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EDITED BY
Rabia Siddique,
Government College University,
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The University of Melbourne, Australia
Murtaza Ali,
Foshan University, China

*CORRESPONDENCE
Waqas Qamar Zaman
drwaqas@iese.nust.edu.pk
Salam A. Ibrahim
ibrah001@ncat.edu

†These authors have contributed equally to this work

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Evaluation of the antimicrobial effects of *Capsicum, Nigella sativa, Musa paradisiaca* L., and *Citrus limetta*: A review

Sonia Abid Bhatti[1†], Muhammad Hammad Hussain[2†], Muhammad Zubair Mohsin[2], Ali Mohsin[2], Waqas Qamar Zaman[1*], Meijin Guo[2], Muhammad Waheed Iqbal[3], Shahida Anusha Siddiqui[4,5], Salam A. Ibrahim[6*], Saeed Ur-Rehman[7] and Sameh A. Korma[8,9]

[1]Institute of Environmental Sciences and Engineering, School of Civil and Environmental Engineering, National University of Sciences and Technology (NUST), Islamabad, Pakistan, [2]State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai, China, [3]School of Food and Biological Engineering, Jiangsu University, Zhenjiang, China, [4]Technical University of Munich Campus Straubing for Biotechnology and Sustainability, Straubing, Germany, [5]German Institute of Food Technologies (DIL e.V.), Quakenbrück, Germany, [6]Food Microbiology and Biotechnology Laboratory, Food and Nutritional Sciences Program, North Carolina A&T State University, Greensboro, NC, United States, [7]School of Biochemistry and Biotechnology, University of the Punjab, Lahore, Pakistan, [8]Department of Food Science, Faculty of Agriculture, Zagazig University, Zagazig, Egypt, [9]School of Food Science and Engineering, South China University of Technology, Guangzhou, China

The extensive use of antibiotics and vaccines against microbial infections can result in long-term negative effects on humans and the environment. However, there are a number of plants that have antimicrobial effects against various disease-causing microbes such as bacteria, viruses, and fungi without negative side effects or harm to the environment. In this regard, four particular plants- *Capsicum*, *Nigella sativa*, *Musa paradisiaca* L., and *Citrus limetta* have been widely considered due to their excellent antimicrobial effect and ample availability. In this review, we discuss their antimicrobial effects due to the presence of thymoquinone, p-cymene, pinene, alkaloids, limonene, camphene, and melanin. These antimicrobial compounds disrupt the cell membrane of microbes, inhibit cellular division, and form biofilm in bacterial species, eventually reducing the number of microbes. Extraction of these compounds from the respective plants is carried out by different methods such as soxhlet, hydro-distillation, liquid-liquid extraction (LLE), pressurized liquid extraction (PLE), solid-phase extraction (SPE), supercritical fluid extraction (SFE), pulsed electric field (PEF), microwave-assisted extraction (MAE), enzyme-assisted extraction (EAE), ultrasound-assisted extraction (UAE), and high-voltage electrical discharge. Suitable selection of the extraction technique highly depends upon the associated advantages and disadvantages. In order to aid future study in this field, this review paper summarizes the advantages and disadvantages of each of these approaches. Additionally, the discussion covers how antimicrobial agents destroy harmful bacteria. Thus, this review offers in-depth knowledge to researchers on the antibacterial properties of *Capsicum, Nigella sativa, Musa paradisiaca* L. peels, and *Citrus limetta*.

KEYWORDS

antimicrobial agent, antimicrobial mechanism, solid waste management, extraction techniques, *Capsicum*, *Nigella sativa*, *Musa paradisiaca* L., *Citrus limetta*

Introduction

A global public health crisis has been caused by the rise in resistant bacteria over the past few decades (Dhingra et al., 2020; Hu et al., 2020; Ibrahim et al., 2021). Although antibiotics are used to treat illnesses by killing bacteria or limiting their growth, they can also have several negative effects (Gyawali and Ibrahim, 2014). For example, because some disease-causing bacteria have acquired resistance to particular antibiotics as a result of their widespread and sustained usage, these treatments are no longer as effective (Namita and Mukesh, 2012). In this regard, antimicrobial agents can eliminate resistant pathogenic microbes without imposing any known side effects. Antimicrobial agents disrupt the cellular structure of microbes, thus inhibiting various infectious diseases (Peterson, 2008) and are used to treat humans with diseases that are primarily due to non-human sources (Organization, 2019). According to WHO (world health organization), the overall consumption of antimicrobial agents is in between 4.4 and 64.4 Defined Daily Doses (DDD) per 1,000 persons per day. As per WHO data, the national estimates of antimicrobial consumption in 65 countries and areas: six from the Region of America, four from the African Region, three from the Eastern Mediterranean Region, 46 from the European Region: and six from the Western Pacific Region are presented in Figure 1 (Organization, 2018).

According to WHO, residents of these low-income countries are more likely to die of communicable and infectious diseases such as malaria, tuberculosis, diarrhea, HIV/AIDS, etc. (Philip, 2017). Therefore, suitable preventive measures against these infectious and parasitic diseases should be adopted. These measures may include self-cleanliness, proper sanitation, safe food preparation and handling, and the use of antimicrobial agents to immunize the body against infectious and parasitic diseases (Mehta et al., 2014). Nature also plays its role in fighting against disease-causing microbes (Ibrahim et al., 2011; Bor et al., 2016). For example, many plants such as *Nigella sativa*, *Capsicum*, *Musa paradisiaca* L., and *Citrus limetta* have attracted tremendous public interest due to their efficient antimicrobial agents (Dutta et al., 2020b; El-Naggar et al., 2020; Ajijolakewu et al., 2021; Vanlalveni et al., 2021; Vijayakumar et al., 2021). Furthermore, whether in underdeveloped, developing, or developed regions of the world, these natural goods are widely accessible. Additionally, these items are cost-effective in their use because they don't need to be prepared using a time-consuming procedure in order to fight infectious bacteria (Ncube et al., 2008).

Nigella sativa, *Capsicum*, peels of *Musa paradisiaca* L., and *Citrus limetta* contain effective antimicrobial agents against various infectious microbes such as the following: pathogenic bacteria (gram-negative and gram-positive), including *S. aureus*, *Candida albicans*, *E. coli*, Gram-positive cocci, *Microsporum canis*, *Trichophyton mentagrophytes*, *Trichophyton interdigitale*, four species of *Trichophyton rubrum*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Listeria monocytogene*, *Bacillus cereus*, *Vibrio parahaemolyticus*, *Pseudo. aeruginosa*, *Salmonella enteritidis*, *Sal. Typhimurium*, and *Shigella flexneri* (Hanafy and Hatem, 1991; Hosseinzadeh et al., 2007; Hannan et al., 2008; Halawani, 2009; Chaieb et al., 2011; Alshareef, 2019). The microbes listed above are known to cause the following diseases: pneumonia, bloodstream infections, wound or surgical site infections, methicillin-resistant *Staphylococcus aureus* (MRSA), urinary tract infection, "traveler's diarrhea," immunologic infections, diarrhea (sometimes bloody), fever, stomach cramps (Awaisheh and Ibrahim, 2009; Awaisheh et al., 2013; Blinov et al., 2022) and various others as reported in earlier conducted studies (Edelson and Unanue, 2000; Otto, 2009; Doernberg et al., 2017; Liu and Ji, 2018). These diseases can be prevented by using *Nigella sativa*, *Capsicum*, peels of *Musa paradisiaca* L., and *Citrus limetta* as antimicrobial agents. We have chosen to focus on these particular natural resources due to their global availability.

Worldwide, *Capsicum* grows on 1.93 million hectares of surface area. China is the world's largest producer of *Capsicum* at ∼16 million tons annually. Mexico is the second largest producer at ∼2.3 million tons, followed by Turkey and Indonesia with 2.2 and 1.8 million, respectively (Penella and Calatayud, 2018). *Nigella sativa* is produced mainly by India on 9,000 hectares. It is also produced in Pakistan, Sri Lanka, Nepal, Bangladesh, Egypt, and Iraq (Huchchannanavar et al., 2019). The annual global production of *Musa paradisiaca* L. is ∼41.265 million tons per year with its primary producers being Brazil, China, Ecuador, the Philippines, and India (Preedy and

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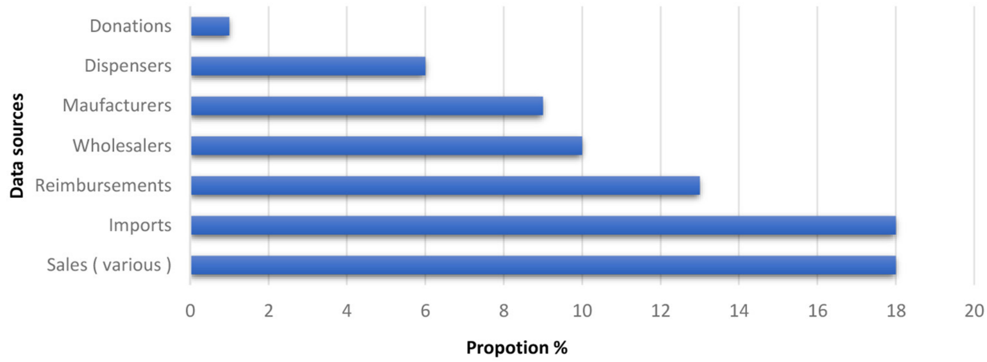

FIGURE 1
National estimates of antimicrobial consumption in 65 countries and areas (Organization, 2018).

