al., 2020 wherein along with the antibacterial and anti-biofilm, the anti-quorum sensing (QS) and antioxidant properties of thyme were explored. The intercellular signalling mechanism, known as quorum-sensing, is the process through which antibiotic resistance, biofilm production, and virulence properties are controlled. In this research, forty-four strains of multi-drug resistant bacteria, of which 11 were variants of ESKAPE pathogens, were tested against four essential oils. These 11 strains were of the species *K. pneumoniae*, *S. aureus*, *A. baumannii*, and *P. aeruginosa*. Along with *Cinnamomum verum* and *Eugenia caryophyllata*, the *T. vulgaris* EO (essential oil) demonstrated remarkable antimicrobial action against all tested MDR strains. The MBC/MIC (minimum bactericidal concentration/minimum inhibitory concentration) ratios of these essential oils were equal to 1 for most bacteria, suggesting a bactericide effect. Biofilm formation was observed in all tested strains but a significant reduction in this mechanism was observed due to sub-inhibitory concentrations of thyme essential oil. Finally, the anti-QS activity of the four EOs was reflected in a decrease in violacein production, with the highest being shown by thyme oil at 99.41%. Notably, no significant differences were observed between the bactericidal action of essential oils against Gram-negative versus Gram-positive bacteria in this study (Alibi et al., 2020).

Once again turning the focus to the role of these ayurvedic plants in nosocomial infections, Amorese et al., 2018 studied the activity of essential oils against *P. aeruginosa* isolated from infected hip implants. Periprosthetic joint infections are one of the most severe and costly complications of joint arthroplasty. These infections are also associated with significant psychological and physical morbidity in patients. In this study, the susceptibility of sixteen strains of *P. aeruginosa* against 11 antibiotics and thyme essential oil. All strains showed multiple antibiotic resistance but were susceptible to aminoglycosides, third-generation fluoroquinolones, third-generation cephalosporins, and some carbapenems. Thymus vulgaris was found to be

effective on eleven strains of *P. aeruginosa* at 8% concentration and the remaining five variants at 16% (Amorese et al., 2018).

Other studies have also reported the antibacterial action of *T. vulgaris* against ESKAPE pathogens such as *E. faecium* and other strains of *S. aureus* and *P. aeruginosa* (Jain & Choudhary, 2022).

Section 3: Phytochemical Analysis of *Thymus vulgaris*

Owing to its commonality, various researchers have studied the phytochemical constituents of *Thymus vulgaris* through methods such as gas chromatography-mass spectroscopy (GC-MS) and high-performance liquid chromatography (HPLC). Overall, the presence of phenolic compounds, terpenoids, flavonoids, steroids, alkaloids, tannins, and saponins was detected in the thyme extracts. Notably, volatile compounds were found to be most prevalent (Abdelli et al., 2017; Al-Asmari et al., 2017; Stefanis et al., 2019). A deeper dive into the essential oils of *T. vulgaris* demonstrates the presence of a list of hydrocarbons, oxides, alcohols/esters, and aldehydes/ketones. Among all of the volatile compounds, major pharmacological activities have been attributed to thymol, carvacrol, geraniol, linalool, α- and β-pinene, p-cymene, and γ-terpinene (Patil et al., 2021). Thymol and carvacrol have been specifically and thoroughly explored in relation to their antibacterial effects. The most frequently reported mechanism of both isomers is bacterial lysis, leading to leakage of intracellular components and cell death. Other mechanisms include the inhibition of efflux pumps, prevention or disruption of biofilm formation, decreasing bacterial motility, and inhibition of membrane ATPases (Kachur & Suntres, 2020).

Similarly, specific phenolic compounds, flavonoids, steroids, tannins, alkaloids, saponins, and other phytoconstituents have also been found. Still, as most of the chemical components are classified under phenolic compounds and essential oils, the specific therapeutic mechanisms and potential of other phytochemicals are yet to be explored (Patil et al., 2021). The antimicrobial effect of thyme essential oils depends on incubation, synergistic ingredient effects, the solvent used for extraction, and more. Hossain et al. demonstrated how the phytoconstituents of thyme extract differ from solvent to solvent, with its butanol extract yielding the greatest number of compounds (Hossain et al., 2022).

