Original Research Article

ANTIMICROBIAL POTENTIAL OF *Pelargonium citrosum* AND *Rosmarinus officinalis* ESSENTIAL OILS.

ABSTRACT

Essential oils derived from aromatic plants have exhibited biological properties and can be used to prevent and treat human diseases. The goal of this work was to investigate the antibacterial and antifungal potential of the essential oils extracted from R. *officinalis and P. citrosum* against Gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis*) and Gram-negative bacteria (*Pseudomonus aeruginosa, Escherichia coli*) and fungus (*Candida albicans*). The antimicrobial activities of the *R. officinalis and P. citrosum* essential oils were carried out using the Disk diffusion method. The results indicated that the essential oils from *P. citrosum* had antimicrobial activity against *Bacillus subtilis* and *Escherichia coli* and *Candida albicans* at a low concentration of 0.5 % v/v and that the activity was concentration dependent. Essential oil from R. *officinalis* on the other hand showed effective antimicrobial activity against *Escherichia coli, Staphylococcus aureus* and *Pseudomonus aeruginosa* and *Candida albicans. P. citrosum* was found to be more effective than Nitrofuractoin and Gentamicin drugs against *Staphylococcus aureus* at a higher concentration of 6% v/v. *R. officinalis* oil extracts also demonstrated similar trends and were comparable to the positive controls against the tested microbes. It was therefore concluded that *R. officinalis and P. citrosum* plant extracts were effective against the tested antimicrobial agents and have potential to be used against the tested microbes.

Key words: Essential Oil, Extract, Chemical Composition, Antimicrobial Activity, *P. citrosum* and *R. officinalis*

INTRODUCTION

Although the antimicrobial properties of essential oils and their components have been reviewed in the past (Shelef, 1983; Nychas, 1995), the mechanism of action has not been studied in great detail. Considering the large number of different groups of chemical compounds present in essential oils, it is most likely that their antibacterial activity is not attributable to one specific mechanism (Burt, 2004).

Growth of microorganisms in food may cause spoilage or foodborne disease (Del Campo *et al.,* 2000). Synthetic additives have been widely used. The trend is to decrease their use because of the growing concern among consumers about such chemical additives (Dua *et al.*,2013). Consequently, search for natural additives, especially of plant origin, has notably increased in the recent years (Abdulrahman *et al*., 2010). Therefore, the development and application of natural products with both antioxidants and antimicrobial activities especially in meat products may be necessary and useful to prolong their storage shelf life and potential for preventing food diseases (Fernandez-Lopez et al., 2004).

Rosemary (*Rosmarinus officinalis*) originally grown in Southern Europe. Its herb and oils are commonly used as spice and flavoring agents in food processing for its desirable flavor, high antioxidant activity and as antimicrobial agent (Lo et al., 2002; Ouattara et al., 1997). Moreno et al. 2006) reported that Rosemary plants were rich sources of phenolic compounds with high antimicrobial activity against both Gram-positive and Gram-negative bacteria. High percent of the antimicrobial activity was attributed to carnosic acid and carnosol. It is clear that Rosemary extracts have bioactive properties, but their antimicrobial activities have not been extensively studied. Antimicrobial activities of plant essential oils have been known for centuries, but their strong flavor limited their use in food (Del Campo et al., 2000). Rosemary contains a number of phytochemicals including rosmarinic acid, camphor, caffeic acid, ursolic acid, betulinic acid, carnosic acid and carnosol (vallverdu-Queralt et al., 2013). In traditional medicine extracts and essentials oil from flowers and leaves are used in the belief that they may be used to treat a variety of disorders. (vallverdu-Queralt *et al*., 2013).

Pelargonium citrosum is a species indigenous to Southern Africa, especially in the Cape Town region, belonging to the Geraniaceae family, which was introduced in the Mediterranean part of Europe since the beginning of the eighteenth century (Pepeljnjak *et al*., 2005). *Pelargonium citrosum* has a woody, straight stem with branches; its leaves are usually alternate, palmately lobed or pinnate, often on long stalks, and sometimes with light or dark pattern; covered with short, rough hairs, which give the plant a strong, pleasant rose-like scent. The erect stems bear five-petaled flowers in umbel-like clusters. The flower has a single symmetry plane which distinguishes it from the Geranium flower, which has radial symmetry.