Watson, 2019). Brazil is the largest *Citrus limetta* producing country globally with 15,912 tons followed by China with 5,450, the United States with 9,237 and the European Union (EU) with 5,999 thousand metric tons (Spreen, 2010). Annually, the global production of *Musa paradisiaca* L. is around 41.3 million tons and a large fraction of it is wasted as the peel. Notably, just in Brazil ∼780 million tons of *Musa paradisiaca* L. -waste is produced annually (Martínez-Ruano et al., 2018). According to Prabhakar and Singh (2019), ∼57% of processed *Citrus limetta* contributes to peel waste Usage of the endocarp of *Musa paradisiaca* L. and *Citrus limetta* creates solid waste problems due to the production of their peels, whereas *Nigella sativa* and *Capsicum* do not create such a problem. In this review, we address the handling of the huge solid waste problem produced by *Musa paradisiaca* L. and *Citrus limetta* by utilizing their peels as an antimicrobial agent against infectious diseases.

At present globally, a gradual increase in the wide spread of infectious diseases is quite evident. Therefore, it is need of time to adopt suitable preventive measures, such as regular use of antimicrobial agents. Therefore, it is proposed in this review paper that *Capsicum, Nigella sativa, Musa paradisiaca* L., and *Citrus limetta* on account of their abundant availability and antimicrobial constituents should be regarded as potential raw materials for the economical commercial production of various antimicrobial agents (Parthiban et al., 2011; Somda et al., 2011; Chitranshi et al., 2020). These natural materials are non-toxic, produce only organic food waste, and are easily available and completely safe for producing antimicrobial agents. Furthermore, the current study also discusses the antimicrobial compounds present in *Capsicum, Nigella sativa,* peels of *Musa paradisiaca* L., and *Citrus limetta* along with the methods employed for their extraction. Moreover, we also explain the overall antimicrobial mechanism of antimicrobial agents present in *Capsicum, Nigella sativa, Musa paradisiaca* L., and *Citrus limetta.* A schematic illustration of the content in this review is presented in Figure 2.

Biological properties of plant

The history of plants and/or plant components' utilization to flavor and conserve food, and to prevent oxidation and treat health problems is dated back to thousands of years ago. Most interestingly, the knowledge of their biological properties has been transmitted over the centuries among human societies. Considering this fact, it has been stated that the phytochemicals are the valuable source of bioactive substances with significant antimicrobial and antioxidant properties (Silva and Fernandes, 2010; Radha and Laxmipriya, 2015).

Anti-microbial agents present in plants

It has long been believed that plants have therapeutic properties. Since prehistory, people have used hundreds of thousands of local plants in infusions and poultices to treat various ailments. The desire to find novel antimicrobials has been prompted by the rise of microbial strains that are resistant to antibiotics and the recurrence of newer, deadlier illnesses. To find possible pharmacological leads, the whole natural resource base is being quickly evaluated (Ref: Antimicrobial Agents from Plants Reshma Reghu, Pramod Sahadevan & Shiburaj Sugathan + Plant Products as Antimicrobial Agents Marjorie Murphy Cowan*). Many plants and/or plant components, including *Nigella sativa, Capsicum,* peels of *Musa Paradisiaca* L., and *Citrus limetta* are ascribed to the presence of anti-microbial agents, which will be discussed herein.

Antimicrobial agent in *Nigella sativa*

It contains more than 100 valuable elements. According to science *N. sativa* contains 20–85% of protein, 38.20% of fat, 7–94% of fiber, and 31.94% of total carbohydrates that have antimicrobial effects. Due to the presence of thymoquinone and

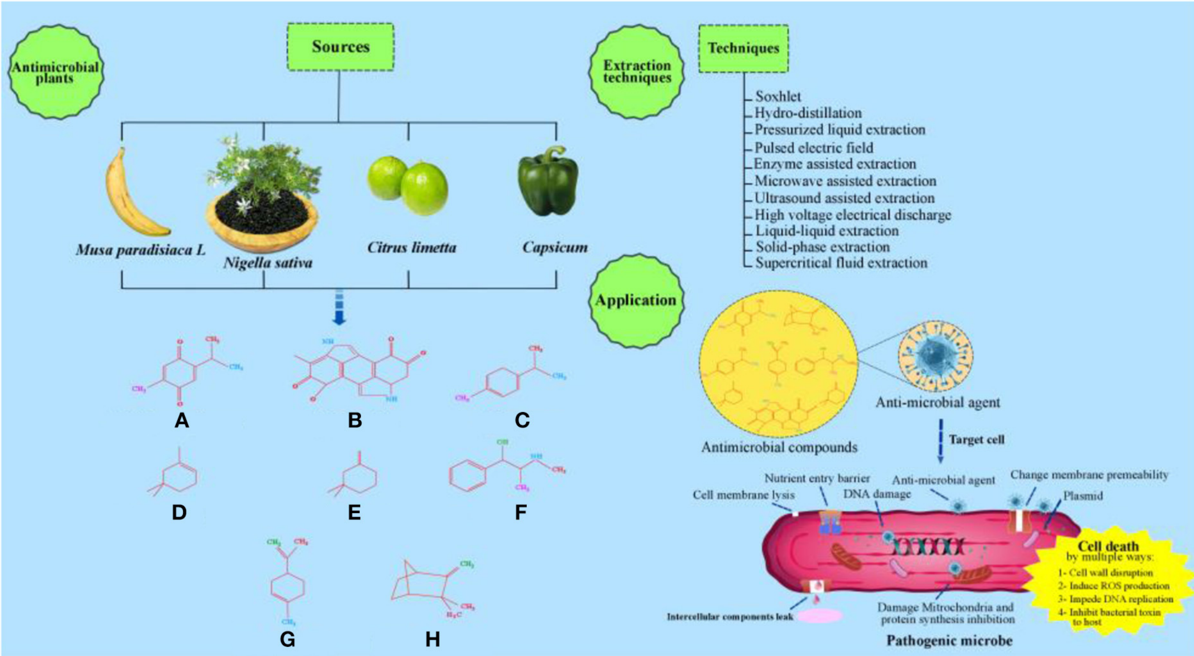

FIGURE 2
Schematic illustration of antimicrobial effects of *Capsicum, Nigella sativa, Musa paradisiaca L and Citrus limetta.* **(A)** Thymoquinone. **(B)** Melanin. **(C)** P-cymene. **(D)** a-Pinene. **(E)** b-Pinene. **(F)** Alkaloid. **(G)** Limonene. **(H)** Camphene.

thymohydroquinone in *N. sativa* it can fight against infectious disease-causing microbes (Bakal et al., 2017). The amount of the most significant bioactive component, thymoquinone, found in the volatile oil obtained from the seeds of *N. sativa* using various extraction techniques can range from 1.06 to 8.8 mg/g of oil, respectively (Yimer et al., 2019).

Antimicrobial agent in *Capsicum*

Capsaicin, also known as 8-methyl-N-vanillyl-6-nonenamide, is a compound found in *Capsicum* plants (chili peppers), which are used for food and medicine. Due to its antibacterial and anti-virulence properties, as well as its multiple pharmacological and physiological benefits (pain alleviation, cancer prevention, and advantageous cardiovascular, and gastrointestinal benefits; Marini et al., 2015), capsaicin has recently attracted a lot of attention. The capsaicinoids (2.5 mg/g; Othman et al., 2011) found in capsicum, which include more than 20 alkaloids (Bakht et al., 2020), are of major medical significance worldwide. Since capsicum has potent antibacterial effects, it is beneficial to utilize it in common food items.

Antimicrobial agent in *Musa paradisiaca* L.

Ethanol (0.5 g/100 g; Gorgus et al., 2016), which has a microbe-killing effect, is present in *Musa paradisiaca* L. at a concentration of 0.5 g/100 g. Secondary metabolites such as flavonoids, tannins, phlobatannins, alkaloids, glycosides, and terpenoids (Imam and Akter, 2011) have been found to be present in banana peel. Ighodaro (2012) evaluated the antibacterial effectiveness of banana peel extract against human pathogenic microorganisms and exhibited good inhibitory activity against *S. aureus*, *Escherichia coli*, and *Proteus mirabilis*. Although banana peel is regarded as waste, peel extracts can be utilized to create antibiotics (Saleem and Saeed, 2020).

Antimicrobial agent in *Citrus limetta* peel

Peel from *Citrus limetta* can be utilized to make solid waste management more efficient as well as to create long-lasting antibacterial products. Citrus peel has been discovered to have thousands more phenolic compounds than citrus pulp (Dutta et al., 2020a), and these phenolic compounds (444.55–502.54 mg/L; Buyukkurt et al., 2019) have antimicrobial properties. In a 2009 study, Kekuda et al. investigated the antifungal efficacy of the peels of three distinct citrus fruits, *C. limetta*, *C. sinensis*, and *C. limon*, against *Aspergillus* species, and found that *C. limetta* was best at preventing the growth of the fungus that was tested (Shabnam et al., 2013).

Plants and/or plant components derived antimicrobial compounds

Thymoquinone, melanin, P-cymene, a-pinene, b-pinene, alkaloid, limonene, and camphene are significantly present in *Nigella sativa, Capsicum, Musa Paradisiaca* L., and *Citrus limetta.* Moreover, these plant-derived chemicals are known to act as antimicrobial agents with numerous applications in the medical and food industries, which will be discussed in the subsequent section.

Thymoquinone

Thymoquinone is a compound that has remarkable anti-sepsis activity at a specific dose. Thymoquinone is the main component present in *Nigella sativa* that is found to be effective against the Avian influenza virus and coronavirus (Ulasli et al., 2014). Another study found that *Nigella sativa* consumption makes it difficult for the coronavirus to survive and replicate in the body (Ahmad et al., 2020). Thymoquinone is reported as an inhibitor for the treatment of coronavirus infections by blocking the entry of virus into the cell (Xu et al., 2021). Studies reported that thymoquinone can serve as an anticancer, anti-inflammatory, antioxidant and as an analgesic compound. Activator of transcription (JAK-STAT), janus kinase/signal transduction, mitogen-activated protein kinase (MAPK), and nuclear factor kappa beta (NF-κβ) signaling pathways can be inhibited by thymoquinone. Thymoquinone causes disruption in reactive oxygen species (ROS), pro-inflammatory cytokines, lipooxygenase (LOX) enzymes, cyclooxygenase (COX), myeloperoxidase, and elastase (Ali A. et al., 2021).

