Antibacterial activity of essential oils pdf

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Antibacterial Activity of Essential Oils Against Human Pathogenic Bacteria

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Abstract: Essential oils are aromatic oily liquids obtained from plant materials. They can be obtained by expression, fermentation or extraction but the method of steam distillation is most commonly used for commercial production. Essential oils are complex mixture comprising many single compounds. Chemically they are derived from terpenes and their oxygenated compounds. Each of these constituents contributes to the beneficial or adverse effects against pathogenic bacteria. In the present study, four Essential oils (Punga oil - *Pongaemia pinnata*, Sesame oil - *Sesamum indicum*, Santhanathi oil - *Santhalum album* and Mustard oil - *Brassica nigra*) were collected and its antibacterial activity was investigated. Maximum inhibitory activity was observed in Santhanathi oil when comparing with the other Essential oils which was studied in the present research. The Santhanathi oil (*Santhalum album*) showed maximum inhibition zone which was recorded against *Bacillus cereus* followed by *Shigella flexneri*, *Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Escherichia coli*. Minimum zone of inhibition was noticed in *Acetobacter baumani*. No zone of inhibition was observed in DMSO blind control. Results of our study recommend the possibility of using the different essential oils as natural antibacterial in foods or pharmaceutical industries. It can be concluded that the plants investigation have opened up a new perspective in pharmaceutical research and they can be used for the development of potential, novel antibacterial agents for the treatment of bacterial diseases. The incorporation of this oil into the drug formulations is also recommended.

Key words: Essential Oils • Bacterial Pathogens • Antibacterial Activity and Well Diffusion Assay

INTRODUCTION

Nature is a source of medicinal agents and an impressive number of modern drugs have been isolated from natural sources. The presence of various life sustaining constituents in plants made scientists to investigate these plants for possibility their uses in treating certain infectious diseases. Traditional medicine has long been accepted as an alternative to western medicinal practice in many countries. Traditional medicine was once regarded as the sole source of treatment, making it a focus in the search for solution to increasing drug resistance among pathogenic microorganisms. The World Health Organization (WHO) has reported that over 80% of the world's population relies on traditional medicine which is largely plant based for their primary healthcare needs [1, 2].

Infectious diseases caused by infective microorganisms like bacteria, fungi, viruses or parasites represent a vital pathological state and one in every of the most causes of morbidity and mortality are included in the list of the ten leading causes of death worldwide [3-5]. In the recent years, the emergence of resistant Gram negative and Gram positive bacteria presents a serious challenge for the antimicrobial medical aid and drastically narrows the treatment choices of human infections [6, 7]. So, there is an imperative ought to resolve newer, safer and more effective natural or artificial antibacterial drug molecules so as to fight the emergence of those newer resistant.

Medicinal and aromatic plants have been used by mankind since ancient time to treat various ailments. Medicinal plants are rich source of bioactive compounds and have played an important role in drug discovery [8]. Recently, there has been a growing interest in the

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ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS EXTRACTED FROM MEDICINAL PLANTS AGAINST MULTI-DRUG RESISTANT *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Multi-drug resistant *Staphylococcus aureus* has been a major contributor to treatment failure in the last decade in Pakistan and indigenous plants focused for screening of new therapeutic options. Antibacterial properties of indigenous medicinal plants essential oils were determined against MDR *S. aureus* isolates from patients, associated healthcare workers and healthy community. Antibacterial activity was determined by agar well diffusion assay. Minimum inhibitory concentrations (MIC) of essential oils exhibiting activity against selected MDR *S. aureus* were calculated using Micro broth dilution method. Five plants essential oils *Carum copticum*, *Anethum sowa*, *Cuminum cyminum*, *Myristica fragrans* and *Zingiber officinale* were tested for antibacterial activity against selected MDR *S. aureus* isolates from three sampling groups. Out of five essential oils, *Carum copticum* was found to be the most effective followed by *Zingiber officinale* and *Cuminum cyminum*. Upon qualitative analysis of five essential oils, *Carum copticum, Cuminum cyminum and Zingiber officinale* essential oils showed zones of inhibition greater than the standards Vancomycin and Linezolid. *Anethum sowa* and *Myristica fragrans* essential oils showed no activity against MDR *S. aureus*. The mean MIC values obtained for *Carum copticum* against selected MDR *S. aureus* isolates from patients, healthcare workers and community were 4.2µl, 1.7 µl and 3.0 µl, respectively. Mean MIC value for *Zingiber officinale* were 10.8 µl, 3.5 µl and 6.8 µl and for *Cuminum cyminum* were 43.8 µl, 23.8 µl and 40 µl. The MIC value of *Carum copticum* essential oil was least against MDR *S. aureus* isolates and considered most effective plant oil.