MATERIALS AND METHODS

Sample collection and extraction

The leaves of *Pelargonium citrosum* and *Rosmarinus officinalis* were collected from Githunguri in Kiambu County, Kenya. The fresh leaves were washed with running tap water and cut into small pieces, air dried at room temperature then pulverized with an in-house mechanical blender. Extraction of essential oils from the ground plant samples was done through hydro-distillation using Clevenger apparatus. The condensed extract was extracted with n-hexane and filtered using Whatman® grade 1 filter paper containing anhydrous Sodium Sulphate to remove any traces of water. The solvent was then removed using a rotary evaporator and the collected essential oil weighed into smaller amber covered vials and stored at 4^{o}C ready for antibacterial and antifungal activities.

Antimicrobial assay

The microorganisms used in this work were: Gram-negative bacteria (*Pseudomonus aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25992), Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis*) and fungi (*Candida albicans* ATC 10231). Nutrient Agar plates were used for the bacteria and yeast cultures. Seeding the culture medium under aseptic conditions was made with a sterilized platinum loop and was followed by incubation.

Disk diffusion method was used to study antimicrobial activity. Different concentrations of the effective plant extracts (0.1, 0.2, 0.5, 2.0, 3.0, 4.0, 5.0 and 6.0%v/v) were prepared separately by dissolving in DMSO, sterilized through Millipore filter and loaded their requisite amount over sterilized filter paper discs (7 mm in diameter). Mueller-Hilton agar was poured into sterile Petri dishes and seeded with bacterial suspensions of the pathogenic strains. The loaded filter paper discs with different concentrations of the effective plant extract were placed on the top of the Mueller-Hilton agar plates. The plates were placed in the fridge at 5°C for 2 hours the incubated at 37°C for 24 hours, to allow diffusion of the sample into the agar. Following this procedure, zones of growth inhibition were measured by Vernier caliper and recorded against the concentrations of the effective plant extracts.

The antimicrobial effect was tested in comparison with reference antibacterial substances (or positive controls), used as microcapsules for antibiograms: Norfloxacin (NX), Ofloxacin (OF), Ceftriaxone (CTR), Sulphamethoxazole (SX), Amoxylclar (AMC), Nitrofuractoin (NIT), Nalidixic acid (NA) and Gentamicin (GEN)

Results and discussion

The antimicrobial activity (zones of inhibition) of *Rosmarinus officinalis* extracts against *Pseudomonus aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus subtilis,* and *Candida albicans* are shown in figure 1 below.