Melanin

Melanin is an efficient green agent that possesses anti-inflammatory, antioxidant, and antimicrobial activity with vast application in the biomedical field (Silvestri et al., 2017; Avossa et al., 2021). Antioxidants protect human bodies from free radicals, which may otherwise result in causing various diseases such as cancer and coronary heart disease (Hamid et al., 2010). Additionally, it is promising as an antibacterial agent against both gram-positive and gram-negative pathogens (Liu et al., 2014).

P-cymene

P-cymene is a natural compound found in many plants. It is used as an important agent in many drugs. It is widely present in more than 200 types of foods, including grape fruit, orange juice, mandarin, raspberries, carrots, butter, oregano, nutmeg, and almost every spice. P-cymene can serve as an analgesic, anti-inflammatory, antioxidant, and anti-tumor agent (Nickavar et al., 2014). P-cymene has anti-nociceptive, anticancer, antidiabetic, antiviral, antiparasitic, anti-inflammatory, antifungal, antioxidant, and antibacterial effects (Balahbib et al., 2021).

Pinene

Pinene is the natural compound found in citrus fruits. Pinene has various potential benefits such as it has anti-inflammatory, antimicrobial, antioxidant, and neuroprotective effects (Di Rauso Simeone et al., 2020). Pinene has anticoagulant, anti-inflammatory, anti-leishmania, antimalarial, antimicrobial, antioxidant, antitumor, analgesic, and antibiotic resistance modulation effects (Park et al., 2021). Pinene has the wound healing process by generating scars with effective tensile strength, accelerating wound closure, acting as an adhesive of primary intention, and contributing to collagen deposition (Salas-Oropeza et al., 2021).

Alkaloid

Alkaloid is a natural compound found in many plants. The presence of alkaloids in plants protects them from the destruction of various insects (Zhang et al., 2005). Indole alkaloids and isoquinoline alkaloids are the primary classes of substances having antibacterial action among the numerous alkaloids, including pyridine alkaloids, indole alkaloids, steroidal alkaloids, and other alkaloids. Alkaloids have antiviral, antibacterial, antifungal, cytotoxic, antioxidant, antifouling activities and anti-infective potential (Barghout et al., 2020; Diaz et al., 2020; Youssef et al., 2021).

Limonene

Limonene is a chemical found mostly in citrus fruits (Rodríguez et al., 2011). Limonene has several health benefits and belongs to the group of terpenes. These chemicals have strong aromas which protect plants from predators. Limonene also has anti-inflammatory and antioxidant effects (Miguel, 2010). Limonene can be used as a greener and safer alternative to market-available antimicrobial agents (Ibáñez et al., 2020).

Camphene

Camphene is a monoterpene found in many plants (Russo and Marcu, 2017). Terpenes are those chemicals that have strong aromas which protect plants from predators (Miguel, 2010). Camphene can fight against various deadly pathogens, such as *Enterococcus spp.* and *S. aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA) and have an antifungal effect against *Trichophyton mentagrophytes* and several species of *Paracoccidioides spp.*, Camphene have activity against *Mycobacterium tuberculosis* and vancomycin resistance

Enterococcus spp. Isolates (de Freitas et al., 2020). Figure 3 shows the chemical structures of thymoquinone, melanin, P-cymene, a-pinene, b-pinene, alkaloid, limonene, and camphene.

Table 1 shows the major antimicrobial compounds present in *Nigella sativa* and *Capsicum* along with the adopted methodology and important findings reported in previous research studies. Whereas, Table 2 shows the major antimicrobial compounds present in *Musa paradisiaca* L. and *Citrus limetta* along with the adopted methodology and important findings reported in previous research studies.

Extraction of antimicrobial compounds

The initial three basic processes in the extraction of antimicrobial compounds reported in the literature are (1) pretreatment, (2) extraction, and (3) purification.

Pretreatment

The first step in the extraction of antimicrobial agents is the preparation of materials employing methods such as washing, grinding, dewatering, heating and microfiltration. Pre-treatment consists of three additional steps: physical treatment, chemical treatment, and biological treatment.

Physical treatment

Physical treatment involves pre-washing, drying, grinding to get a homogeneous sample, heating, steaming, freeze-drying, and often boosting the kinetics of analytical extraction as well as increasing the contact of the sample surface with the solvent system. When making the extract from plant samples, proper precautions must be taken to ensure that any potential active ingredients are not lost, altered, or destroyed (Fabricant and Farnsworth, 2001; Cos et al., 2006; Sasidharan et al., 2011).

Chemical treatment

Chemical treatment involves the use of salts, acids, bases, and solvents. The chemical extraction method relies on the kinds of functional groups each component in the mixture possesses. Chemical processes can separate or purify materials when the right reagents are used. There are several chemical tests that can be used to identify the presence of alkaloids, flavonoids, tannins, saponins, flavones, sterols, and terpenes, which have been briefly discussed in the later part.

Identification of alkaloids

The extracts are individually filtered, dissolved in diluted hydrochloric acid, and analyzed for the presence of alkaloids. Typically, Mayers and Wagner's tests are used to identify alkaloids. In Mayer's test, the appearance of a yellow color on using Mayer reagent indicates the presence of alkaloids. Whereas, in Wagner's test a brown-reddish brown is developed upon using Wagner's reagent that indicates the presence of alkaloids (Trease and Evans, 1989; Wallis, 1989; Pandey and Tripathi, 2014; Beena et al., 2016; Dhawan and Gupta, 2017; Nortjie et al., 2022).

Identification of flavonoids

For the identification of flavonoids lead acetate and sulphuric acid test are usually performed. In Lead acetate test; the extracts are mixed with a few drops of a lead acetate solution. The presence of flavonoids is indicated by a precipitate that is yellow in color. In the sulfuric acid test; after a couple of drops of sulfuric acid are added to the extracts, an orange color appears, indicating the presence of flavonoids (Trease and Evans, 1989; Wallis, 1989; Pandey and Tripathi, 2014; Beena et al., 2016; Dhawan and Gupta, 2017; Nortjie et al., 2022).

Identification of steroids

Likewise, on adding a few drops of acetic anhydride to the extracts causes samples to turn violet then blue and lastly to green, which is a sign of the presence of steroids (Trease and Evans, 1989; Wallis, 1989; Pandey and Tripathi, 2014; Beena et al., 2016; Dhawan and Gupta, 2017; Nortjie et al., 2022).

Identification of terpenoids

The identification of terpenoids is usually done by Salkowski's test. This test is conducted by adding 3 mL of concentrated sulfuric acid to an extract of 5 mg of the targeted plant component. Eventually, the presence of terpenoids is indicated by the appearance of reddish-brown color (Trease and Evans, 1989; Wallis, 1989; Pandey and Tripathi, 2014; Beena et al., 2016; Dhawan and Gupta, 2017; Nortjie et al., 2022).

Identification of anthraquinones

For the identification of anthraquinones, Bontrager's test is usually performed. The Bontrager's test involves boiling 5 mg of the extract in a water bath for a short period of time with 10% HCl. After filtering, it is allowed to cool followed by the addition of $CHCl_3$ in an equal amount to the filtrate. The liquid is then heated before a few drops of 10% NH_3 are added. Anthraquinones are present when a pink color forms, indicating their presence (Trease and Evans, 1989; Wallis, 1989; Pandey and Tripathi, 2014; Beena et al., 2016; Dhawan and Gupta, 2017; Nortjie et al., 2022).

Identification of phenols

For the identification of phenols, ferric chloride test and lead acetate test are usually performed. In the Ferric chloride test; 10 mL extract is mixed with a few drops of ferric chloride. The presence of phenol is indicated by a bluish-black color. In the Lead acetate test; 10 mg of extract is added with a few drops of lead acetate solution. The presence of phenol is indicated

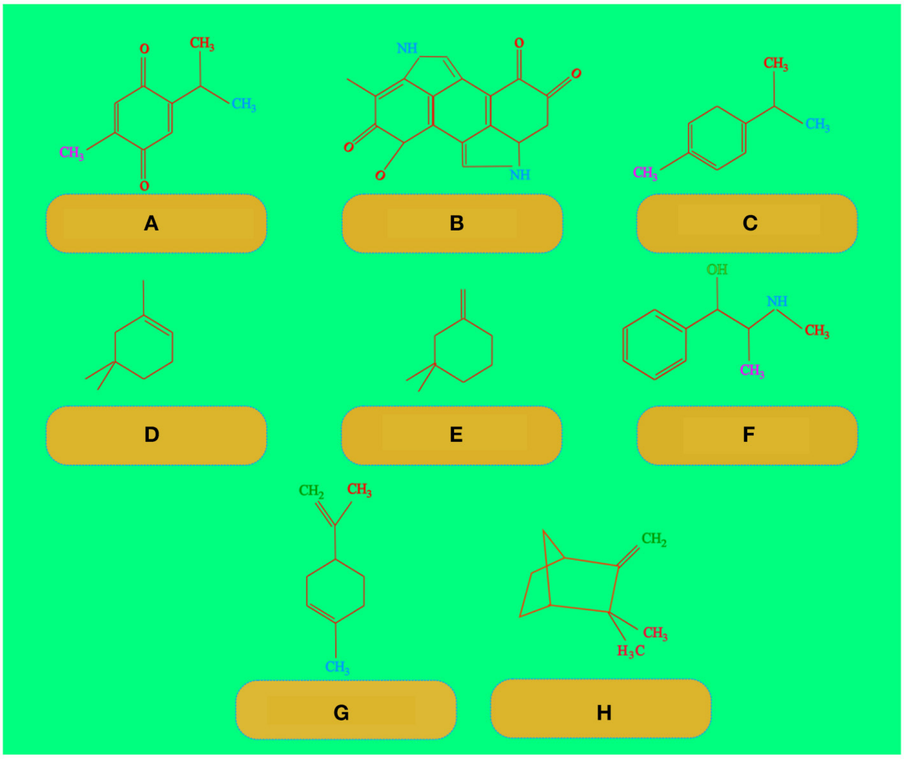

FIGURE 3
Chemical structures of **(A)** thymoquinone, **(B)** melanin, **(C)** P-cymene, **(D)** a-pinene, **(E)** b-pinene, **(F)** alkaloid, **(G)** limonene, and **(H)** camphene.

by a yellow color (Trease and Evans, 1989; Wallis, 1989; Al-Owaisi et al., 2014; Pandey and Tripathi, 2014; Beena et al., 2016; Dhawan and Gupta, 2017; Nortjie et al., 2022).

