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Full Length Research

Determination of antibacterial activity of essential oils from mint (*Mentha spicata*) leaves on selected pathogenic bacteria

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ABSTRACT: Drug resistant pathogenic microorganisms account for the highest proportion of death today. This study was aimed at extracting essential oils (EOS) from mint. Sensitivity tests of selected pathogenic microorganism were also carried out. EOS were extracted using distillation method. Phytochemical screening of the essential oils for the presence of tannins, alkaloids, glycosides, flavonoids, resins, phenols and steroids was carried out using standard procedures. Sensitivity test of *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (clinical isolate), *Escherichia coli* ((ATCC 25922), *Erwinia carotovora* (ATCC 33244), *Xanthomonas campestris* (ATCC 33913) and *Bacillus subtilis* (ATCC 6633) to the EOS was carried out using agar well diffusion technique. The minimum inhibitory concentration of the test pathogens by the essential oils was carried out using two-fold serial dilution. The yield of essential oils varied from 1.0±0.01% in first replicate to 3.0±0.03% in the fourth replicate. All the tested phytochemical compounds were present in the EOS of mint. There was no significant difference between the zones of inhibition of the three replicates (F=0.34 P=0.74). In addition, there was no significant difference between the MIC's of the three replicates (F=0.33 P=0.72). Mint from Egerton University has EOS that can be extracted using distillation method. The EOS were bioactive against the selected bacterial pathogens which creates a need for their mass production.

Keywords: Egerton, sensitivity, pathogenic bacteria.

INTRODUCTION

In recent years, multiple drugs resistance has developed due to indiscriminate use of existing antimicrobial drugs in treatment of infectious diseases (Zaidi and Dahiya, 2015). In addition, these antibiotics are sometimes associated with adverse effects in their hosts. This creates a need for alternative antibiotic medicines for the treatment of infectious diseases from other sources such as plants (Huang et al., 2014). Natural products of higher plants may be a new source of antibiotic agents possibly with novel mechanism of action (Sonam et al., 2017).

Antibiotic resistance is one of the most serious health threats that result from selective pressure exerted by antibiotic misuse and abuse (Winanda et al., 2016). The problem has prompted researchers into identification of new biocide with broad spectrum of activity to control these pathogenic bacteria. Traditionally, EOS were used in folk medicine to extend the shelf life of foods. They were also used for treatment of minor ailments besides showing a cooling effect when used on muscle pain nerve relief (Bajera et al., 2017). Plants produce enormous array of functional relevant secondary metabolites (phytochemical) which exhibit diversity of medicinal properties (De Sousa et al., 2015). EOS from several plant species are able to control microorganisms related to skin, dental carries and food spoilage including both gram positive and gram negative bacteria (Diao et al., 2014).

Mint is widely used in food, cosmetics and in pharmaceutical industry (Knezevic et al., 2016). It is chemo

preventive and anti-mutagenic (Ceylan et al., 2014). It has been proven helpful in symptomatic relief of common cold. It also decreases symptoms of irritable bowel syndrome and decrease digestive symptoms such as dyspepsia and nausea. In addition, it is used topically as an analgestic and to treat headache (Hua et al., 2017). It is mosquito repellent and has anti-nematodal, antiviral, antifungal properties (Lan et al., 2016). Thus, the aim of this study was to extract and determine the sensitivity of selected pathogenic bacteria to EOS from mint.

MATERIALS AND METHODS

Study area

Egerton University Main Campus is located 180 kilometers North West of Nairobi and about 30 Kilometers from Nakuru. The University lies on a 144 hectare piece of land donated by Lord Maurice Egerton of Tatton (1874 – 1958). The coordinates of the University are: 0°22'11.0"S, 35°55'58.0"E (Latitude: -0.369734; Longitude: 35.932779). The University lies at an altitude of approximately 2,250 meters above sea level (GoK, 2014).

Collection of plant sample

Mint (*Mentha spicata*) leaves were collected by hand picking from field 7 in Egerton University. The leaves were placed in new sterile polythene bags and transported to Department of Biological Sciences Laboratories, Egerton University, Njoro, Kenya. The samples were stored in refrigerated conditions at 4^{o}C awaiting processing.