Overall, despite their proven antibacterial action, there is limited use of thyme essential oils and other phytochemicals in the pharmaceutical industry. Thus, information regarding optimal solvents, extraction conditions, and the synergistic effects of these compounds with each other and antibiotics must be gathered and compiled to enable the application of *T. vulgaris* in the medicinal community.

D. ***Saussurea lappa***

Section1: Morphology and Common Uses

Saussurea lappa, better known as kuth or kushta, belongs to the Asteraceae family and is another perennial herb widely used in Ayurveda. In India, it can be found in the Kashmir and Alpine Himalayan ranges at 8,000 to 12,000 feet. It is a robust, erect plant that can grow 25 to 80 cm high. Its roots already have various applications in the pharmaceutical industry and are

stout, brownish with longitudinal streaks or furrows and can grow to 60 cm long. They have a distinct, sweet, and pleasant odour but taste bitter. It has simple alternating leaves that are radical, irregularly toothed and have long lobately winged stalks. Dark purple or black flowers occupy its axillary and terminal heads and the plant produces two kinds of fruits - Achenes and Pappas. Pappas is about 1.7 m long, feathery and brown while Achenes is 3 mm long, curved and compressed (Alaagib & Ayoub, 2015; Gautam & Asrani, 2018).

In India, this herb has been mentioned in the Atharvaveda and is regarded as an ancient Vedic plant god. It was supposedly derived from a heavenly plant originating from the Himalayas. It was used to strengthen and stabilize digestion, increase fertility, ease pain, and even as an ointment for ulcer treatments (Tharak & Zuhra, 2012). Research has now confirmed its pharmacological properties, which are in line with its traditional uses. Another secondary review delved deep into the antitumor, antibacterial, anti-inflammatory, hepatoprotective, antiulcer, cholagogic, and immunomodulatory properties. The same paper also reported the various miscellaneous activities of this medicinal plant including cardiovascular, anticonvulsant, antiparasitic effects, and more (Gautam & Asrani, 2018). Keeping its commonality and a wide array of applications in mind, the following review was compiled to assess its effectiveness against ESKAPE pathogens.

Section 2: Antibacterial Action of *Saussurea lappa*

Naseer et al. 2022 evaluated the antibacterial effects of *Saussurea lappa* against the multidrug-resistant human pathogenic bacteria *K. pneumoniae*, methicillin-resistant *S. aureus*, *P. aeruginosa*, *E. coli* and Extended Spectrum Beta-Lactamase (ESBL). Using the agar well diffusion method, the authors assessed the dose-dependent antimicrobial effects of the ethanolic *S. lappa extract*. The plant was found to be effective against the four aforementioned ESKAPE pathogens and its ethanol extract exhibited a concentration-dependent zone of inhibition with the largest zone of inhibition corresponding to the highest concentration and *vice versa*. The effect of *S. lappa* varied from pathogen-to-pathogen. At a concentration of 2000 µg/mL, its ethanol extract was bacteriostatic against *S. aureus* and *P. aeruginosa* but effective against *K. pneumoniae and A. baumannii.* However, the herb extract turned bactericidal against *S. aureus* and *P. aeruginosa* at concentrations of 6000 µg/mL and 4000 µg/mL, respectively.

Another research explored the effectiveness of different *S. lappa* extracts against six ATCC (American Type Culture Collection) and three MDR strains: *S. aureus*, *K. pneumoniae* and *P. aeruginosa.* The roots, leaves, stems, and flowers of *S. lappa* were extracted using methanol and chloroform solvents. Their bactericidal activities were evaluated using the agar well diffusion method. The results of this study revealed the potent antibacterial action of the plant extracts against the ATCC and MDR strains. Overall, the greatest effect was observed for the chloroform leaf extract against MDR *P. aeruginosa*. While all solvent extracts inhibited the activity of MDR *K. pneumoniae*, the methanolic stem extract was ineffective against MDR *P. aeruginosa* and all methanolic extracts were bacteriostatic to the *S. aureus* strains. Additionally, the results showed that the leaf and root extracts of this plant were most inhibitory against the bacteria, especially the MDR ones (Naseer et al., 2022).