Biological treatment

Biological treatment uses bacteria and fungi to extract antimicrobial agents. The antibacterial activity of an extract or a pure chemical can be assessed or screened using a number of biological laboratory techniques. Disk-diffusion and broth or agar dilution procedures are the most well-known and fundamental techniques (Magaldi et al., 2004; Pfaller et al., 2004; Roden et al., 2005; Das et al., 2010; Bensah and Mensah, 2013; Kokollari et al., 2015; Fierascu et al., 2019).

Disk-diffusion method

The underlying idea behind this procedure is that an antibiotic concentration gradient is created when an antibiotic-impregnated disc is placed on agar that has already been inoculated with the test bacterium. The antibiotic is present in high concentrations near the disk's edge and gradually decreases as the space between them expands until the or3ganism is no longer inhibited by the antibiotic, at which time it is free to proliferate. If the antibiotic suppresses bacterial growth, a clear zone or ring forms around the antibiotic disc incubated overnight (Digrak et al., 1999; Parekh et al., 2005; Klančnik et al., 2010; Novaković and Janković, 2011).

Broth and agar dilution

Agar dilution implies adding various quantities of the antimicrobial drug to a nutrient-rich agar medium before applying a set number of cells to the agar plate's surface. In broth dilution, Bacteria are introduced into a liquid growth medium while being exposed to various concentrations of an antimicrobial agent, which is frequently assessed in 96-well microtiter plate format. After 16–20 h of incubation, growth is evaluated and the MIC value is recorded. This 3-day regimen is only applicable to aerobic bacteria (Griffin et al., 2000; Wiegand et al., 2008; Wu C. et al., 2015; Albano et al., 2020).

Extraction techniques

The second step is the extraction of required compounds from the material. The best extraction process must involve the thorough extraction of all the necessary metabolites or chemicals. If it's going to be done repeatedly, it needs to be quick, easy, and repeatable. The choice of an appropriate extraction technique mostly depends on the work that has to be done

TABLE 1 *Nigella sativa* and *Capsicum* as an antimicrobial agent.

Name of material	Antimicrobial agent	Adopted methodology	Findings	References
Nigella sativa	Thymoquinone and melanin	Paper disc diffusion method	A clear antibacterial effect on the growth of *Staphylococcus* was obtained	Bakathir and Abbas, 2011; Alshareef, 2019
	Fixed oil and thymoquinone of *N. Sativa*	-	Effective against many microbes including *Escherichia coli*, *Shigella niger*, *Staphylococcus Albus*, *Salmonella typhi*, and *Vibrio cholera*. Most effective against *Aspergillus species*	Ali and Blunden, 2003
	Thymoquinone, p-cymene, p-tert-butylcatechol, and pinene	Disc diffusion method	Thymoquinone is the main component that causes antimicrobial effects	Roy et al., 2006
	Essential oil of *N. sativa*	Plate diffusion method	Most effective against *Bacillus subtilis*	El-Kamali et al., 1998
	Crude alkaloid and water extract of the seed	-	Gram-negative was most effective than Gram-positive	Morsi, 2000
Capsicum	Low pH of fruit	Disk diffusion method and Well diffusion method	Extracts of *Capsicum* fruits are suitable for antibacterial activities	Careaga et al., 2003; Tano et al., 2008; Koffi-Nevry et al., 2012
	Isopropanol extracts	Growth inhibition test	Cinnamic and M-coumaric acids from Chili extracts are effective against bacterial species.	Dorantes et al., 2000
	Capsaicin-induced alterations in the pH	-	Prevent microbial infections	Tellez et al., 1993
	Presence of Capsaicin	Inhibit CT production	Effective against *V. cholera*	Omolo et al., 2014
	Presence of Capsaicin and Capsaicinoids	Disk diffusion method	Capsaicinoids are main component that causes antibacterial effect.	Das et al., 2018

and on the target metabolites to be known. There are various extraction processes like; Conventional techniques include solvent extract, soxhlet extraction, vortexing, maceration, centrifugation, hydro-distillation, cold pressing, and pressing and solid-liquid dynamic extraction. Non-conventional techniques include; pressurized liquid extraction, microwave-assisted method, ultrasound-assisted method, pulsed electric field, enzyme-assisted extraction, and supercritical fluid extraction. Conventional techniques are more time-consuming and less environmental friendly while non-conventional are fast and environment friendly techniques (Koubala et al., 2008; Azmir et al., 2013a,b; Deng et al., 2015).

Extraction technologies for extracting antimicrobial agents from *Nigella sativa, Capsicum*, peels of *Musa paradisiaca* L., and *Citrus limetta*

This review presents an analysis of several extraction techniques to extract antimicrobial agents from different plant sources, especially *Nigella sativa*, *Capsicum*, peels of *Musa paradisiaca*, and *Citrus limetta*.

Soxhlet extraction

This extraction technique involves putting solid material inside the thimble present in the extractor. The solvent condenses and fills the thimble as the vapor rises. This process of extraction is repeated again and again until the desired quantity is achieved. This technique was firstly used for the determination of fat in milk (Voon et al., 2012; Parashar et al., 2014; Chen and Urban, 2015). Five types of soxhlet techniques are mainly used for solvent extraction i.e., conventional soxhlet, high-pressure soxhlet extraction, ultrasound-assisted soxhlet extraction, automated soxhlet extraction, and microwave-assisted soxhlet extraction (Redfern et al., 2014; Wu C. et al., 2015; Wu G. et al., 2015). Conventional soxhlet extraction is a continuous process with slow processing time and an environmental unfriendly technique (Azmir et al., 2013b; Lopresto et al., 2014). High-pressure soxhlet extraction is used to fractionate polyethylene. The automated soxhlet technique saves

TABLE 2 *Musa paradisiaca* L. and *Citrus limetta* as an antimicrobial agent.

Name of material	Antimicrobial agent	Adopted methodology	Findings	References
Musa paradisiaca L.	Alcoholic extracts of *Musa paradisiaca* banana fruit peel	Agar diffusion method	Effective against *Staphylococcus* and *Pseudomonas*	Ahmad and Beg, 2001; Karuppiah and Mustaffa, 2013
	Presence of Phenolic and Flavonoid compounds	Minimum Inhibition Concentration (MIC) and paper disc diffusion method	ß-sitosterol, 12-hydroxy strearic, malic and succinic acid from banana green peel shows antibacterial activities	Fidrianny et al., 2014
	Presence of fatty acids	Fermentation	A suitable environmental condition should be provided for high ethanol production	Brooks, 2008
	Methanol extract of *M. paradisiaca* peels	Zone of inhibition test (ZIT), minimum bacterial concentration (MBC) and minimum inhibitory concentration (MIC)	Methanol extracts of Banana peels are more effective against bacteria than its ethanol extracts.	Okorondu et al., 2010
Citrus limetta	Aqueous and organic extracts of *C. limetta* peels	Solvent extraction procedure	Methanolic extract of *Citrus limetta* have antibacterial activity mostly against *Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus*	Shakya et al., 2019; Dutta et al., 2020a
	Ethyl acetate and butanol fractions of *C. limetta* peels extract	Agar diffusion method	Ethyl acetate stops the growth of bacteria and butanol fractions stops the quorum sensing control pigment production	Khan et al., 2017
	Limonene (95.98%) Camphene (1.79%) and Terpenes	Hydro-distillation, Disc diffusion method	Have strong antibacterial activity against food-borne pathogens	Javed et al., 2013
	Peel extracts	Well diffusion assay	Pomegranate peel extract showed maximum antibacterial activity among Orange, Banana, Sweet lime, Apple, Papaya, and Mango	Prakash et al., 2013
	Acetone extracts	Disc diffusion method	Effective against *Staphylococcus aureus Bacillus subtilis*, and *Bacillus* cereus	Al-Snafi, 2016

time, sample consumption and is used for agricultural, food and industrial samples. Ultrasound-assisted soxhlet extraction is mainly used to extract fat from sunflowers, grapes and soya beans. However, this technique does not affect the quality of extracted oil due to the adoption of mild conditions in the process (De Castro and Priego-Capote, 2010; Parashar et al., 2014).

The soxhlet technique was used to extract 6.77 ± 1.2, 3.66 ± 1.43, and 4.81 ± 1.8% w/w of thymoquinone from *Nigella sativa* (Kausar et al., 2017). Capsaicin was extracted from *Capsicum* by using this technique (Chuichulcherm et al., 2013). *Citrus limetta* which is a major phyto compound was extracted from *Capsicum* by using this technique (Baranitharan et al., 2020).