Keywords: Multiple drug resistant *Staphylococcus aureus*, Post-operative wounds, Healthcare workers, Healthy volunteers from Community, Essential oils, Agar well diffusion assay, Minimum inhibitory concentration.

INTRODUCTION

Emergence of highly resistant bacteria has seriously affected the impact to combat infections by use of current antibiotics (Simoes *et al*., 2010). Clinical infections due to Methicillin resistant *Staphylococcus aureus* with decreased susceptibility to Vancomycin are increasing worldwide (Sakoulas and Moellering, 2008). Linezolid was alternative as compared to Vancomycin more effective choice of treatment (Kaleem *et al*., 2011). Serious complications have been reported with Linezolid on long term use in replacement for Vancomycin (Spellberg *et al*., 2004). It resulted in screening for novel and better molecules for adequate management of patients presented with multiple drug resistant infections and to handle the load of such pathogens in future (Harvey, 2008). Due to emergence of multiple drug resistant pathogens the clinical effectiveness of many current antibiotics has declined posing a serious threat as options available are scarce (Sangeetha *et al*., 2011). The problem of growing resistance requires to surge for new treatment options and to explore the medicinal plants for their bioactive molecules with antimicrobial properties. These include alkaloids, flavonoids, tannins, phenolic compounds, steroids, resins, fatty acids and gums which are known to be physiologically active. This is the most important motivational aspect that has always encouraged researchers to explore for new antimicrobial substances from medicinal plants being one of the largest reservoirs to be quest (Rios and Recio, 2005).

Essential oils are gaining attention having high potential as antimicrobials with minimum risk of resistance (Prabuseenivasan *et al*., 2006). These are the secondary metabolites used as defensive agents by the plants and have been known through centuries for their beneficial effects *i.e.* antibacterial, antifungal, antiviral, insecticidal and antioxidant (Burt, 2004; Schafer and Wink, 2009). Essential oils have shown potential results as antimicrobials against clinical isolates as well as food-borne pathogens upon in vitro testing (Burt, 2004).

Present study was conducted to evaluate *in-vitro* antibacterial activity against multi-drug resistant *Staphylococcus aureus* isolates from patients, health-care staff members and community.

MATERIALS AND METHODS

Antibacterial activity of five selected medicinal plants essential oils was evaluated against multi-drug resistant *Staphylococcal aureus* isolates from patients,

Antibacterial activity of essential oils on *Xanthomonas vesicatoria* and control of bacterial spot in tomato

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Abstract – The objective of this work was to evaluate the effects of plant essential oils (EOs) on the growth of *Xanthomonas vesicatoria*, on bacterial morphology and ultrastructure, and on the severity of tomato bacterial spot. EOs from citronella, clove, cinnamon, lemongrass, eucalyptus, thyme, and tea tree were evaluated in vitro at concentrations of 0.1, 1.0, 10, and 100% in 1.0% powdered milk. The effect of EOs, at 0.1%, on the severity of tomato bacterial spot was evaluated in tomato seedlings under greenhouse conditions. The effects of citronella, lemongrass, clove, and tea tree EOs, at 0.1%, on *X. vesicatoria* cells were evaluated by transmission electron microscopy. All EOs showed direct toxic effect on the bacteria at a 10%-concentration in vitro. Under greenhouse conditions, the EOs of clove, citronella, tea tree, and lemongrass reduced disease severity. EOs of clove and tea tree, and streptomycin sulfate promoted loss of electron-dense material and alterations in the cytoplasm, whereas EO of tea tree promoted cytoplasm vacuolation, and those of citronella, lemongrass, clove, and tea tree caused damage to the bacterial cell wall. The EOs at a concentration of 0.1% reduce the severity of the disease.

Index terms: alternative control of plant disease, cell ultrastructure, pathogenic bacteria.