Figure 1: Antimicrobial activity of *Rosmarinus officinalis* essential oils

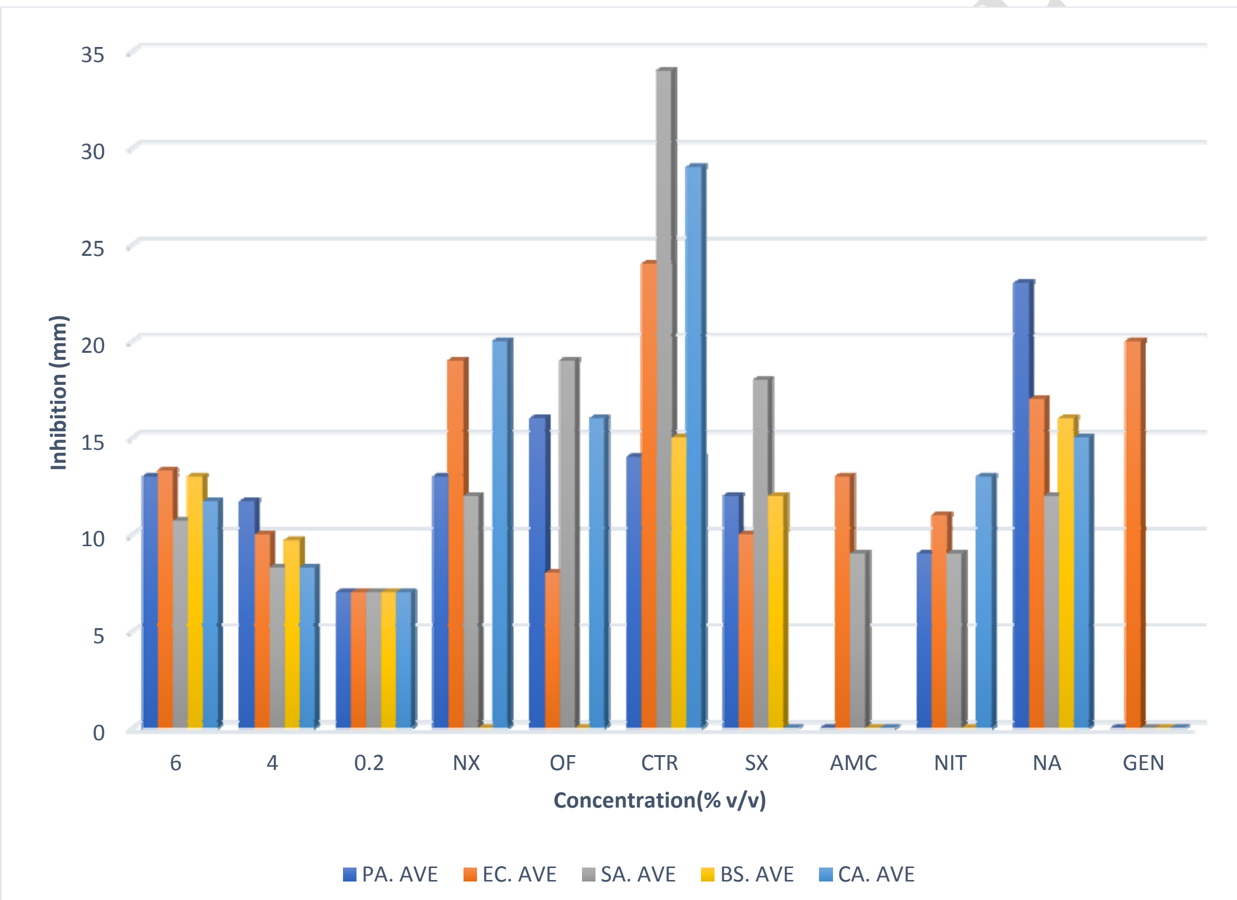

Figure 1: Antimicrobial activity of Rosmarinus officinalis essential oils

Key: PA; *Pseudomonus aeruginosa, EC: Escherichia coli, SA: Staphylococcus aureus, BS: Bacillus subtilis,* and *CA: Candida albicans, NX*-Norfloxacin, OF-Ofloxacin, CTR- Ceftriaxone, SX- Sulphamethoxazole, AMC- Amoxylclar, NIT- Nitrofuractoin, NA- Nalidixic acid, GEN-Gentamicin.

R. officinalis extract gave the highest zone of inhibition against *Escherichia coli* at a concentration of 6%v/v. The lowest effective concentration was 0.5%v/v against *Pseudomonus aeruginosa* and *Escherichia coli* at 7.3 ± 0.6 mm inhibition zone diameter while the effective concentrations against *Staphylococcus aureus* was 3%v/v and 2% v/v for *Bacillus subtilis* and *Candida albicans* respectively.

Figure 2 below shows the antimicrobial activity of *P. citrosum* essential oils against *Pseudomonus aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus subtilis,* and *Candida albicans*