Hydro distillation

Hydro distillation is a conventional method that is used for extracting bioactive compounds from different plant species. This process involves packing plant materials in a still chamber, adding an appropriate amount of water, and then heating it to a boil. Alternative methods include injecting direct steam straight into the plant sample. By using indirect cooling with water, the water and oil vapor combination is condensed. Oil and bioactive substances are automatically separated from the water in a separator when the condensed mixture flows from the condenser. Its usage is constrained by restrictions on high-temperature applications for heat-sensitive phenolic compounds, although it has several benefits such as the lack of organic solvents in the process, the requirement for no dehydration of the plant materials, and faster extraction times (Vankar, 2004; Sa-Nguanpuag et al., 2011; Erkan et al., 2012; Chenni et al., 2016; Elyemni et al., 2019).

Through this technique, thymoquinone and capsaicin were extracted from *Nigella sativa* (El Khoury et al., 2019) and *Capsicum,* respectively (Jiang et al., 2013). Hydro distillation is the most common method for obtaining thymoquinone from *Nigella sativa* (Liu et al., 2011; Oskouei et al., 2018).

Liquid–liquid extraction

In liquid-liquid extraction separation of required material from liquid solution is made through contact with another insoluble liquid. The required material is separated based on its solubility in different liquids (Chen et al., 2017; El Blidi et al., 2019; Goyal and Singh, 2022).

It is best suited for liquid samples. The technology is simple and can be used at ambient temperature. However, the technology is time-consuming, requires expensive chemicals, and takes a lot of labor (Espinosa-Alonso et al., 2006).

Liquid-liquid extraction is an energy e?cient technique (Blahušiak et al., 2018) and can be used in biorefineries for lipid extraction from microalgae (Du et al., 2017) and for fractionation of pyrolytic bio-oils (Cesari et al., 2019). Earlier research study also reports lignin recovery *via* liquid extraction (Stiefel et al., 2017). Moreover, the fractionation of citrus essential oils (separating the terpenic hydrocarbons and oxygenated compounds) is achieved through liquid-liquid extraction (Gonçalves et al., 2016).

Solid-phase extraction

The solid-phase extraction technique is the simplest technique for extracting and pre-concentrating trace levels of contaminants from samples. The extraction efficiency of solid-phase extraction is enhanced by using nanomaterials as sorbents which provide more pollutants capturing sites (Jacobsen et al., 2004; Silva et al., 2016; Azzouz et al., 2018).

The only difference between solid-phase extraction and liquid-liquid extraction is the medium used for separation; in solid-phase extraction, one phase is a liquid phase and the other is a solid phase. The solid surface consists of adsorbent material that absorbs specific required material through the solution. The separation rate of solid-phase extraction technique is faster than liquid-liquid extraction technique. It is a simple technique. It can be employed for polar compounds. It is not suitable for volatile samples (Abd-Talib et al., 2014). It is an expensive technique than the liquid-liquid extraction technique (Hernanz et al., 2008). Different methods were used to extract thymoquinone from *Nigella sativa* including Solid-phase extraction (Kausar et al., 2017).

Supercritical fluid extraction

Suppose the temperature and pressure of a specific liquid and gas are above the critical point. In that case, the supercritical fluid extraction technique should be used for extracting certain required materials from samples like essential oils. Certain parameters such as temperature, pressure, the flow rate of fluids, and the sample size significantly affect extraction yield (Santoyo et al., 2006; Ma et al., 2018; Yousefi et al., 2019; Zizovic, 2020; Santos et al., 2021).

The supercritical fluid extraction technique saves time, it is sustainable and suitable for volatile samples. However, it is not suitable for drugs and polar substances. It is also a costly technique (Mendiola et al., 2007; Abbas et al., 2008).

From mandarin, orange, lemon, grapefruit peel and sour orange peel phenolic compounds were extracted by using supercritical fluid extraction (Omar et al., 2013; Trabelsi et al., 2016). Super critical fluid extraction is used for obtaining thymoquinone from *N. sativa* (Liu et al., 2011; Oskouei et al., 2018).

Pressurized liquid extraction

Pressurized liquid extraction is mainly used for extracting phenolic compounds from plants. Liquid solvents are used in it with specific temperatures and pressure. It is suitable for solid samples and polar compounds. However, pressurized liquid extraction is time-consuming technology that requires less solvent. Also due to the 10 g maximum sample weight limit, it is not appropriate for situations where very low-level analyte detection is required (Jacobsen et al., 2004; Juan et al., 2010; Mustafa and Turner, 2011; Alañón et al., 2015; Alvarez-Rivera et al., 2020). It is mainly used to recover bioactive compounds from grapes (Pereira et al., 2019). Extraction of phenolic compounds (alkaloids) from *Citrus limetta* through pressurized liquid extraction was carried out by Olabinjo et al. (2020).

Pulsed electric field

The pulsed electric field is an efficient non-thermal technique that involves the application of high-voltage pulses. These pulses cause rupturing of plant materials thus extraction of required components occurred from plant cells. Though it is a sustainable technology, it takes a long time to process. This technology requires process parameters like energy inputs, treatment temperature and field strength to be maintained (Liang et al., 2002; Wu et al., 2005; Nguyen and Mittal, 2007; Puértolas et al., 2012; Garner, 2019). According to a study, *Citrus limetta* peels were subjected to pulsed electric fields in order to extract phenolic components (Luengo et al., 2013). In another study, phenolic compounds were extracted from orange, grapefruit, and lemon peel through a pulse electric field (El Kantar et al., 2018). Much more components were extracted from the *N. sativa*'s oil when PEF was applied as a pretreatment. The major constituent was thymoquinone and cymene, whereas D-Limonene and β-Pinene were also present in small quantities (El-Dakhakhny et al., 2000; Bakhshabadi et al., 2018).

Enzyme-assisted extraction

Specific enzymes are used in enzyme-assisted extraction to target the cell walls of plant materials, destroying them and liberating antimicrobial compounds. The effectiveness of the procedure as a whole can be improved by combining this strategy with a number of other techniques (Nadar et al., 2018; Bilal et al., 2019; Habeebullah et al., 2020). For instance, Naghshineh et al. (2013) found enzymatic extraction with high pressure treatment led to a higher level of pectin extraction form lime peel powder (Naghshineh et al., 2013). This technology has high extraction rates and is sustainable. However, it has a high enzyme cost and is not appropriate for industrial use. Its operations must be handled with caution (Puri et al., 2012; Singh et al., 2016). Camphene is the predominant essential oil of *Aristolochia manshurientsis Kom* extracted from enzyme-assisted extraction followed by hydrodistillation (Zhao et al., 2018).

Microwave-assisted extraction

Organic compounds can be extracted from plant material with more ease and effectiveness using microwave technology than using traditional methods. It is a technology that saves time. It is a very efficient technology with a high yield. This technology is sustainable because it requires less heat (Huie, 2002; Kaufmann and Christen, 2002; Wang and Weller, 2006; Chen et al., 2007; Cravotto et al., 2008; Sticher, 2008; Zhang et al., 2009). It involves a simple and economical process as compared to supercritical fluid extraction. It has a difficult operation as compared to ultrasonic-assisted extraction (Hayat et al., 2009). Extraction of Capsaicin through *Capsicum* was done through microwave-assisted extraction by Chuichulcherm et al. (2013). A study reported that phenolic compounds (alkaloids) were extracted from *Citrus limetta* through microwave-assisted extraction (Arafat et al., 2020). In another study, phenolic compounds were extracted from orange peel through microwave-assisted extraction (Nayak et al., 2015). Microwave-assisted extraction is used for obtaining thymoquinone from *N. sativa* (Liu et al., 2011; Oskouei et al., 2018). Various alkaloids were extracted from plants using microwave-assisted extraction (Bergs et al., 2013; Ngo et al., 2017; Aqil et al., 2020).

Ultrasound-assisted extraction

Ultrasound-assisted extraction is a technique that involves throwing sound energy to plants and agitating it thus extracting the required materials through a plant cell. In this technology, low energy and chemicals are required. High yield can be obtained from it, and it is a time-saving technology. Proper optimization in ultrasound frequency, the nominal power of the device, input power, propagation of cycle, and system geometry is required for maximum yield (Azmir et al., 2013a; Barba et al., 2015; Sneha et al., 2022). Studies report the extraction of Capsaicin from *Capsicum,* phenolic compounds (alkaloids) from orange peel and citrus fruit peel through ultrasound-assisted extraction (Chuichulcherm et al., 2013; Omar et al., 2013; Boukroufa et al., 2015; Nishad et al., 2019). Different methods were used to extract thymoquinone from *Nigella sativa* including Ultrasound-assisted extraction (Kausar et al., 2017).

High-voltage electrical discharge

A high-voltage electrical discharge is a non-thermal method that relies on ambient temperature and uses pulsed rapid discharge voltages. It is a technique that saves time and uses less energy. However, at the industrial and pilot level, this technology has not yet been utilized (Barba et al., 2015; Li et al., 2019; Rajha et al., 2019; Žuntar et al., 2019). Extraction of phenolic compounds (alkaloids) from grapefruit peel (El Kantar et al., 2019) and from the mandarin peel (Buniowska et al., 2015), through high voltage electric discharge was studied in respective studies.

