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Fractionation of Essential Oils and the Effect of Various Fractions on *Salmonella typhi* and *Staphylococcus aureus*

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Abstract: The sensitivity potential of the bacteria *Salmonella typhi* and *Staphylococcus aureus* to *Eucalyptus citrodora* (Lemon) and *Eucalyptus camadulensis* (menthol) fractions was investigated. The whole extract samples were first evaluated for anti-microbial properties using the cup plate method. The whole oil extract samples showed high antimicrobial activity on the *Salmonella typhi* and *Staphylococcus aureus*. The whole extracts were then fractionated and each fraction was collected at its boiling point range. The sensitivity potential of the test organisms was then carried out in these fractions collected. The results obtained indicated that *Salmonella typhi* is highly sensitive to the fraction 3 (bpt) 152-154°C of *Eucalyptus camadulensis* and moderately sensitive to all the other fractions of *Eucalyptus camadulensis*. However all the fractions obtained from *E. citrodora* except fraction (I) and the residual extract were very effective on the *Salmonella typhi*. Meanwhile *Staphylococcus aureus* showed very high sensitivity to all the fractions obtained from the two whole extracts of *E. citrodora* and *E. camadulensis*.

Key words: Fractionation, essential oils, *Salmonella typhi*, *Staphylococcus aureus*

INTRODUCTION

Essential oil is the volatile oil produced by steam, or water distillation of vegetable plant materials. The vapours are condensed to yield a water condensate and an essential oil that can be separated off, usually by gravity. The essential oil, may be rectified in order to achieve a specific distinctive odour effect (Burfield, 2000). Essential oils are a complex mixture of hundred of chemical ingredients. This complex can be broken into simpler component or single separated chemical products, which are raw material for further industrial transformation and these industrial chemical ingredients are used as building blocks to introduce a particular flavour or aroma into another product, such as pharmaceutical products, perfumes and toiletries, hygiene products and pesticides. Eucalyptus oil is also reported by some researchers (Bienvenu, 2003; Burfield, 2000) to possess therapeutic effect and is used in pharmaceutical preparations and aroma therapy.

Man had used various parts and extracts of plants and herbs as antimicrobial agents from earliest times (Al-Shamma and Mitcher, 1979). In the continued search for antimicrobial agents from plants, Kubo *et al.* (1992) reported that the steam distillation of both fresh green olive leaves and commercial olive oils were found to exhibit a broad spectrum of activity against some micro organisms. A great number of antimicrobial agents from plant sources already exist for various purposes and applications (Nwaeze *et al.*, 1986). However, the continued search for new ones should be a sustained process, since the target microorganisms sometimes often evolve into new genetic variants, which subsequently become resistant to existing agents (Udeh *et al.*, 2001).

The anti microbial and the antibacterial activities have been reported on the methanolic extract of *Eucalyptus* species (Babayi *et al.*, 2004; Mehraban *et al.*, 2005; Rogerio *et al.*, 2004); there has been little or no documented scientific evidence on their Fractionated samples on the test organisms.

Eucalyptus citrodora and *Eucalyptus camaldulensis* are abundant in tropical West Africa including Nigeria and have a lot of applications in traditional and herbal medicine. The genus, *Eucalyptus* contains more than 400 species and only few of these are used to produce *Eucalyptus* oil (Bienvenu, 2003).

Although antimicrobial effects of *E. citrodora* and *E. camaldulensis* have been reported, there has been no report on fraction of these oils and their effect on the microorganisms. Thus the aim of this study is to fractionate the oil into their various components and test the same on the *Staphylococcus aureus* and *Salmonella typhi*.

MATERIALS AND METHODS

Stock Cultures

Pure isolates of test microorganisms *Salmonella typhi* and *Staphylococcus aureus* were obtained from the department of Microbiology, Ahmadu Bello University, Zaria. These organisms were preserved on sterile nutrient agar and kept at 4°C in a refrigerator pending analysis.

Whole Extract/Fractionated Oil Samples

The *Eucalyptus citrodora* and *Eucalyptus camaldulensis* leaves were collected from *Eucalyptus* plantation, Samaru Zaria. Essential oils were obtained from the fresh leaves of *Eucalyptus citrodora* and *Eucalyptus camaldulensis* through simple distillation. The whole extract obtained was then separated into different fractions using a glass fractional distillation column. Each fraction was collected at its different boiling point. The experiment was carried out at National Research Institute for Chemical Technology, Zaria, Kaduna State, Nigeria.

Sensitivity Potential Tests

Prior to commencement of the sensitivity potential tests, *Staphylococcus aureus* and *Salmonella typhi* were subcultured from the sterile nutrient agar slants into sterile nutrient broth and incubated at 37°C for 24 h. The antimicrobial sensitivity test was carried out using the cup plate method described by Rogerio *et al.* (2004). Nutrient agar was used as the medium. Twenty eight gram per liter of the agar was autoclaved along side with the petri dishes at 121°C for 15 min (Cheesbrough, 1984). Sterile plates containing the nutrient agar were aseptically swabbed with a loopful of the test organisms using a sterile drug cotton swab. Wells were then punched into the agar with the aid of sterile cork borers. The wells measured 3 mm (internal diameter) and were 2.5 mm deep. The wells were carefully made without disturbing the medium. Three drops of the essential oil (fractions) were then put inside the wells using a sterile Pasteur pipette. The plates were incubated at 37°C for 24 h and the zones of inhibition were measured with a transparent meter rule. The same procedure was followed with the different essential oil fractions. The experiments were carried out in triplicates. Control experiment with the whole extract was carried out.