Atividade antibacteriana de óleos essenciais sobre *Xanthomonas vesicatoria* e controle da mancha-bacteriana do tomateiro

Resumo – O objetivo deste trabalho foi avaliar o efeito de óleos essenciais (OEs) no crescimento de *Xanthomonas vesicatoria*, na morfologia e na ultraestrutura bacteriana, e na severidade da mancha-bacteriana do tomateiro. OEs de citronela, cravo-da-índia, canela, capim-limão, eucalipto, tomilho e árvore-de-chá foram avaliados in vitro nas concentrações de 0,1, 1,0, 10 e 100% em leite em pó a 1,0%. O efeito dos OEs, a 0,1%, na severidade da mancha-bacteriana do tomateiro foram avaliados em plantas de tomateiro em casa de vegetação. Os efeitos dos óleos de citronela, capim-limão, cravo-da-índia e árvore-de-chá, a 0,1%, nas células de *X. vesicatoria* foram avaliados por meio de microscopia eletrônica de transmissão. Todos os OEs apresentaram efeito tóxico direto sobre as bactérias na concentração de 10% in vitro. Em casa de vegetação, os OEs de cravo-da-índia, citronela, árvore-de-chá e capim-limão reduziram a severidade da doença. Os OEs de cravo-da-índia e árvore-de-chá, e o sulfato de estreptomicina promoveram perda de material eletro-denso e alterações no citoplasma, enquanto o OE de árvore-de-chá promoveu vacuolização do citoplasma, e os de citronela, capim-limão, cravo-da-índia e árvore-de-chá causaram danos à parede celular bacteriana. Os OEs, na concentração de 0,1%, reduzem a severidade da doença.

Termos para indexação: controle alternativo de doenças de plantas, ultraestrutura celular, bactéria fitopatogênica.

Introduction

Diseases of tomato (*Solanum lycopersicum* L.) are responsible for significant yield losses. Bacterial spot, caused by a number of species of bacteria of the genus *Xanthomonas* (Dowson) (Jones et al., 2000), is among the most important of these diseases.

Several studies have shown that it is possible to use essential oils to control plant diseases (Pereira et al., 2011). Medicinal plants contain substances that are able to play an important role in plant-pathogen interaction, by activating plant defense mechanisms (Schwan-Estrada et al., 2003; Lucas et al., 2012). Antifungal substances, similar to fungicides, may also be present in those plants, acting directly on the pathogens (Rosato et al., 2007). Medice et al. (2007) observed that essential oils from eucalyptus [*Corymbia citriodora* (Hook.) K.D.Hill & L.A.S. Johnson], thyme (*Thymus vulgaris* L.), neem (*Azadirachta indica* A. Juss.), and citronella [*Cymbopogon nardus* (L.) Rendle.] completely inhibited the germination of urediniospores of Asian soybean rust (*Phakopsora*

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RESEARCH ARTICLE Open Access

Comparison of the antibacterial activity of essential oils and extracts of medicinal and culinary herbs to investigate potential new treatments for irritable bowel syndrome

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Abstract

Background: Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder, which may result from alteration of the gastrointestinal microbiota following gastrointestinal infection, or with intestinal dysbiosis or small intestinal bacterial overgrowth. This may be treated with antibiotics, but there is concern that widespread antibiotic use might lead to antibiotic resistance. Some herbal medicines have been shown to be beneficial, but their mechanism(s) of action remain incompletely understood. To try to understand whether antibacterial properties might be involved in the efficacy of these herbal medicines, and to investigate potential new treatments for IBS, we have conducted a preliminary study *in vitro* to compare the antibacterial activity of the essential oils of culinary and medicinal herbs against the bacterium, *Escherichia coli*.

Methods: Essential oils were tested for their ability to inhibit *E. coli* growth in disc diffusion assays and in liquid culture, and to kill *E. coli* in a zone of clearance assay. Extracts of coriander, lemon balm and spearmint leaves were tested for their antibacterial activity in the disc diffusion assay. Disc diffusion and zone of clearance assays were analysed by two-tailed t tests whereas ANOVA was performed for the turbidometric assays.

Results: Most of the oils exhibited antibacterial activity in all three assays, however peppermint, lemon balm and coriander seed oils were most potent, with peppermint and coriander seed oils being more potent than the antibiotic rifaximin in the disc diffusion assay. The compounds present in these oils were identified by gas chromatography mass spectrometry. Finally, extracts were made of spearmint, lemon balm and coriander leaves with various solvents and these were tested for their antibacterial activity against *E. coli* in the disc diffusion assay. In each case, extracts made with ethanol and methanol exhibited potent antibacterial activity.