Comparative analysis of different extraction technologies for extracting bioactive compounds

Selecting an extraction technique presents one of the biggest challenges in getting bioactive compounds from plants because many factors need to be taken into account, including the solvent's polarity, the chemical makeup of the bioactive compound, yield, selectivity, temperature, solubility, extraction time, and cost. Natural plant extracts can be obtained in a variety of ways, but the elements of the process profoundly affect the final product's quality. Among the extraction techniques, the supercritical extraction and/or the extraction utilizing pressurized fluids can be highlighted. In these procedures, a highly compressible fluid is used as a solvent in low- or mid-temperature conditions. The utilization of supercritical fluids is a possible technique for the extraction and fractionation of natural resources, particularly for food and pharmaceuticals. The key advantages of supercritical fluid extraction include high selectivity, ease of solvent removal, and use of mild temperatures (Correa et al., 2017). Using *Capsicum frutescens* L. (Chuichulcherm et al., 2013), investigated the extraction of capsaicinoids using microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE), in comparison to the conventional soxhlet method. They concluded that the UAE approach was the best technique for extracting capsaicinoids from *C. frutescens*. Therefore, even though the amounts of capsaicinoids in MAE and UAE were 5.28 and 4.01 mg/g of *C. frutescens*, respectively, UAE needed the least amount of energy and was deemed the best approach. The low-quality oil produced by traditional solvent extraction methods necessitates intensive purifying procedures, which cause thermal deterioration and the loss of important components. There has been a shift toward more environmentally friendly extraction techniques, such supercritical fluid extraction, as a result of the use of organic solvents and the need to recover the solvent (SFE). SFE is evolving into a successful and widely utilized method for extracting valuable natural compounds from challenging materials (Ghahramanloo et al., 2017). Particularly, maceration, microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) have been considered as popular extraction techniques; Zhang and his coworkers concluded that MAE was the most effective with a yield of 7.04 $\pm$ 0.14 mg/g chelerythrine and 17.10 $\pm$ 0.4 mg/g sanguinarine with 5 min of extraction time (Zhang et al., 2005). High hydrostatic pressure (HHP), ultrasonics and pulsed electric fields (PEF) were used for extracting bioactive compounds from grapes and the finding of this study was; the total phenolic content of samples was 50% higher than in the control samples (Corrales et al., 2008). Typically, phenolics have antibacterial and antioxidant properties. Due to their unique molecular structure, can offer superior platforms for making antimicrobial products (Siddiqui et al., 2022). High pressure (HP), pulsed electric fields (PEF), and low pasteurization (LPT) were used for extracting, pulsed electric field and low pasteurization; the former was the most effective treatment for the extraction of bioactive components from orange juice (Plaza et al., 2011). Conventional soxhlet extraction, MAE and ultrasound assisted extraction (UAE) were used for extracting bioactive compounds from agiorgitico red grape pomace. This study concluded that UAE was the most effective one (Drosou et al., 2015). There have been several research investigations on the extraction of bioactive substances using traditional and non-conventional (green) methods, including soxhlet extraction, microwave, supercritical fluid extraction, ultrasound extraction, etc. Each methodology has pros and cons. For instance, the greatest yield (26.52%) and total phenolic content (15,263.32 mg Eq gallic/100 g DW) from citrus reticulata blanco cv. sainampueng peel were obtained *via* the extraction of bioactive components under the ideal UAE conditions. UAE therefore demonstrated greater extraction efficiency in terms of yield under the same extraction circumstances, which was 1.77 times higher than MAE (Saini et al., 2019).

Purification

Purification of antimicrobial agents is generally the final step. There are various purification methods that can be used to purify antimicrobial agents. The choice of a specific purification method in any study depends on the degree of purification required for specific antimicrobial agents. In the case of polar compounds, purification is done mainly by filtration, ultrafiltration, paper chromatography, thin-layer chromatography, gas chromatography and high-performance liquid chromatography. Whereas, non-polar compound purification is mainly performed by; liquid-liquid extraction, reversed phase purification chromatography and through Hydrophobic interaction chromatography (Thumar et al., 2010; Galanakis, 2012; Ingle et al., 2017; Tang et al., 2018; Martinenghi et al., 2020).

Filtration

Filtration is a technique that involves pouring a combination of solid and liquid onto a membrane or a filter paper, which permits the passage of liquid (the filtrate) and results in the collection of the solid, this process is used to separate solids from liquids. During filtration, the size of the materials that can be removed relies on the filter's pore size. With just one filter, this technique can filter out a variety of undesirable particles of various sizes and is comparatively inexpensive (McGaw et al., 2002; Jin et al., 2007; Mushore and Matuvhunye, 2013; Sahle and Okbatinsae, 2017).

Ultrafiltration

Ultrafiltration (UF) is a type of membrane filtration, that forces a liquid against a semi-permeable membrane by hydrostatic pressure. Water and solutes <0.005 micro meter pass through the barrier, whereas suspended solids and solutes >0.1 micro meter are trapped. Compared to traditional methods, ultrafiltration technology can accomplish more work in 50% less space (Pampanin et al., 2012; Wang et al., 2013; Tang et al., 2018; Kellogg et al., 2019; Martinenghi et al., 2020).

Paper chromatography

In this method, separations are performed using a piece of paper that serves as both a support and a medium for separation. The filter paper is placed in the chromatographic chamber with the solvent, and the sample is put close to the bottom of the filter paper. By means of capillary action, the solvent flows while also transferring soluble molecules. Paper chromatography has the benefit that separations can be done easily on sheets of filter paper, which serves as both a substrate and a medium for separation. The high degree of reproducibility of Rf (retention factor) values can also be obtained on paper through this technique (Thumar et al., 2010; Galanakis, 2012; Ingle et al., 2017; Muhamad et al., 2017; Tang et al., 2018; Martinenghi et al., 2020).

Thin-layer chromatography

Using this method, samples are separated based on their interactions with a thin layer of adsorbent that is adhered to the plate of low molecular weight substances. Compounds are separated using various adsorbents. The particular benefit of TLC over paper chromatography is its adaptability, speed, and sensitivity (Thumar et al., 2010; Galanakis, 2012; Tang et al., 2018; Martinenghi et al., 2020).

Gas chromatography

Utilizing this method, volatile substances are separated. Through its distribution in the gas phase, the chemical species' rate of kinetics is identified. While the liquid phase is still, the gas phase is moving. The chemical species' rate of migration is estimated based on how widely distributed it is in the gas phase. For instance, a species that disperses 100% of itself into the gas phase will migrate at the same rate as the gas that is flowing, whereas a species that disperses 100% of itself into the stationary phase will not move at all. In gas chromatography, a sample is vaporized and then injected into the chromatographic column's head. The flow of the inert, gaseous mobile phase carries the sample through the column. A liquid stationary phase that is adsorbed onto the surface of an inert solid is present in the column itself (Thumar et al., 2010; Galanakis, 2012; Ingle et al., 2017; Tang et al., 2018; Sreedharan et al., 2019; Martinenghi et al., 2020).

High-performance liquid chromatography

HPLC is an analytical technique used for the separation and measurement of organic and inorganic solutes in any samples, particularly biological, pharmaceutical, food, environmental, industrial, etc. This method divides compounds by how they interact with the solvent of the mobile phase and the solid particles of a densely packed column. To help in identifying the chemicals, the Diode Array Detector examines the analytes' absorption spectra (Thumar et al., 2010; Galanakis, 2012; Ingle et al., 2017; Tang et al., 2018; Martinenghi et al., 2020).

Reverse-phase chromatography

In reverse-phase chromatography (RPC), molecules are separated from one another through hydrophobic interactions between the ligands attached to the stationary phase and the solute molecules in the mobile phase of the liquid chromatography. Better solubility for polar analytes is provided by reverse-phase chromatography, which also uses non-toxic solvents, provides a means for removing impurities and mobile phase additives, and provides rapid sample recovery with minimal solvent evaporation (Nair and Kanfer, 2006; Thumar et al., 2010; Galanakis, 2012; Tang et al., 2018; Martinenghi et al., 2020).

Hydrophobic interaction chromatography

Based on their hydrophobicity, molecules are separated using hydrophobic interaction chromatography (HIC). Due to the use of less denaturing conditions and matrices, HIC is a good separation approach for removing proteins while preserving biological activity. This technique can improve the mass spectrometry's sensitivity of detection. When compared to reversed-phase liquid chromatography, the flow rate in this approach can be larger because the mobile phase's viscosity is low. Low viscosity mobile phases enable the use of columns that are twice as long as those required for reverse phase HPLC. The sample separates very quickly due to the eluent's viscosity, which is also correlated with a lower separation impedance. Compared to reverse phase HPLC, basic solutes can be loaded more readily (Thumar et al., 2010; Galanakis, 2012; Sagar et al., 2012; Tang et al., 2018; Martinenghi et al., 2020).

Antibiotic resistance mechanisms in disease causing bacteria

Microbial infections are on the rise globally, and the human population is at risk due to microbes because these microbes are the major cause of worldwide mortalities, as shown in Table 3. Basically, disease causing bacteria have resistance against various antibiotics due to myriad mechanisms. These mechanisms are: (1) Bacteria can have resistance through transformation, transduction, and conjugation phenomenon. (2) Through the processes of phosphorylation, adenylation, or acetylation bacteria deactivate the antibiotics. (3) Bacteria prevent the interaction of drugs and antibiotics. (4) They can cause efflux of the antibiotic from the cell (Munita and Arias, 2016; Reygaert, 2018; Abushaheen et al., 2020). Recently, resistance to antibiotics has been the biggest challenge which threatens human communities. Meanwhile, the occurrence of the evolution of resistance endangers the effectiveness of existing antibiotic drugs (Baym et al., 2016; Marston et al., 2016). Therefore, several strategies have been proposed to overcome the antimicrobial resistance. One of the recommended methods to achieve this goal is the usage of natural compounds that are derived from plants. These plant extracts seem to be ideally suited to overcome the emergence of antibiotic resistance in multidrug resistance bacteria (Ncube et al., 2008; Rossiter et al., 2017).

Anti-microbial properties of *Capsicum*, *Nigella sativa*, peels of *Musa paradisiaca* L., and *Citrus limetta*

Plants have a plethora of constituents that impose antimicrobial effects (Gyawali et al., 2011; Mickymaray et al., 2016; Dewapriya et al., 2018; Casciaro et al., 2019; Ali M. Y. et al., 2021). Two major groups of antibiotics extracted from plants are; (1) phytoanticipins and (2) phytoalexins. Phytoanticipins inhibit microbial actions, whereas Phytoalexins are generally antioxidants (Sukalingam et al., 2017, 2018). Moreover, various secondary plant metabolites are known to cause a significant antimicrobial effect which will be discussed in later section.