Figure 2: Antimicrobial activity of *P. citrosum* essential oils

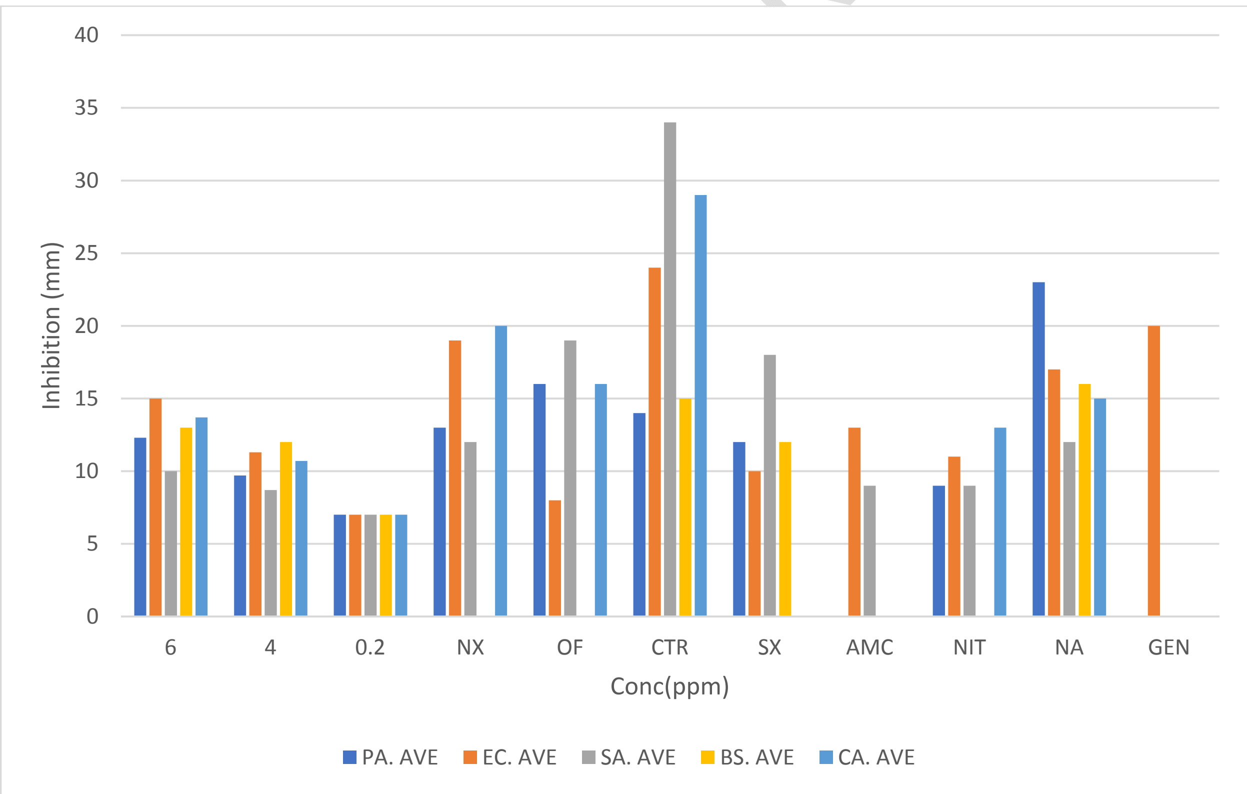

Figure 2: Antimicrobial activity of P. citrosum essential oils

Key: PA; *Pseudomonus aeruginosa, EC: Escherichia coli, SA: Staphylococcus aureus, BS: Bacillus subtilis,* and *CA: Candida albicans, NX*-Norfloxacin, OF-Ofloxacin, CTR- Ceftriaxone, SX- Sulphamethoxazole, AMC- Amoxylclar, NIT- Nitrofuractoin, NA- Nalidixic acid, GEN-Gentamicin.

At a concentration of 6% v/v, the zone of inhibition diameter against *Escherichia coli* was 15.0 ±1.0 mm compared to inhibition zone diameter of 10.0 ±1.0 mm for *Staphylococcus aureus*. Similar trends were observed, whereby *R. officinalis* and P. *citrosum* oils were effective at low concentrations of 0.5% v/v against *Pseudomonus aeruginosa, Escherichia coli* and *Bacillus subtilis,* and at a concentration of 2% v/v, against *Candida albicans* and 3% v/v against *Staphylococcus aureus.*

At 6%v/v concentration, the inhibition zone diameter measured for *P. citrosum* oils against *Staphylococcus aureus* was higher than for positive controls, namely, Nitrofuractoin and Gentamicin drugs. The antimicrobial activity of *Pelargonium citrosum* against *Bacillus subtilis* was found to be higher than that exhibited by Sulphamethoxazole, Norfloxacin, Amoxylclar and Ofloxacin. *R. officinalis* oils also demonstrated similar trends as the standards against various microbes, for instance, at 6%v/v, the zone of inhibition of *R. Officinalis* against *Escherichia coli* was 15.0 ±1.0 mm, which was slightly higher than that of Norfloxacin, Sulphamethoxazole, Nitrofuractoin and Nalidixic acid standards.