Conclusions: Many of the essential oils had antibacterial activity in the three assays, suggesting that they would be good candidates for testing in clinical trials. The observed antibacterial activity of ethanolic extracts of coriander, lemon balm and spearmint leaves suggests a mechanistic explanation for the efficacy of a mixture of coriander, lemon balm and mint extracts against IBS in a published clinical trial.

Keywords: Irritable bowel syndrome, IBS, Small intestinal bacterial overgrowth, SIBO, Herbal medicine, Antibacterial, Antimicrobial, Essential oil

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Full Length Research Paper

Antibacterial and antioxidant activities of *Origanum compactum* essential oil

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In the present study, essential oil of *Origanum compactum* was analysed and its chemical composition was identified by gas chromatography coupled to mass spectrometry (GC-MS). Among thirty two assayed constituents, carvacrol (30.53%), thymol (27.50%) and its precursor γ-terpinene (18.20%) were found to be the major components. The oil was investigated for its *in vitro* antibacterial activity against a panel of standard reference strains using well diffusion and broth dilution methods. In solid medium, the oil was found to be remarkably active against all tested strains except *Pseudomonas* which showed resistance. In liquid medium the Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentration (MBCs) ranged from 0.0078 to 0.25% (v/v). The antioxidant activity was investigated by three different methods; 1,1-diphenyl-2-picryl-hydrasyl (DPPH) radical scavenging assay, β-carotene bleaching test and reducing power. The results of this study revealed evidence that the essential oil of *O. compactum* possesses a good antioxidant effect with all assays; the antioxidant activity is dependent on the oil concentration and can be attributed to the phenolic compounds present in the oil.

Key words: Essential oil, *Origanum compactum*, chemical analysis, antibacterial activity, antioxidant activity.

INTRODUCTION

The reactive oxygen species (ROS) are a group of highly reactive molecules including the free radicals such as superoxyde ion (O_2^-) and hydroxyl radical (OH) as well as the no free radicals such as the hydrogen peroxide (H_2O_2). In human body, ROS are produced through normal aerobic respiration and during inflammatory process. Furthermore, aggressions especially such as radiations, stress, pollution, alcoholism and nicotinism increase the production of ROS (Yildirm et al., 2000). Natural protection against ROS is provided by enzymatic system (superoxyde dismutase, catalase and the selenium glutathion peroxidase) or by chemical molecules (scavengers and antioxidants) (Berger, 2006). The imbalance between ROS production and the defence mechanisms leads to oxidative modification in the intracellular molecules or the cellular membrane. Such alterations can be involved in high number of diseases including diabetes, cancer and cardiovascular diseases. (Kaplan et al., 2007). Moreover, ROS can cause lipids peroxidation in food during manufacturing process and storage which consequently leads to the loss of the food quality and safety (Mau et al., 2004).

Antioxidants are capable to prevent or delay oxidative processes by inhibiting the initiation or propagation of an oxidative chain reaction. They are important in the prevention of many oxidative-stress related diseases (Gerber et al., 2002) and are used to maintain the nutritional quality and to increase the shelf life of food. However, the most frequently used synthetic antioxidants in food industry such as 2,3-tert-butyl-4-methoxy phenol (BHA) and 2,6-di-tert-butyl-4-methyl phenol (BHT) have been suspected to cause undesired health effects (Namiki, 1990). Consequently, a large body of research is now focused on the antioxidant action of natural substances, especially those derived from plants.

On the other hand, there is an impetus for researches for natural antimicrobial agents to use as alternative for food preservation and human remedies. In fact, the excessive and inappropriate use of antibiotics in agriculture or in human's medication to treat infectious

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In vitro antibacterial activity of some plant essential oils. Antibacterial activity of essential oils from australian native plants. Antibacterial and antifungal activity of ten essential oils in vitro. In vitro antibacterial activity of essential oils against streptococcus pyogenes. Antibacterial activity of essential oils encapsulated in chitosan nanoparticles. Antibacterial activity of terpenes and terpenoids present in essential oils. Antibacterial activity of essential oils from plants of the genus origanum. Antibacterial activity of essential oils pdf.