Antimicrobial mechanism of bioactive compounds

These secondary metabolites are grouped into three types; (1) phenolic compounds, (2) terpenes, and (3) alkaloids (Crozier et al., 2006; Mickymaray, 2019; Roaa, 2020). These antimicrobial extracts cause cell wall disruption and lysis, cell wall construction, inhibit biofilm formation, inhibit microbial DNA replication, inhibit energy synthesis, inhibit bacterial

TABLE 3 Infectious diseases caused by microbes.

Infectious disease	Microbe that causes the disease	Death rate	References
Methicillin-Resistant *Staphylococcus Aureus* (MRSA), Vancomycin-Resistant Enterococci (VRE), and *Clostridium difficile*	Gram-positive bacteria	76%	Doernberg et al., 2017; Sizar and Unakal, 2020
Pneumonia, bloodstream infections, wound or surgical site infections, and meningitis	Gram-negative bacteria	14%	Centers for Disease Control and Prevention, 2011; Sizar and Unakal, 2020
Candida can cause infections if it grows out of control or if it enters deep into the body (for example, the bloodstream or internal organs like the kidney, heart, or brain)	*Candida albicans*	19–24%.	Akpan and Morgan, 2002; Magill et al., 2014
Cholecystitis, Bacteremia, Cholangitis, Urinary tract infection (UTI), traveler's diarrhea, and other clinical infections such as neonatal meningitis and pneumonia.	*E. coli*	4.2%	Makvana and Krilov, 2015; Khalil et al., 2018
Dermatophytosis	*Trichophyton interdigitale*	4–8%	Kardjeva et al., 2006; Mügge et al., 2006; Zhang et al., 2019
Nosocomial infections	*Staphylococcus epidermidis*	20%	Otto, 2009; Turnidge et al., 2009
Food-borne bacterial illness cause to unborn babies, newborns and people with weakened immune systems	*Listeria monocytogene*	15–20%	Edelson and Unanue, 2000
Diarrheal and toxico-infections	*Bacillus cereus*	619 death from 1998 to 2015	Jessberger et al., 2020; McDowell et al., 2020
Superficial and ear infections in humans	*Vibrio alginolyticus*	35–50%	Reilly et al., 2011
Human infectious diseases including keratitis, burn infections, urinary tract infections (UTIs), sepsis, as well as acute and chronic infections of human airways.	*Pseudo. aeruginosa*	18–61%	Kang et al., 2003; Cao et al., 2017
Gastroenteritis and focal infections	*Salmonella typhimurium*	3 million each year	Coburn et al., 2007; Jung et al., 2020
Diarrhea (sometimes bloody), fever, and stomach cramps.	*Shigella flexneri*	13.2%	Khalil et al., 2018; Duncan-Lowey et al., 2020

toxins to the host, and induce reactive oxygen species production. Despite all these effects, these antimicrobials also prevent antimicrobial resistance and synergetics to antibiotics, which helps kill pathogenic organisms (Tariq et al., 2019; Zheng et al., 2020; Vaou et al., 2022), as shown in Figure 4.

Promote cell wall disruption and lysis

Cell disruption mechanism takes place *via* two approaches that are mechanical and non-mechanical approach (Figure 5). The detail of the antimicrobial mechanism is given in separate subsequent paragraphs. Firstly, compounds that functionalized this disruption are delineated as follows.

Phenolic compounds belong to the family of aromatics containing a hydroxyl functional group, which in reaction with the microorganisms disrupts their cell wall (Ganesan and Xu, 2017a, 2018). These aromatics are moved to the microbe's cell surface, and thus they disrupt their cell walls. Flavonoids are phenolic compounds that form a complex relationship with the bacterial cell wall and thus disrupt its structure (Ganesan and Xu, 2017b,c). Rutin, naringenin, sophoraflavanone, and tiliroside are the flavonoids that disrupt *S. aureus* and *S. mutans* microbes (Tsuchiya and Iinuma, 2000; Sanver et al., 2016). Terpenes contain isoprene that disrupts microbial membranes (Guimarães et al., 2019; Moghrovyan et al., 2019).

Induction of reactive oxygen species production

Reactive oxygen species are formed by the partial reduction of molecular oxygen that helps in antimicrobial activity and provides a defense mechanism against various microbes. The method of catechins involves augmentation of the production of oxidative stress that disrupts cell walls (Fathima and Rao, 2016; Van Acker and Coenye, 2017; Zou et al., 2017). Sources of reactive oxygen species, antioxidant defenses, and subsequent biological effects depending on the level of reactive oxygen species production are shown in Figure 6.

Inhibition of biofilm formation

Biofilms developed by bacteria are 100–1,000 times more resistant to the antimicrobial drugs (Kahaliw et al., 2017; Mishra et al., 2020). In some studies, it was indicated that flavonoids

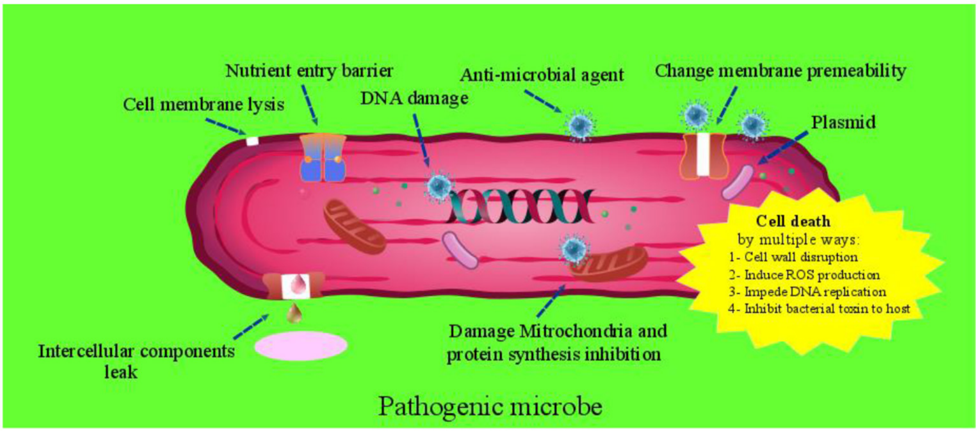

FIGURE 4
Antimicrobial mechanism of bioactive compounds.

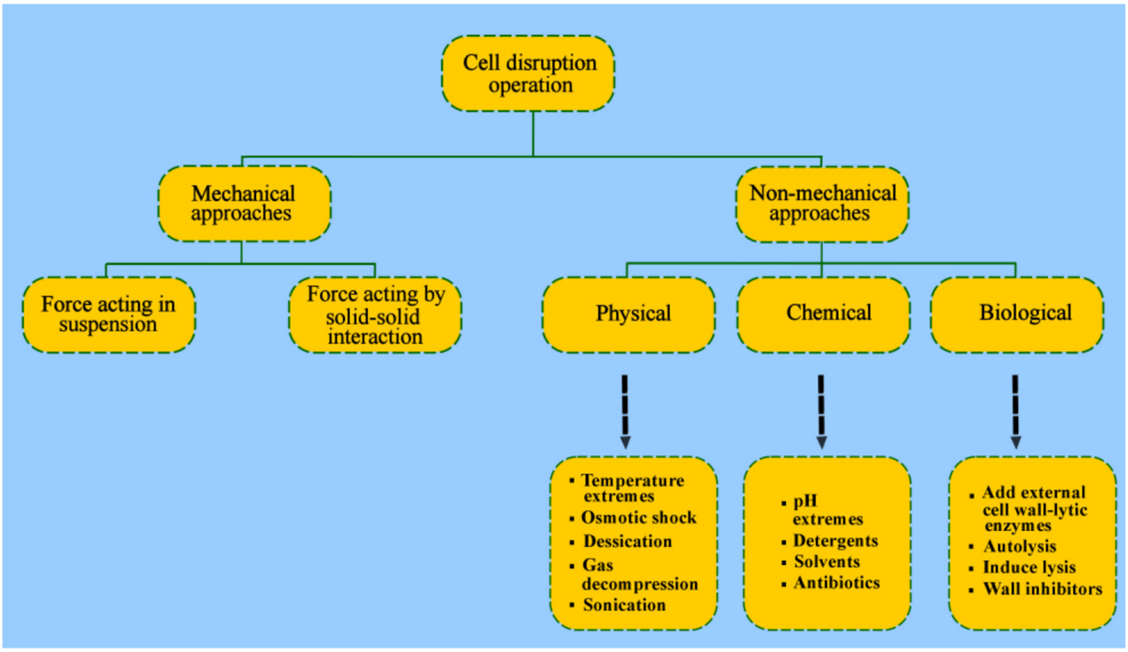

FIGURE 5
Antimicrobial mechanism by promoting cell wall disruption and lysis (Moo-Young, 2019).

aggregate the multicellular composites; thus, inhibiting bacteria growth. Flavonoids such as; (1) galangin, (2) isovitexin, (3) 3-O-octanoyl-epicatechin, and (4) 5, 7, and 40-trihydroxyflavanol cause the aggregation of *S. aureus* and *S. mutans*; therefore inhibiting their growths (Awolola et al., 2014; Rabin et al., 2015).

Inhibition of cell wall construction

The bacterial cell wall is the main target of any antimicrobial agent because the cell wall is the main source of biosynthesis of lipids, respiration and the transport mechanism. For all these functions, membrane integrity plays a major role in bacterial species, so the membrane disruption leads to bacterial death (Lazar and Walker, 2002; Klis et al., 2006; Reygaert, 2014).

Inhibition of bacterial toxins against the host

Catechins and some other flavonoids cause disruption of bacteria cell walls thus resulting in an inability to discharge toxins (Lee et al., 2011; Chang et al., 2019).