These findings were similar to those found by other researchers, that is, the antibacterial activity of Rosemary extracts against Listeria strains in a study by Tanja et al., (2008) that showed that the Rosemary extracts had the antilisterial activity against all of the eleven strains which were tested. According to Moreno et al., (2006) Rosemary plants are rich sources of phenolic compounds with high antioxidative and antimicrobial properties.

CONCLUSIONS

The penetration of new, natural, antimicrobial and antifungal agents, without negative impact on the environment, as substitutes to those obtained by chemical synthesis is a lasting necessity and also a challenge to scientists. The biological properties revealed by *P. citrosum* and *R. officinalis* in this study validates their potential commercial exploitation in industry. However, more research should be done to ascertain the safety of these essential oils for use as antimicrobial agents.

REFERENCES

Abdulrahman, M.S., Thangaraj, S., Salique, S.M., Khan, K.F., Natheer, S.E., 2010. Antimicrobial and biochemical analysis of some spices extracts against food spoilage pathogens. Int. J. Food Safety 12, 71–75

Burt S. 2004. Essential oils: their antibacterial properties and potential applications in food – a review. International Journal of Food Microbiology, 94: 223-253.

Del Campo J., Amiot M.J., Nguyen-The C. 2000. Antimicrobial effect of rosemary extracts. Journal of Food Protection, 10: 1359-1368.

Dorman, H.J.D., A. Peltoketo, R. Hiltunen and M.J. Tikkanen, 2003. Characterisation of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs. J. Food Chem., 83: 255-262.

Dua, A., Gaurav, G., Balkar, S., Mahajan, R., 2013. Antimicrobial properties of methanolic extract of cumin (Cuminum cyminum) seeds. Int. J. Res. Ayurveda Pharm. 4 (1), 104–107.

Fernandez-Lopez J., Zhi N., Aleson-Carbonell L., PerezAlvarez J.A., Kuri V. 2004. Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. Meat Science, 69: 371-380.

Iwasa, M., Makino S.I. Asakura H. Kobori H. and Morimoto,Y. (1999). Detected of *Escherichia Coli* O157:H7 from *Musca domestica* (Diptera: Muscidae) at a Cattle Farm in Japan. *Journal of Medical Entomology*, 36.108-112.

Lo A.H., Liang Y.C., Lin-Shiau S.Y., Ho C.T., Lin J.K. 2002. Carnosol, an antioxidant in rosemary, suppresses inducible nitric oxide synthase through down–regulating nuclear factor–κB in mouse macrophages. Carcinogenesis, 23: 983-991.

Moreno S., Scheyer T., Romano C.S., Vojnov A.A. 2006. Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. Free Radical Research, 40: 223-231.

Nychas G.J.E. 1995. Natural antimicrobials from plants. New methods of food preservation. Gould G.W. (ed.). London, Blackie Academic and Professional: 58-89 Shelef L.A. 1983. Antimicrobial effects of species. Journal of Food Safety, 6: 29-44.

Ouattara, B., Sabato, S. F., & Lacroix, M. (2001). Combined effect of antimicrobial coating and gamma irradiation on shelf life extension of pre-cooked shrimp (Penaeus spp.). International Journal of Food Microbiology, 68(1-2), 1-9.

Pepeljnjak, S., Kosalec, I., Kalodera, Z., & Blazevic, N. I. K. O. L. A. (2005). Antimicrobial activity of juniper berry essential oil (Juniperus communis L., Cupressaceae). acta pharmaceutica-zagreb-, 55(4), 417.

Tanja S., Barbara J., 2009. Antimicrobial activity of rosemary extracts (Rosmarinus officinalis L.) against different species of Listeria. Acta agriculturae Slovenica, 93 - 1, 51 – 58.

Vallverdú-Queralt, A., Regueiro, J., Martínez-Huélamo, M., Alvarenga, J. F. R., Leal, L. N., & Lamuela-Raventos, R. M. (2014). A comprehensive study on the phenolic profile of widely used culinary herbs and spices: Rosemary, thyme, oregano, cinnamon, cumin and bay. Food Chemistry, 154, 299-307.