The strong antimicrobial of *S. lappa* has also been reported in Omer et al. 2019 where the effect of its ethanolic and aqueous root extracts against *S. aureus* and *Salmonella sp.* The water

and ethanol extracts were only bactericidal against *S. aureus*. Further, the ethanolic extract was generally more effective than the aqueous one with a mean inhibition zone of between 18 mm to 20 mm as compared to the water extract's 15 mm (Omer et al., 2019).

In fact, the roots of this plant seem to possess potent antimicrobial activity as their methanolic, cold water, and hot water extracts showed inhibitory effects against *S. aureus, P aeruginosa,* and *E. faecalis* (Khalid et al., 2011). Overall, the methanolic extract exhibited the greatest bactericidal action with a maximum inhibition zone of 18 mm against *P. aeruginosa* and *S. aureus.* Only the hot water and methanolic extracts managed to inhibit all strains, while the cold water one showed no activity against *E. faecalis* (Khalid et al., 2011).

Both previously mentioned researches attributed these trends to the presence of greater numbers of bioactive compounds in specific extracts, making the consideration of the solvent extraction method crucial for the use of this plant in pharmaceuticals.

Section 3: Phytochemical Analysis of *S. lappa*

Hasson et al., 2013 accompanied their assessment of *S. lappa's* microbial action against MDR bacteria with a thorough phytochemical review meant to detect the presence of 20 bioactive compounds. Overall, sixteen active components were reported across the chloroform and methanol root, leaf, stem, and flower extracts. The presence of flavonoids, alkaloids, proteins, β-carotene, coumarins, saponins, carbohydrates, fats, and quinones was confirmed in all extracts while glycosides, emodins, and phlorotannins were found in none.

The greatest number of phytoconstituents were found in the methanol root extract at 14, followed by 13 in the chloroform root and leaf extracts. Hasson et al., 2013 attributed the high effectiveness of the methanol root extract of *S. lappa* to the bioactive compound richness and possible synergistic action of two or more compounds against *S. aureus*. Andrographolide, 4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, and isosteviol methyl ester are known for their strong pharmacological activities and were present exclusively in ethanol extracts. The potential of andrographolide as a pharmaceutical agent has also been confirmed by Banerjee et al. 2017, which showed how an *S. aureus* strain was found to be sensitive to this compound at a MIC of 100 µg/ml, which is comparable to the amount detected in the ethanol extract (Hasson et al., 2013).

This same research also briefly mentioned the role of fatty acid methyl esters and sesquiterpenes in defence against their microorganisms. Similarly, other studies have also reported the bioactive potential of sesquiterpenes such as costunolide, Isodihydrocostunolide, cynaropicrin in drug development.

III. CONCLUSION

In conclusion, this study highlights the significant antibacterial potential of *Salvadora persica, Caesalpinia pulcherrima, Thymus vulgaris,* and *Saussurea lappa* against multi-drug resistant and ESKAPE pathogens. The presence of various bioactive phytochemicals further supports their therapeutic potential. These findings provide valuable insights for the development of alternative and effective strategies to combat drug-resistant bacterial infections using indigenous

Indian medicinal plants. Further research and clinical studies are warranted to explore their application in the development of novel antimicrobial agents or adjunct therapies.

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Conflict of Interest
The authors declared that they have no conflict of interest.

References:

1. Abdelli, W., Bahri, F., Romane, A., Höferl, M., Wanner, J., Schmidt, E., & Jirovetz, L. (2017). Chemical Composition and Anti-inflammatory Activity of Algerian *Thymus vulgaris* Essential Oil. *Nat Prod Commun, 12*(4), 611-614.

2. Ahmad, I., & Beg, A. Z. (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *Journal of Ethnopharmacology, 74*(2), 113-123. doi:https://doi.org/10.1016/S0378-8741(00)00335-4

3. Alaagib, R. M. O., & Ayoub, S. M. H. (2015). On the chemical composition and antibacterial activity of *Saussurea lappa* (Asteraceae). *The Pharma Innovation Journal, 4*, 73-76.