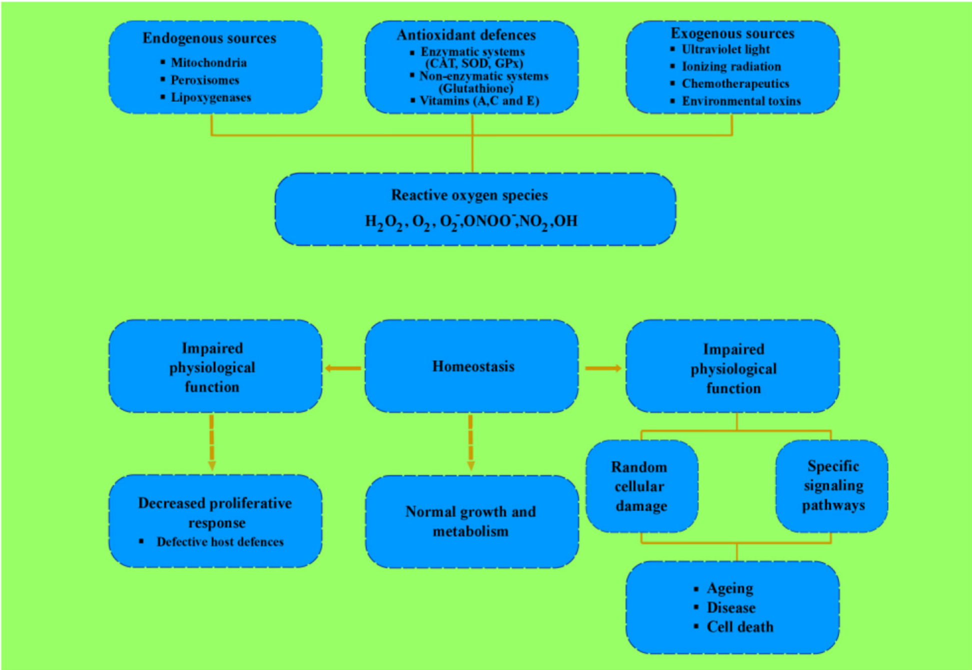

FIGURE 6
Endogenous and exogenous sources of ROS, antioxidant defenses, and biological effects that depends on ROS production.

Compounds such as catechins, pinocembrin, kaempferol, kaempferol-3-O-rutinoside, gallocatechin gallate, quercetin glycoside, genistein, and proanthocyanidins neutralize bacterial toxic factors initiating from *N. gonorrhoeae*, *V. cholerae*, *E. coli*, *B. anthracis*, *V. vulnificus*, *S. aureus*, and *C. botulinum* (Shahnazari et al., 2011; Ahmed et al., 2016).

Inhibition of energy synthesis

The production of energy or adenosine triphosphate (ATP) is important for the development of bacteria because it is the main source of a living system. The reaction of flavonoids such as isobavachalcone and 6-prenylapigenin with *S. aureus* results in disruption of the bacterial cell wall (Freudenberg and Mager, 1971; Kuete et al., 2011; Xie et al., 2015).

Inhibition of microbial DNA replication

Antimicrobial agents inhibit DNA replication thus stopping the further division of microbes. Moreover, bioactive compounds present in plants such as kaempferol, quercetin, apigenin, genistein, nobiletin, myricetin, chrysin, tangeritin, and 3, 6, 7, 30, 40-pentahydroxyflavone are DNA disrupters (Gotoh et al., 2008; van Eijk et al., 2017; Vijayakumar et al., 2018).

Antimicrobial mechanism of antimicrobial agents

Antimicrobial mechanisms of antimicrobial agents present in *Nigella sativa*, *Capsicum*, *Musa paradisiaca* L., and *Citrus limetta* are discussed below;

Antimicrobial mechanism of thymoquinone

Thymoquinone plays a major role in fighting against microbes. Basically, thymoquinone causes resistance in the biofilm formation in bacterial species because biofilm formation is the important activity of microbes in their virulence strategy. Thymoquinone also causes hindrance in the oxidative activity of microbes thus reducing the number of microbes (Harzallah et al., 2011; Forouzanfar et al., 2014; Khan, 2018; Fan et al., 2021).

Microbes cause many infectious diseases like AIDS, dengue, MERS, MRSA, and VRE, etc. Thymoquinone prevents the murine cytomegalovirus (CMV) replication in the liver and spleen of the infected mice. Medication with thymoquinone increases the number of $CD4^+$ T cells which plays a major role in building the immune system. Thymoquinone increases levels of serum interferon-γ (IFN-γ) and macrophages. Thymoquinone also fights against various fungal pathogens like *Candida albicans* and *Aspergillus fumigatus* (Khan, 2018; Wang et al., 2021; Qureshi et al., 2022).

Antimicrobial mechanism of melanin

Melanin constitutes a heterogenous group of phenolic polymers, which plays a major role in fighting against microbes. Melanin is an important antimicrobial agent which stops the proliferation of pathogens around the infected area. Melanin can fight against food pathogens; thus, contributes a major role in the food and health sector. Melanin also helped in antimicrobial drug discovery. Melanin extracted from hair and further dopped in metal ions demonstrated the potential of antibacterial activity (Mackintosh, 2001; Alviano et al., 2004; Nosanchuk and Casadevall, 2006; Eliato et al., 2021).

Antimicrobial mechanism of P-cymene

P-cymene is an important antimicrobial agent which is found in more than 100 plants and contributes to the food and medicine sector. P-cymene is used in the preparation of fungicides and pesticides. P-cymene affects the bacterial membrane, pH and ATP thus contributing to the file of antimicrobes. Studies reported that p-cymene inhibits the growth of *E. coli, L. monocytogenes, S. enterica*, *S. aureus, Vibrio parahaemolyticus*, and *Streptococcus mutans* (Kordali et al., 2008; Marchese et al., 2017; Miladi et al., 2017; Balahbib et al., 2021).

Antimicrobial mechanism of pinene

Pinene belongs to the group of terpenes and has antimicrobial properties. Pinene inhibits microorganisms by interfering in their metabolic process and modulation of gene (Tyc et al., 2017; do Amaral et al., 2020). Pinene has its antimicrobial activity (Dhar et al., 2014; Zhang et al., 2021) against *C. neoformans, C. neoformans phospholipase* and *esterase*, *C. Albicans*, *R. oryzae*, and MRSA (Silva et al., 2012; Kovač et al., 2015).

Antimicrobial mechanism of alkaloid

Alkaloids disrupt the cell membrane of microbes, inhibit cellular division, inhibit efflux pump, and inhibit biofilm formation thus act as a lead compound in the development of antimicrobial agents. Studies reported that alkaloids can kill *Pseudomonas aeruginosa, Streptococcus mutans, faecalis* and *pyogenes, Lactobacillus acidophilus, Candida albicans*, and *Staphylococcus aureus* (Orhan et al., 2010; Özçelik et al., 2011; Mittal and Jaitak, 2019; Othman et al., 2019; Zielińska et al., 2019; Huang et al., 2022).

Antimicrobial mechanism of limonene

Limonene belongs to the family of monoterpenes. Limonene by accumulating in the membrane of microbes causes disruption in it. Limonene is also one of the main causes of the dissipation of proton motive force in microbes. Limonene being an antimicrobial agent has a broad application in the food sector. Studies reported that limonene can kill gram-positive and gram-negative bacterial species. Limonene is also found to be effective against fungal pathogens (Cai et al., 2019; Han et al., 2019, 2020; Gupta et al., 2021).

Antimicrobial mechanism of camphene

Camphene is a lipophilic antimicrobial compound that by penetrating in cell membranes of bacterial species becomes the reason of cell death. Camphene inhibits microorganism by disruption in many cell activities, and the breakdown of the membrane thus cellular components and ions start leaking from the bacterial body. Also, camphene is the major source of depletion of ATP in bacterial species. Studies reported that camphene has antibacterial, antiviral, and antifungal activities (Zhou et al., 2016; Er et al., 2018; de Freitas et al., 2020; Hachlafi et al., 2021).

Conclusion

Microbial diseases are a major threat to the human population. The worldwide death rates due to microbes are: *Pseudomonas aeruginosa* 61%, *E. coli* 4.2%, *Staphylococcus epidermidis* 20%, *Candida albicans* 24%, *Shigella flexneri* 13.2%, *Sal. Typhimurium* 3 million each year, and *Vibrio alginolyticus* 50%. However, these death rates can be reduced by using antimicrobial agents on a regular basis. This review evaluated the antimicrobial effects of four plant resources- *Capsicum*, *Nigella sativa*, *Musa paradisiaca* L., and *Citrus limetta*. These natural resources contain thymoquinone, p-cymene, pinene, alkaloid, limonene, camphene, and melanin, which serve as antimicrobial agents by disrupting the cell membrane of microbes, inhibiting the cellular division and inhibiting the formation of biofilm in bacterial species, thus reducing the number of microbes. Soxhlet, hydro-distillation, solid-phase extraction (SPE), liquid-liquid extraction (LLE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), pulsed electric field (PEF), microwave-assisted extraction (MAE), enzyme-assisted extraction (EAE), ultrasound-assisted extraction (UAE), and high-voltage electrical discharge are the various techniques used for extracting critical antimicrobial agents from *Capsicum*, *Nigella sativa*, peels of *Musa paradisiaca* L., and *Citrus limetta.* A brief description of all these techniques was given along with their advantages and disadvantages in order to ease the selection of appropriate technology for the extraction of naturally available antimicrobial compounds.

Author contributions

SB, WZ, MH, and AM equally contributed to the concept of the study, its framework, the coordination and activities of subgroups, and writing of the manuscript. MM, MG, MI, SS, SI, SU-R, and SK equally contributed to the activities of subgroups and the review of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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