Extraction of EOS

Extraction of EOS was carried out using steam distillation method (Martuccia et al., 2015). A sample of 400 g of fresh mint leaves were loaded into 2-litre round bottom flask containing 1.5 litres of water and placed on a heating mantle having a power rating of 450 watts and timed (Figure 1). The samples were boiled with water to release the oil held within the matrix of leaves. The volatile oils evaporated along with the water into the condenser connected to the flask at 100^{o}C. The condensed steam and oils were collected in a separating funnel where the essential oil and water were separated. The water was drained off gently and the oils collected in a 10 mL measuring cylinder and measured. The measurements were recorded at an interval of 15 minutes for 1 h (Nikoli'c et al., 2014).

Phytochemical screening of the EOS

The presence or absence of the phytochemical constituents in the EOS was analysed using standard procedures for tannins, alkaloids, glycosides, flavonoids, resins, phenols and steroids as described by Rajinder et al. (2015).

Test for tannins

About 0.5 mL of the essential oils was dissolved in 1ml of water. Two drops of ferric acid solution were added. A blue or green blue colour indicated a positive test (Zhang et al., 2016).

Test for alkaloids

About 2 mL of the essential oils were dissolved in 2 mL of 2% HCL and heat in a water bath for 10 minutes. Five drops of Meyer's reagent were added to the filtrate of the crude extract. Observation for appearance of turbidity was done (Yang et al., 2018).

Test for glycosides

To 2 mL of the essential oils, 1 mL of glacial acetic acid was added followed by few drops of ferric chloride and concentrated sulphuric acid. Observation for appearance of red brown indicated positive results (Sfeir et al., 2014).

Test for flavonoids

To 2 mL of the essential oils, few drops of concentrated HCL and Mg ribbon was added. Appearance of pink tomato red colour indicated a positive test (Yang et al., 2018).

Test for resins

To 1mL of the essential oils, 1mL of distilled water was added. Presence of turbidity indicated positive results (Costa et al., 2015).

Test for phenols

The essential oils were heated with 4 drops of alcoholic ferric chloride solution. Formation of a blue black or green colour indicated a positive test (Yang et al., 2018).

Test for Steroids

About 1 mL of the essential oils was dissolved in 5 mL chloroform. An equal amount of concentrated sulphuric acid was added from the side of the test tube. For positive

Figure 1. Essential oil distiller.

results, the upper layer turns red and sulphuric acid layer turns yellow with a green fluorescence (Radelli et al., 2015).

Test pathogens

The following test pathogens were used in the study such as *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (clinical isolate), *Escherichia coli* (ATCC 25922), *Erwinia carotovora* (ATCC 33244) and *Xanthomonas campestris* (ATCC 33913).

Preparation of standard inoculum

The test pathogens were inoculated into Muller Hinton Broth (MHB) supplemented with 5% of sheep blood. Incubation was carried out at 37°C for 15 h. The turbidity of the resulting suspension was diluted with MHB to match McFarland standard (Zhang et al., 2017).

Determination of antibacterial activity

The antibacterial activity of essential oils from mint leaves was determined using agar well diffusion method. Sterile Muller Hinton Agar was separately inoculated with the test pathogens using spread plate technique. Wells of 8 mm diameter were made in the agar with a cork borer and filled with the essential oils. Incubation was carried out at 37°C for 24 h. The diameter of zones of inhibition was determined in mm using a ruler. Distilled water was used as negative control while 10 µg ml^{-1} vancomycin was used as positive control (Yang et al., 2018).

Minimum inhibitory concentration

Minimum inhibitory concentration was determined by two-fold serial dilution method. About 1 mL of sterile Mueller Hinton Broth was placed in 11 sterile test tubes. Using a micropipette, 1 ml of the mint EOS was placed in the second test tube. Serial dilution was carried out up to the 11th test tube. 0.1 mL of the standardized pathogens were separately added from the 1st test tube up to the 10th test tube. The 1st test tube was used as the negative control and the 11th test tube as the positive control. Incubation was carried out at 37°C for 24h. Growth was observed by visual inspection and by measuring optical density (OD) at 630 nm using a spectrophotometer after the visual reading (Sonam et al., 2017).

Statistical analysis

Data was presented using frequency tables. The relationship between time and yield of EOS was

Table 1. Percentage yield of essential oils from mint.

Replicate	weight	Distilled water(L)	Heating time (Min)	Temperature (°c)	Yield (%)
1	100	1	15	100	1.0±0.01
2	200	1	30	100	1.5±0.02
3	300	1	45	100	2.5±0.01
4	400	1	60	100	3.0±0.03

Table 2. Phytochemical compounds of essential oils from mint.