View PDFVolume 7, Issue 4, April 2021, e06835 rights and content Background. Emerging of multidrug-resistant bacteria can compromise the effectiveness of antibiotics used to treat skin infections. Those bacteria imposed public health problems and questioning medical care in the 21st century. In this circumstance, essential oils of medicinal plants origin are supreme sources of structural and functionally divergent compounds, which inhibited the growth of common wound colonizing MRSA and ESBL producing P. aeruginosa. The aim of this study was to evaluate the combined antibacterial activity of essential oils extracted from Rumex abyssinicus, Cucumis pustulatus, and Discopodium penninervium against multidrug-resistant (MDR) isolates of skin ulcers. Methods. Essential oils (EOs) were extracted from aerial parts of R. abyssinicus, C. pustulatus, and D. penninervium with steam distillation. A mixture of each oil (1 : 1) was adsorbed to a disc and placed on Mueller Hinton Agar. Then, minimum zone of inhibition and bactericidal concentration of EOs was measured after incubeted for 18–24 hours at 37 °C. Their combined antibacterial effect was determined by the fractional inhibitory concentration index. Results. The antibacterial activity of mixed oil varied in their doses and bacteria species, of which a mixture of essential oil of R. abyssinicus and D. penninervium had inhibition zone (32 mm); its MIC and MBC values range from 1-2 µl/ml against MRSA. It had an inhibition zone (36 mm), MIC value 4 µl/ml, and MBC (8 µl/ml) against ESBL producing P. aeruginosa, whereas combined effects of R. abyssinicus and C. pustulatus had MIC values ranging from 2–8 µl/ml for E. coli and K. pneumoniae and 2 µl/ml for MRSA. There was a strong synergistic effect between R. abyssinicus and D. penninervium and promising antibacterial effect more specifically on MRSA and P. aeruginosa. Conclusion. This in vitro study of the combined effect of EOs has significant antibacterial activity on wound colonizing bacteria and reduces delaying wound healing as that of modern drugs tested in parallel. Hence, further structural elucidation of active compounds helps us to properly design or synthesis of topical antibiotics for wound care.1. BackgroundModern humans have universally used medicinal plants for healing properly many ailments [1, 2]. Evolving of the powerful analytical tools based upon proteomics, metabolomics, and genomics can aid to discover novel compounds from medicinal plants. The compounds of plant origin have divergent chemical structures and functionally relevant precursor molecules to discover antibiotics [2, 3]. Besides this, widely ethnobotanical and ethnopharmacological studies of essential oils from plants contributed to finding various compounds, of which leptospermone, tricyclene, flavesone, myrcene, carvacrol, p-cymene, eugenol, γ-terpinene, phenylpropanoids, β-selinene, and calamenene are some important compounds inhibiting the growth and biofilm formation of pathogen bacteria and used as immunomodulatory compounds [4, 5]. For instance, carvacrol caused collapse of the proton-motive force and depletion of the ATP pool, with consequent cell death [6]. Therefore, essential oils are applicable as precursors in the pharmaceutical industries for development of antibiotics [7, 8].Nowadays, there is an increasing attention in exploring potential therapeutic bioactive to treat serious ailments caused by multidrug-resistant bacteria such as chronic wound [3, 4, 8, 9]. WHO reported that there were more than 8.2 million people infected with wounds with or without infection. This caused $28.1–96.8 billion lost to treat acute and chronic wounds. In this regard, the United States lost $25 billion per year for healthcare expenditure for nonhealing ulcer [10]. Likewise, a study conducted in Europe showed that there were 1-5–2 million people agonized from wounds. Unless and otherwise invented for new novel treatment, it imposed serious economic impact and costs $15–22 billion per year in coming five years. It has become major public health problem that caused psycho-social consequence on infected patients [9, 10]. Emerging of MRSA, VRE, and ESBL producing P. aeruginosa that identified as common wound colonized MDR bacteria has been worsen medical-surgical care and other