4. Al-Asmari, A. K., Athar, M. T., Al-Faraidy, A. A., & Almuhaiza, M. S. (2017). Chemical composition of essential oil of *Thymus vulgaris* collected from Saudi Arabian market. *Asian Pacific Journal of Tropical Biomedicine, 7*(2), 147-150. doi:https://doi.org/10.1016/j.apjtb.2016.11.023

5. Al-Ayed, M. S. Z., Asaad, A. M., Qureshi, M. A., Attia, H. G., & Al Marrani, A. H. (2016). Antibacterial Activity of *Salvadora persica* L. (Miswak) Extracts against Multidrug Resistant Bacterial Clinical Isolates. *Evidence-Based Complementary and Alternative Medicine, 2016*, 7083964. doi:10.1155/2016/7083964

6. Alibi, S., Ben Selma, W., Ramos-Vivas, J., Smach, M. A., Touati, R., Boukadida, J., . . . Ben Mansour, H. (2020). Anti-oxidant, antibacterial, anti-biofilm, and anti-quorum sensing activities of four essential oils against multidrug-resistant bacterial clinical isolates. *Current Research in Translational Medicine, 68*(2), 59-66. doi:https://doi.org/10.1016/j.retram.2020.01.001

7. Aljarbou, F., Almobarak, A., Binrayes, A., & Alamri, H. M. (2022). *Salvadora persica's* Biological Properties and Applications in Different Dental Specialties: A Narrative Review. *Evidence-Based Complementary and Alternative Medicine, 2022*, 8667687. doi:10.1155/2022/8667687

8. Alireza, R. G., Afsaneh, R., Seied Hosein, M. S., Siamak, Y., Afshin, K., Zeinab, K., . . . Amir Reza, R. (2014). Inhibitory activity of *Salvadora persica* extracts against oral bacterial strains associated with periodontitis: An in-vitro study. *J Oral Biol Craniofac Res, 4*(1), 19-23. doi:10.1016/j.jobcr.2014.01.001

9. Amorese, V., Donadu, M., Usai, D., Sanna, A., Milia, F., Pisanu, F., . . . Doria, C. (2018). In vitro activity of essential oils against *Pseudomonas aeruginosa* isolated from infected hip implants. *The Journal of Infection in Developing Countries, 12*(11), 996-1001.

10. Anand, U., Jacobo-Herrera, N., Altemimi, A., & Lakhssassi, N. (2019). A Comprehensive Review on Medicinal Plants as Antimicrobial Therapeutics: Potential Avenues of Biocompatible Drug Discovery. *Metabolites, 9*(11), 258. Retrieved from https://www.mdpi.com/2218-1989/9/11/258

11. Bakshi, D. N. G., Sensarma, P., & Pal, D. C. (1999). *A lexicon of medicinal plants in India*.Naya Prokash, Calcutta.

12. Bhatia, P., Sharma, A., George, A. J., Anvitha, D., Kumar, P., Dwivedi, V. P., & Chandra, N. S. (2021). Antibacterial activity of medicinal plants against ESKAPE: An update. *Heliyon, 7*(2), e06310. doi:https://doi.org/10.1016/j.heliyon.2021.e06310

13. Brown, E. D., & Wright, G. D. (2016). Antibacterial drug discovery in the resistance era. *Nature, 529*(7586), 336-343. doi:10.1038/nature17042

14. Bud, R. (2007). Antibiotics: the epitome of a wonder drug. *BMJ, 334*(suppl 1), s6-s6. doi:10.1136/bmj.39021.640255.94

15. Denissen, J., Reyneke, B., Waso-Reyneke, M., Havenga, B., Barnard, T., Khan, S., & Khan, W. (2022). Prevalence of ESKAPE pathogens in the environment: Antibiotic resistance status, community-acquired infection and risk to human health. *International Journal of Hygiene and Environmental Health, 244*, 114006. doi:https://doi.org/10.1016/j.ijheh.2022.114006

16. Exner, M., Bhattacharya, S., Christiansen, B., Gebel, J., Goroncy-Bermes, P., Hartemann, P., . . . Trautmann, M. (2017). Antibiotic resistance: What is so special about multidrug-resistant Gram-negative bacteria? *GMS Hyg Infect Control, 12*, Doc05. doi:10.3205/dgkh000290

17. Frieri, M., Kumar, K., & Boutin, A. (2017). Antibiotic resistance. *Journal of Infection and Public Health, 10*(4), 369-378. doi:https://doi.org/10.1016/j.jiph.2016.08.007

18. Gautam, H., & Asrani, R. (2018). Phytochemical and pharmacological review of an ethno medicinal plant: *Saussurea Lappa*. *Vet Res Int, 6*, 1-9.