S. No	Test	Present/absent
1	Tannins	+
2	Alkaloids	+
3	Glycosides	+
4	Flavonoids	+
5	Resins	+
6	Polyphenols	+
7	Steroids	+

Table 3. Sensitivity tests of bacterial pathogens to mint essential oils.

Pathogen	Zone of inhibition (mm)		
	Replicate 1	Replicate 2	Replicate 3
Staphylococcus aureus	18±0.01	16±0.02	17±0.01
Escherichia coli	15±0.02	13±0.02	14±0.01
Erwinia carotovora	14±0.01	14±0.02	14±0.01
Bacillus subtilis	18±0.02	16±0.02	17±0.01
Xanthomonas campestris.	23±0.01	20±0.02	22±0.01
Klebsiella pneumoniae	20±0.02	20±0.02	20±0.01

F=0.34 P=0.74.

determined using Pearson Correlation. The means of sensitivity test and minimum inhibitory concentrations were compared using ANOVA.

RESULTS

Yield of EOS from mint

The yield of essential oils varied from 1.0±0.01% in the first replicate to 3.0±0.03% in the fourth replicate (Table 1). The heating time ranged from 15 minutes to 60 minutes. There was a relationship between heating period and yield of essential oils (r > 1).

Phytochemical screening of essential oils from mint extracts

The results on phytochemical tests of the extracts are presented in Table 2. All the tested phytochemical compounds were present in the essential oils of mint.

Antibacterial activity

The zones of inhibition in *S. aureus* varied from 16±0.02 mm in replicate 2 to 18±0.01 mm in replicate 1, *E. coli* (13±0.02 mm in replicate 2 to 15±0.02 mm in replicate 1), *E. carotovora* (14±0.01 mm in replicate 1 and 3 to 14±0.02 mm in replicate 2), *B. subtilis* (16±0.02 mm in replicate 2 to 18±0.02 mm in replicate 1), *X. campestris* (20±0.02 mm in replicate 2 to 23±0.01 mm in replicate 1) and in *K. pneumoniae* (20±0.01 mm in replicate 3 to 20±0.02 mm in replicate 1 and 2) (Table 3). There was no significant difference between the zones of inhibition of the three replicates (F=0.34 P=0.74) (Figure 2).

Minimum inhibition concentration of the EOS from mint

The minimum inhibitory concentration in *S. aureus* varied from 0.3±0.02 mg/mL in replicate 2 to 0.4±0.02 mg/mL in replicate 1, *E. coli* (0.4±0.02 mg/Ml in replicate 2 to 0.5±0.02 mg/mL in replicate 3), *E. carotovora* (0.5±0.01