health services [9–11]. In such case, essential oils of medicinal plants origin are suitable candidates to develop topical ointments for wound care and beyond [8, 12].Essential oils have many compounds that can aid wound healing. In this regard, essential oil able to immunomodulating potential to typically activate both humoral and cell-mediated immune response. Other compounds such as carvacrol have bactericidal property through inhibition of protein and nucleic acid synthesis of MRSA and ESBL producing Enterobacteriaceae [5, 12, 13]. Some of them prevent biofilm formation and multiplication, inhibitory effect on inflammatory edema formation, and leucocyte chemotaxis around the wound. Therefore, essential oils are improving the quality wound care, decrease morbidity, and mortality and overcoming nonhealing trajectory and low therapeutic response of chronic wound infection [8, 12, 13].Many studies showed that combined effect of essential oils had powerful antibacterial activity on wound colonizing pathogen multidrug-resistant bacteria [3, 12, 13]. The EOs of Cinnamomum verum and piperacillin mixture had synergistic activity against beta-lactamase TEM-1 E. coli. This combination made wrecked the outer membrane or QS inhibition in bacteria cells [14]. Interestingly, a study conducted on Qingre Baidu mixture could inhibit the biofilm formation of S. aureus and P. aeruginosa. It reduces AI-2 level and upregulating expression of HIF-1α, HIF-2α, and HIF-3α, which increased the levels of VEGF, thereby promoting angiogenesis and wound healing in chronic and refractory wounds [15]. Another study showed that EOs of Pilgerodendron uviferum and Melaleuca alternifolia can inhibit efflux pump mechanism of the S. aureus NorA. These essential oils can inhibit efflux pump and characterize a potentially safe and affordable ingredients to develop skin friendly ointment to wound colonizing multidrug-resistant bacteria [16–20].Currently, using combined conventional antibiotics and essential oils or essential oils themselves as wound care ointments are promising strategies to overcome multidrug-resistant bacteria. MRSA and ESBL producing E. coli have resisted for amoxicillin, tetracycline, piperacillin, ofloxacin, and oxacillin [21–23]. Many studies showed that combined effect of Leptospermum scoparium and Tri-EDTA; Mentha piperita and Micromelum integerrimum and; cinnamon bark oil and cinnamaldehyde had synergistic antibacterial activity on MSSA, E. coli, MRSA, ESBL, and P. aeruginosa [24, 25]. From these viewpoints, we purposed to evaluate combined antibacterial activity of EOs extracted from R. abyssinicus, C. pustulatus, and D. penninervium against bacteria isolated from wounds. Essential oils had immunomodulatory compounds that serve as a baseline data to formulate and designing novel compounds and find out the scientific rationale for the combined effects of untapped medicinal plants used by different societies.2. Materials and Methods2.1. Study Area and Design2.1.1. Study AreaThis study was conducted in the Seweyna woreda, Bale Zone. This woreda is located 437 km away from Robe (zonal town) in the northern east direction and 750 km from the capital city, Addis Ababa. An elevation extends from 400–1850 m above sea level and is located at coordinates latitude 7°19′60.00″ N and longitude 41°19′60.00″ E. Major rivers include the Mekenisa, Dare, Manduba, and Kurkura. According to the national land survey, Seweyna is covered by abundant pasture (46.3%), arable or cultivable (24.4%) and forest or heavy vegetation (24.1%). This district is the remotest area with no infrastructure (transport, hospital, and power supplies). There has been one health center and residents depend on traditional knowledge of TMP to treat ailments such as skin diseases, diabetes, STI cancer, hypertension, and impotence.2.1.2. Study Design and PeriodAn in vitro experimental study was carried out to evaluate the antibacterial effect of combined essential oils, R. abyssinicus, C. pustulatus, and D. penninervium, against MDR and their reference strains at KMU, Core Laboratory, from January to April, 2020.2.2. Medicinal Plants Selection CriteriaNowadays, pastoralist communities are deprived of modern medical care and depend on the traditional medicinal plants to treat many human and