19. Hasson, S. S. A., Al-Balushi, M. S., KhazinaAlharthy, Al-Busaidi, J., MunaSulimanAldaihani, Othman, M. S., . . . AhmedIdris, M. (2013). Evaluation of anti–resistant activity of Auklandia (*Saussurea lappa*) root against some human pathogens. *Asian Pacific Journal of Tropical Biomedicine, 3*(7), 557-562. doi:https://doi.org/10.1016/S2221-1691(13)60113-6

20. Helander, I. M., Alakomi, H.-L., Latva-Kala, K., Mattila-Sandholm, T., Pol, I., Smid, E. J., . . . von Wright, A. (1998). Characterization of the Action of Selected Essential Oil Components on Gram-Negative Bacteria. *Journal of Agricultural and Food Chemistry, 46*(9), 3590-3595. doi:10.1021/jf980154m

21. Hossain, M. A., Alrashdi, Y. B. A., & Al Touby, S. (2022). A review on essential oil analyses and biological activities of the traditionally used medicinal plant Thymus vulgaris L. International Journal of Secondary Metabolite, 9(1), 103-111.

22. Hosseinzadeh, S., Jafarikukhdan, A., Hosseini, A., & Armand, R. (2015). The application of medicinal plants in traditional and modern medicine: a review of *Thymus vulgaris*. *International Journal of Clinical Medicine, 6*(09), 635-642.

23. Jain, N., & Choudhary, P. (2022). Phytochemistry, Traditional Uses and Pharmacological Aspect of *Thymus vulgaris*: A Review. *Indian Journal of Pharmaceutical Sciences, 84*(6), 1369-1379.

24. Javed, H., Erum, S., Tabassum, S., & Ameen, F. (2013). An overview on medicinal importance of *Thymus vulgaris*. *Journal of Asian Scientific Research, 3*(10), 974-982.

25. JM, S. P., & Sreeja, N. (2018). Priliminary phytochemical, antioxidant and antimicrobial analysis of *Caesalpinia pulcherrima*.

26. Kachur, K., & Suntres, Z. (2020). The antibacterial properties of phenolic isomers, carvacrol and thymol. *Critical Reviews in Food Science and Nutrition, 60*(18), 3042-3053. doi:10.1080/10408398.2019.1675585

27. Khalid, A., Urrehman, U., Sethi, A., Khilji, S., Fatima, U., Khan, M., . . . Murtaza, G. (2011). Antimicrobial activity analysis of extracts of *Acacia modesta, Artimisia absinthium, Nigella sativa and Saussurea lappa* against Gram positive and Gram negative microorganisms. *African Journal of Biotechnology, 10*, 4574-4580.

28. Khan, F., javed, s., Hanif, A., Faizi, S., Yousuf, M., & Kazmi, S. (2021). Antimicrobial activity of phenolic compounds isolated from *Caesalpinia pulcherrima*.

29. Khare, T., Anand, U., Dey, A., Assaraf, Y. G., Chen, Z.-S., Liu, Z., & Kumar, V. (2021). Exploring Phytochemicals for Combating Antibiotic Resistance in Microbial Pathogens. *Frontiers in Pharmacology, 12*. doi:10.3389/fphar.2021.720726

30. Khatak, M., Khatak, S., Siddqui, A. A., Vasudeva, N., Aggarwal, A., & Aggarwal, P. (2010). *Salvadora persica*. *Pharmacogn Rev, 4*(8), 209-214. doi:10.4103/0973-7847.70920

31. Kumar, D., Singh, J., Baghotia, A., & Kumar, S. (2010). Anticonvulsant effect of the ethanol extract of *Caesalpinia pulcherrima* (L.) Sw., Fabaceae, leaves. *Revista Brasileira de Farmacognosia, 20*, 751-755. doi:10.1590/S0102-695X2010005000014