RESULTS AND DISCUSSION

There are at least not less than 10 fractions obtained from *E. camaldulensis* (Table 1). The results of this investigation revealed that both bacteria are highly susceptible to the whole extract of *Eucalyptus citrodora* and *Eucalyptus camaldulensis*. This is in support with what was reported on the methanolic extract of these plants on *Staphylococcus aureus* and *Salmonella typhi* by Babayi *et al.* (2004). Also, Udeh *et al.* (2001), reported that *Staphylococcus aureus* showed indications of high susceptibility to the oil of *Eucalyptus citrodora* even at high dilutions of the crude oil so also Lemon

Table 1: The inhibitory effect of different fractions of *Eucalyptus camaldulensis* (Menthol) oil on *Salmonella typhi*

Fractions	Boiling point (°C)	Zones of inhibition after 24 h of incubation at 37°C
1	76-78	33.30±2.90
2	80-150	28.75±1.77
3	152-154	No growth
4	154-158	66.25±1.77
5	160-162	62.67±10.79
6	164	60.83±7.15
7	166	45.33±14.50
8	168	29.00±15.22
9	169	40.33±10.50
10	170	50.50±7.78
Residual extract	Residual extract	14.00±0.71
Whole extract	Whole extract	54.83±9.12

Between 80-148°C, No fraction was collected; Whole extract: The whole oil extracted; Residual extract: The oil remaining after the fractionation

Table 2: The inhibitory effect of different fractions of *Eucalyptus citriodora* on *Salmonella typhi* after 24 h

Fractions	Boiling point (°C)	Zones of inhibition (mm)
1	98	52.5
2	186	No growth
3	196	No growth
4	198	No growth
5	202	No growth
6	203	No growth
7	204	No growth
8	206	No growth
9	Residual oil	44.5
10	Whole extract	No growth

Whole extract: The whole oil extracted; Residual extract: The oil remaining after the fractionation

Table 3: The inhibitory effect of *Eucalyptus camaldulensis* (different fractions) on *Staphylococcus aureus* after 24 h

Fractions	Boiling point (°C)	Zones of inhibition (mm)
1	76-78	No growth
2	80-150	No growth
3	152-154	No growth
4	154-158	No growth
5	160-162	No growth
6	164	No growth
7	166	No growth
8	168	No growth
9	169	No growth
10	Residual oil	No growth
11	Whole extract	No growth

Whole extract: The whole oil extracted; Residual extract: The oil remaining after the fractionation

grass (*Cymbopogon citratus*). It was also observed that some of the oils are highly inhibitory such that they inhibit growth absolutely of the microorganisms after 24 h of incubation. The extracts obtained from these plants possess a low or moderate inhibitory effect to growth of the test organisms. The result in Table 1 shows that, *Salmonella typhi* is remarkably susceptible to fraction 3 (Boiling point 152-154°C) of the *Eucalyptus camaldulensis*. This suggests that fraction 3 contains the main active component of the oil responsible for its antimicrobial property on *Salmonella typhi*.

On the other hand, *Staphylococcus aureus* is highly susceptible to all the fractions of *Eucalyptus camaldulensis*. The entire fractions and the whole extract of *Eucalyptus camaldulensis* are highly inhibitory to the growth of *Staphylococcus aureus* (Table 3). However, the oil fractions and the whole of *Eucalyptus citriodora* showed very high inhibitory effect on *Salmonella typhi* and *Staphylococcus aureus* tested (Table 2 and 4). It was also recorded that the residue of the fractions of

Table 4: The inhibitory effect of different fractions of *Eucalyptus citriodora* on *staphylococcus aureus* after 24 h

Fractions	Boiling point (°C)	Zones of inhibition (mm)
1	98	No growth
2	186	No growth
3	196	No growth
4	198	No growth
5	202	No growth
6	203	No growth
7	204	No growth
8	206	No growth
9	Residual oil	1.8
10	Whole extract	No growth

Between 80-148°C, No fraction was collected; Whole extract: The whole oil extracted; Residual extract: The oil remaining after the fractionation

E. citriodora showed little or no inhibitory effect. These observations tend to suggest that the fractions of these oils (except the residue) possess antibacterial properties (high inhibitory effect) on *Salmonella typhi* and *Staphylococcus aureus* and thereby, these organisms are highly sensitive to the fractions obtained from *Eucalyptus citriodora* and *Eucalyptus camaldulensis*. The present study has demonstrated that the oils either in their whole form or at their different fractions have significant inhibitory effect on the test organisms.

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