animals' ailments such as skin diseases, diabetes, hepatitis, and cancer. These medicinal plants were selected based on traditional knowledge of healers to treat bacterial infections such as eczema, gonorrhea, syphilis, pneumonia, scabies, and other skin infections commonly with R. abyssinicus, C. pustulatus, and D. penninervium.2.3. Plant Collection and ExtractionPlant samples of R. abyssinicus, C. pustulatus, and D. penninervium were collected from sideways of Mekenisa and Kurkura rivers of Seweyna woreda. Each plant was euthanatized and deposited at the National Herbarium with Rumex abyssinicus voucher number (E-55/07), Cucumis pustulatus (E-45/07), and Discopodium penninervium (E-18/07) in the Department of Biology, Faculty of Natural and Computational Science, AAU. Well-grown aerial part of each plant was collected and extracted its essential oils with steam distillation using AMIO-37/04 model for 3–5 hours and stored in brown colored bottle vials at 4 °C as described by Eshetu [3].2.4. Culture Media and Multidrug Bacteria2.4.1. Culture MediaNutrient agar, TSY broth, MacConkey, MHA, MHB, BA, mannitol salt agar, chocolate agar, and other reagents were used to grow bacteria collected from wounds.2.4.2. Test OrganismsThe reference bacterial species of E. coli (ATCC25922), K. pneumoniae (ATCC700603), E. faecalis (ATCC 29212) and S. aureus (ATCC 25923), P. aeruginosa (ATCC 27853), and their MDR strains were isolated from wounds of out-patients attending Menelik Hospital, Addis Ababa. Ciprofloxacin (5 µg), gentamycin (10 µg), cephalotaxine (30 µg), cefotaxime (5 µg), ceftazidime (10 µg), cefoxitin (30 µg), ceftriaxone (30 µg), amikacin (30 µg), cefuroxime (5 µg), ceftriaxone (30 µg), cloxacillin (5 µg), and augmentin (30 µg) were antibiotics as described in CLSI guideline [26].2.5. Screening for Multidrug-Resistant BacteriaMultidrug-resistant bacteria were isolated from wounds. All bacterial cultures were first grown on 5% BA plates at 37°C for 18–24 hrs before inoculation onto the MHA. Few colonies (3–5) of similar morphology of the respective bacteria were transferred with a sterile inoculating loop to a liquid medium until adequate growth of turbidity with McFarland in 0.5. Then, the bacterial suspension was streaked on MHA plates using a sterile swab in such a way as to ensure thorough coverage of the plates and a uniform thick lawn of growth following incubation. The susceptibilities of clinical isolates were tested by using the MHA containing a range of antimicrobial agents. Dilutions of overnight broth cultures were inoculated onto antibiotic-containing plates to yield final inoculums of approximately 106 CFU per spot for S. aureus, E. faecalis, K. pneumoniae, E. coli, and P. aeruginosa according to disk diffusion methods as recommended by CLSI guidelines [3, 26].2.5.1. Screening for Gram-Negative BacteriaSelected multidrug-resistant Gram-negative bacteria such as ESBL producing E. coli and P. aeruginosa were detected by double disk synergy test (DDST) as described by Jarlier [27]. Mueller Hinton Agar was inoculated with standardized inoculum using sterile cotton swab. Augmentin (20 µg amoxicillin and 10 µg of clavulanic acid, AMC) disk was placed in the center of the plate and test disks of cephalosporins (ceftazidime 30 µg, ceftriaxone 30 µg, and cefotaxime 30 µg) and aztreonam 30 µg disks were placed at 20 mm distance (center to center) from the amoxicillin-clavulanic acid disk before incubation. The plate was incubated overnight at 35°C. Enhancement of the zone of inhibition of any one of the four drug disks toward amoxicillin-clavulanic acid suggested the presence of extended-spectrum beta-lactamases. K. pneumoniae was screened for its resistance to more than two different classes of antibiotics following disk diffusion method as CLSI guidelines [3, 26, 28].2.5.2. Screening for Drug Resistant Gram-Positive BacteriaIn this study, cefoxitin was used as the marker of mecA/mecC mediated by methicillin resistant S. aureus and vancomycin (VRE) drug of choice for disk diffusion method as recommended by CLSI guidelines. On the other hand, the concentration values (MIC and MBC) and fractional inhibitory concentration index were determined by MHB broth microdilution method as described by Eshetu [3, 26, 29, 30].2.6. Statistical AnalysisStatistical