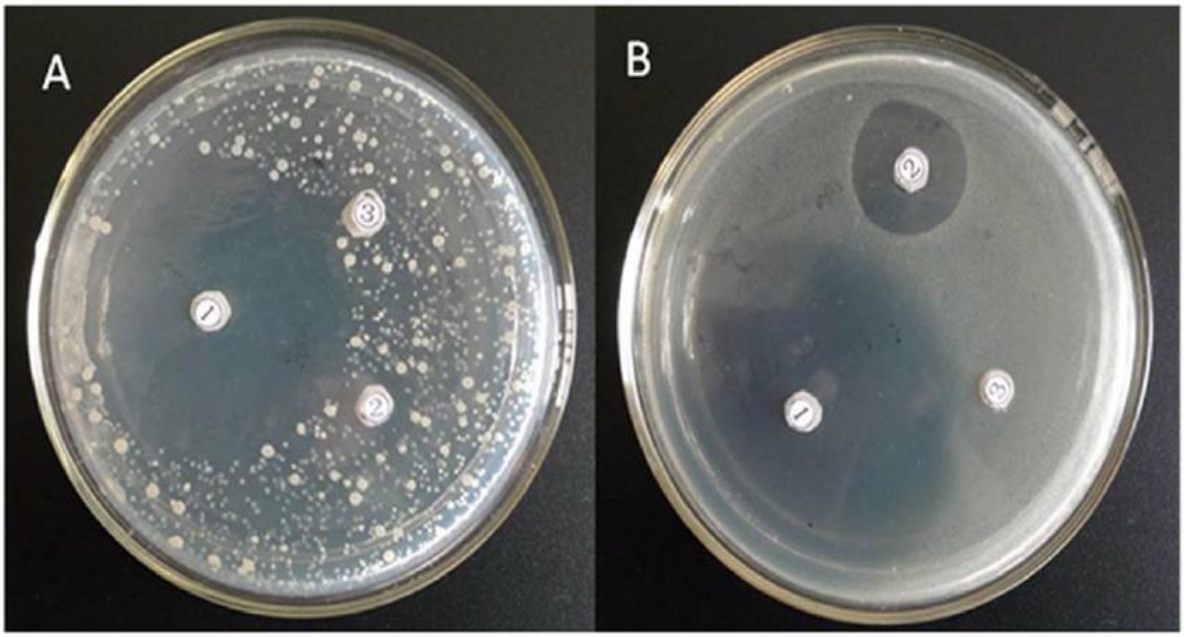

Figure 2. Zones of inhibition for *Staphylococcus* (A) and *E. coli* (B).

Table 4. Minimum Inhibitory Concentration (MIC) of essential oils from mint when tested against selected bacterial pathogens.

Type of bacteria	MIC (mg/ml)		
	Replicate 1	Replicate 2	Replicate 3
Staphylococcus aureus	0.4±0.02	0.3±0.02	0.4±0.01
Escherichia coli	0.5±0.01	0.4±0.02	0.5±0.02
Erwinia carotovora	0.5±0.01	0.5±0.01	0.5±0.02
Bacillus subtilis	0.7±0.02	0.6±0.02	0.6±0.01
Xanthomonas campestris	0.4±0.02	0.4±0.02	0.5±0.02
Klebsiella pneumoniae	0.3±0.01	0.4±0.01	0.4±0.01

F=0.33 P=0.72.

mg/mL in replicate 1 and 2 to 0.5±0.02 mg/mL in replicate 3), *B. subtilis* (0.6±0.01 mg/mL in replicate 3 to 0.7±0.02 mg/mL in replicate 3), *X. campestris* (0.4±0.02 mg/ML in replicate 1 and 2-0.5±0.02 mg/mL in replicate 3) and in *K. pneumoniae* (0.3±0.01 mg/ML in replicate 1 to 0.4±0.01 mg/mL in replicate 2 and 3) (Table 4). There was no significant difference between the MICs of the three replicates (F=0.33 P=0.72).

DISCUSSION

The induction period for extraction of essential oils was between 0 to 15 minutes. Induction period, is the time required to rupture the cells of the plant material in order to release the oil and transport it to the condenser (Si et al., 2016). The yield of essential oil increased with time. These results agreed with a previous study carried out in Brazil (Radaelli et al., 2015). The similarity in results in the two studies may have been caused by the two studies being carried out in similar environments (Babar et al., 2015).

However, the results of this study demonstrated that the essential oils from mint were dominated by tannins, alkaloids, glycosides, flavonoids, resins, phenols and steroids. This was contrary to findings by Plant and Stephens (2015), since flavonoids and steroids were absent in the latter study. The difference in results may have originated from differences in soil physicochemical characteristics which the plants were growing in. According to El Babili et al. (2014), the soil constituents influence the phytochemical accumulated by mint.

The antibacterial activity of mint was assessed using agar well diffusion method by measuring the diameter of

growth inhibition of the test pathogens by the essential oils from mint (Hercules and Chrissanthy, 2017). Gram positive bacteria are known to be more susceptible to essential oils than gram negative due to variation in the structure of the cell wall (Lang and Buchbauer, 2012). However, the results of this study differed with this observation since there was no significant difference in the zones of inhibition between gram positive and gram negative bacteria (Freires et al., 2015). These results disagreed with a previous study by Aishwarya (2015). This may have arisen from differences in the composition in the essential oils produced by the plant samples (de Aguiar et al., 2018).

However, the minimum inhibitory concentrations of the essential oils obtained in the current study (Table 4) partially agreed with a study carried out in Mauritius (Aumeeruddy-Elalfi et al., 2015). This may have been caused by similarity of the active ingredients of the essential oils in the two studies. In addition, Shaaban et al. (2014) asserted that strains of the test pathogens under investigation influence the minimum inhibitory concentration of an antibiotic. Huerta et al. (2016) attributed minimum inhibitory concentration of essential oils from mint to the evolutionary stage of test pathogens.

Conclusions

Mint from Egerton University has essential oils that can be extracted using distillation method. The essential oils inhibited growth of the selected bacterial pathogens.

Recommendations

The essential oils from mint should be extracted in large scale. There is need to determine the mechanism through which the essential oils inhibit growth of pathogenic microorganisms. In addition, sensitivity test of fungal pathogens to the essential oils need to be carried out.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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