32. Naseer, S., Iqbal, J., Naseer, A., Kanwal, S., Hussain, I., Tan, Y., . . . Mahmood, T. (2022). Deciphering chemical profiling, pharmacological responses and potential bioactive constituents of *Saussurea lappa* Decne. Extracts through *in vitro* approaches. *Saudi Journal of Biological Sciences, 29*(3), 1355-1366. doi:https://doi.org/10.1016/j.sjbs.2022.01.040

33. Oliveira, D. M. P. D., Forde, B. M., Kidd, T. J., Harris, P. N. A., Schembri, M. A., Beatson, S. A., . . . Walker, M. J. (2020). Antimicrobial Resistance in ESKAPE Pathogens. *Clinical Microbiology Reviews, 33*(3), 10.1128/cmr.00181-00119. doi:doi:10.1128/cmr.00181-19

34. Omer, R., Faisal, H., Koua, F., Abdelhag, I., & Ismail, A. (2019). Gas chromatography/mass spectrometry profiling of the costus plant *Saussurea lappa* (Decne.) C.B. Clarke root extracts and their anti-bacterial activity. *Journal of Applied Pharmaceutical Science, 9*, 73-081. doi:10.7324/JAPS.2019.90509

35. Pankaj, N., Nanda, D., Batsa, R., & Nainwal, P. (2014). A review on phytochemical and pharmacological aspects of *Caesalpinia pulcherrima*. *international journal of research in Ayurveda and Pharmacy, 2*, 416-421.

36. Parekh, J., & Chanda, S. (2007). In vitro Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants. *Turkish Journal of Biology, 31*, 53-58.

37. Patil, S. M., Ramu, R., Shirahatti, P. S., Shivamallu, C., & Amachawadi, R. G. (2021). A systematic review on ethnopharmacology, phytochemistry and pharmacological aspects of *Thymus vulgaris* Linn. *Heliyon, 7*(5), e07054. doi:https://doi.org/10.1016/j.heliyon.2021.e07054

38. Pulipati, S., Pallavi, G., Sujan, B., Babu, K., & Babu, P. (2012). Evaluation of antibacterial activity of fresh and dry flower extracts of *Caesalpinia Pulcherrima* L., IJBPR, Volume 3, Issue 3, Page 360-365, June 2012.

39. Qureshi, W., Saeed, F., Ajaz, M., & Rasool, S. (2022). *In vitro* antimicrobial, antibiofilm and antiphage activity of thyme (*Thymus vulgaris*). *Pakistan Journal of Botany, 54*. doi:10.30848/PJB2022-3(43)

40. Refahy, L. A., Farghaly, T. A., Abdel-Aziz, M. S., & Mohamed, T. (2015). Antimicrobial, Antioxidant and Cytotoxic Potential of *Caesalpinia pulcherrima* Flower.

41. Santajit, S., & Indrawattana, N. (2016). Mechanisms of Antimicrobial Resistance in ESKAPE Pathogens. *Biomed Res Int, 2016*, 2475067. doi:10.1155/2016/2475067

42. Stahl, B., & Venskutonis, R. (2012). Thyme. Handbook of Herbs and Spices, second ed., *Woodhead Publishing, London, UK*, 499-525.

43. Stefanis, I., Hadjipavlou-Litina, D., Bilia, A.-R., & Karioti, A. (2019). LC-MS- and NMR-guided isolation of monoterpene dimers from cultivated *Thymus vulgaris* varico 3 hybrid and their antityrosinase activity. *Planta Med, 85*(11/12), 941-946. doi:10.1055/a-0927-7041

44. Swamy, H. C. S., Asha, M. M., Chaithra, M., Vivek, M. N., Yashoda, K., & Kekuda, T. R. P. (2014). Antibacterial activity of flower extract of *Caesalpinia pulcherrima, Delonix regia* and *Peltaphorum ferrugineum* against urinary tract pathogens. *International Research Journal of Biological Sciences, 3*(4), 80-83.

45. Tatke, P. (2017). Evaluation of antioxidant, antimicrobial and wound healing potential of *Salvadora persica* twig extracts. *World Journal of Pharmaceutical Research*, 1186-1199. doi:10.20959/wjpr20174-8175

46. Tharak., M., & Zuhra, K. (2012). Comprehensive *in-vitro* pharmacological activities of different extracts of *Saussurea lappa*. *European Journal of Experimental Biology, 2*.