data were reading values of inhibition zones and concentration values (MIC and MBC) analyzed using SPSS, version 21. Each experiment value is expressed as mean ± S.D. Statistical significance was determined by student t-test.Significance was determined by student's t-test. Values with were considered significant.3. Results3.1. Antibacterial Effect of Modern AntibioticsMajority of bacteria isolated from wounds were resistant to two or more antibiotics. In this regard, methicillin resistant S. aureus (MRSA) and ESBL producing P. aeruginosa were major wound colonizing bacteria. From Gram-positive bacteria, MRSA and VRE were resisted for amikacin, cefoxitin, amoxicine, ampicillin, and cefotaxime (Table 1). On the other hand, Gram-negative bacteria ESBL producing E. coli and P. aeruginosa were isolated from surgical wounds. Those Gram-negative bacteria have been undermining in the case of wound care, whereas these bacteria strains were unpredictably resisted for classical modern third-generation cephalosporins and penicillin classes (Table 1). In such cases, gene or gene products of those bacteria transfer intra- or interbacteria species compromised treatment options and worsen the future of medical care.Modern drugsDrug susceptibility test for multidrug bacteria strains isolated from wound samplesEf1Ef2Ef3A1A2A3Ec1Ec2Ec3Ar1Ar2Ar3K1K2K3CiprofloxacinRRIRIRRRRRSIRIRCefoxitinRIRRRRRNNNNNNNNCefuroximeSRINNNRRRRRRRIRCeftriaxoneSIRNNNRRRRRRRRCeftazidimeNNNNNNRIRRRRRSIChloramphenicolRRRSIRRRSRRRSRRRAmpicillinISSRRRSRRRSRRRAugmentinRISRISRIRRSRRISAmikacinRRRRRRNNSRRRIRSErythromycinRIRRRIRIRRRRSRTetracyclineRSIRRRIIRRRRSRIPenicillinRIRRRRRRRRRRIRVancomycinRRRIRSNNNNNNNNNGentamycinRISRRRSRRSRISRRA = methicillin resistant S. aureus; Ar = ESBL producing P. aeruginosa; Ec = ESBL producing E. coli; N = not done; Ef = Vancomycin resistant E. faecalis; K = multidrug-resistant K. pneumoniae; I = intermediate (17–20 mm IZ); R = resistance (≤16 mm IZ); S = sensitive (≥21 mm IZ) according to CLSI guidelines [25].3.2. Antibacterial Effect of Essential OilsThis study revealed that testing essential oils had broad bactericidal activities. They inhibited growth of E. faecalis, E. coli, P. aeruginosa, K. pneumoniae, and S. aureus with zones of IZ ranging from 12–26 mm (Table 2). Thus, oils demonstrated an antibacterial effect against wound colonization MRSA and ESBL producing Gram-negative bacteria. Their effectiveness varied with the concentration and type of bacterial species. For instance, R. abyssinicus inhibited the growth of MRSA (23 mm) and ESBL producing E coli (22 mm) and P. aeruginosa (20 mm) at its 20 µl/disc, whereas D. penninervium inhibited the growth of MRSA (21 mm) and ESBL producing E coli (18 mm) and P. aeruginosa (22 mm) at its 20 µl/disc. These essential oils had an inhibitory effect on both reference strains and MDR bacteria with IZ ranging from 9–26 mm in diameter and MIC and MBC values ranging from 2–32 µl/ml. The EOs of R. abyssinicus, C. pustulatus, and D. penninervium showed IZ in diameter values 23 mm, 19 mm, and 21 mm on the MRSA, respectively. Vancomycin resistant E. faecalis were highly susceptible for tested EOs as compared to other species.Essential oils, 50 µl/ml/disc Inhibition zone in diameter (mm), pathogen bacteria isolated from wounds Gram-positive bacteria Gram-negative bacteriaS. aureusE. faecalisE. coliK. pneumoniaeP. aeruginosaATCCMRSAATCCVREATCCESBLATCCMDRATCCESBLR. abyssinicus2023 ± 0.823 ± 3.025 ± 1.325 ± 1.521 ± 0.722 ± 0.2d25 ± 0.525 ± 0.921 ± 0.820.91017 ± 0.418 ± 0.120 ± 0.919 ± 1.011 ± 0.312 ± 1.417 ± 1.717 ± 0.114 ± 1.214 ± 2.1510 ± 1.79 ± .0.914 ± 1.013 ± 1.5NINI11 ± 0.610 ± 1.18 ± 2.09 ± 0.1D. penninervium2020 ± 2.021 ± 0.522 ± 0.122 ± 1.019 ± 0.818 ± 0.126 ± 0.726 ± 0.421 ± 0.922 ± 0.3103 ± 0.114 ± .0.317 ± 0.416 ± 0.714 ± 0.715 ± 1.018 ± 0.117 ± 0.577 ± 0.78 ± .0.911 ± 0.19 ± 1.39 ± 1.68 ± 1.012 ± 1.99 ± 0.1NINIC. pustulatus2019 ± 0.119 ± 0.220 ± 0.121 ± 0.112 ± 0.712 ± 1.122 ± 0.322 ± 0.419 ± 0.319 ± 0.41010 ± 0.59 ± 0.212 ± 0.614 ± 0.6NINI13 ± 0.413 ± 0.98 ± 0.411 ± 0.95NINI9 ± 0.68 ± 0.3NINI7 ± 0.610 ± 0.6NINIPositive controlSRSRSRSRSRNegative control (5% DMSO)NINININININININININIMean ± SD, C = ciprofloxacin; NI = no inhibition zone; NT = not tested; ATCC = susceptible >21 mm IZ, resistant 21 mm IZ, resistant