A quantitative in vitro comparison of essential oil bactericidal effects: a comparison of manufacturers.

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ABSTRACT

OBJECTIVE: To discover if the price of an essential oil makes a quantifiable difference in the effective quality of the oil.

METHODS: Ten essential oil companies were selected, based primarily on reputation and secondarily based on price. The ten companies are as follows: Essential Oil University, Anatolian Treasure (by Appalachian Valley Natural Products), Nature's Gift Aromatherapy Products, Aura Cacia, Liberty Natural Products, Mountain Rose Herbs, Young Living, Uncle Harry's Natural Products, Original Swiss Aromatics, Elizabeth Van Buren. Five known antibacterial oils (clove, cinnamon, eucalyptus, tea tree, and oregano) were bought from each company, and were tested against three bacteria (s. aureus, s. pneumoniae, and e. coli). The diameter of growth inhibition after 60 hours of incubation was then measured and compared.

RESULTS: The more affordable oils were not in any way outperformed by the expensive oils.

CONCLUSION: The price of an essential oil has no correlation with its effective bacteriocidal properties

INTRODUCTION

"Essential oils" have been used for millennia for the treatment of disease. In the modern day, numerous companies exist which claim that their oils are superior to competitors. It is not uncommon to find companies that sell their product for over ten times the cost of their competitors for the same oil. But is this inflated price really worth it? Is the price inflated to yield higher dividends? This study seeks to get to the bottom of this question and objectively find out which oil has the best value based upon price and effectiveness. As public usage of essential oils increases, it is not uncommon for consumes purchase oil based on advertisement. This makes the comparison of quality and cost useful and appropriate for today's consumers.

Multiple companies exist which produce varying qualities of essential oils, and many of them claim that their product is "top quality" or "the best," however, the consumer never truly knows the real quality of the oil. The oils can easily be cut with almond or olive oil and the consumer would never know. Also, extraction and storage mechanisms vary from company to company, and some methods may promote faster oxidation or oil degradation than other methods. And lastly, some companies sell the same quantity of specific oil at a cost that as much as ten times that of another company. As a result, the big question remains: Is the high-priced oil really worth the cost?

The objective of this study is to quantitatively discover which company actually provides the best product at the lowest cost. The quantitative analysis focuses on specific oils that are known to be antibacterial (i.e. oregano, cinnamon, rosemary, lemongrass, etc). With a micropipet, a designated amount of oil was dropped onto a small sterile disc and placed on a nutrient agar petri dish that had swabbed with one of three specific bacteria. For variation, multiple antibacterial oils from each company will be tested, and multiple bacteria will be used. The experimenters will be blinded, and each trial was repeated three times. The diameter of the zone of inhibition was measured at its widest point with a micrometer.

While it is difficult to quantitatively assess the quality of most oils, the oils that are specifically antibacterial can be objectively studied. The results of this study would provide a summary of which company produces the top quality product for the money.

METHOD

Companies

The following essential oil companies were chosen as participants in this study: Young Living, Uncle Harry's, Aura Cacia, Liberty Natural Products, Elizabeth van Buren, Mountain Rose Herbs, Original Swiss Aromatics, Essential Oil University, Nature's Gift, and Appalachian Natural Products. The companies were chosen primarily on the basis of popularity, however, it was important in this study to compare oils of a wide variety of price ranges. The types of oils chosen for the study were selected based on versatility and previously published research proving the efficacy of the oil as related to antibacterial, antimicrobial, or antifungal properties [2,3,5,6]. As the demand for alternatives to antibiotics increases, there is the potential for essential oils to take a place in the treatment of infections [1,4,7,8].

The Apparatus

The materials used include the following: essential oils, 450 (85mm) petri dishes, nutrient agar, micropipette (1-200 µl), 150 micropipette tips, 450 ¼" blank discs, 450 cotton swabs, Bunsen burner, tweezers, 50ml pipette, incubator, micrometer and three bacterial strains: *E. coli*, *S. aureus*, and *S. pneumoniae*

Procedure

Five antibacterial oils were bought from each company: *Cinnamomum zeylanicum* (cinnamon leaf), *Eugenia aromatical* (clove bud), *Eucalyptus globulus* (eucalyptus), *Origanum vulgare* (oregano), and *Melaleuca alternifolia* (tea tree). Young Living did not have cinnamon leaf, but only cinnamon bark, which the experimenters felt was comparable enough to the other manufactures' oils to provide validity with testing. It was important to check the Latin names when ordering because there are a few different species of some of the plants. The oils were all ordered on the same day within a few hours of each other, and the companies all shipped the oils almost immediately after ordering. All of the oils are ordered at the same time to avoid any possibility that old oils might degrade. The oils were kept sealed until the day of the experiment. 50 separate oil vials were used (five different oils from ten different companies), and each oil was tested three times with three different bacteria. Therefore, a total nine petri dishes were used for each of the 50 oils, which added up to 450 total petri dishes. It was crucial to not let the companies know about our study, to avoid receipt of oils enhanced or altered by the company, this allows the experimenters to reflect the quality of the oil bought by the average consumer.

On the day of the experiment, nutrient agar was prepared as per the manufacturer's directions, autoclaved at 121oC for 15 minutes, pipette into each of the 450 petri dishes, sufficient enough to cover the bottom of the dish. A 25ml pipette was used so that the amount of agar in each plate was the same at 10ml. The agar set for 20 minutes to cool and solidify. The plates were labeled with previously determined abbreviations, as follows: <bacteria>, <company number>, <trial number>, <oil number>; such that "A 1-1 t" would indicate *S. aureus*, Essential Oil University, trial 1, tea tree oil. The cultures of bacteria were gram stained and examined under a microscope to confirm the presence of the expected bacteria and the absence of contamination in each culture. *S. aureus* is gram-positive and the bacteria were round typically forming clusters. *S. pneumoniae* is gram-positive and exists as rods. *E. coli* is gram negative and exists as rods typically in a chain of two or more. Each culture was found to be uncontaminated and gram stained as expected.

With proper sterilization procedure, the three different bacteria were inoculated and swabbed onto the corresponding petri dishes. One sterile cotton applicator was used with each inoculation and the plates were streaked for lawn growth. Eleven blank ¼" cotton discs were placed on an empty, sterile petri dish and 12-µl of essential oil was dropped on each blank disc. To help reduce any errors in the precision of the micropipette, a visual inspection of the 11 discs were made, and two discs were discarded: the disc that appeared the "most saturated" and the disc that appeared the "least saturated" relative to the other discs. Using sterilized tweezers the disc was then placed on the appropriate inoculated petri dish. The plates were then collected and incubated at 37 degrees Celsius for approximately 60 hours. After an incubation period of around 60 hours, the minimum diameter of inhibited growth was measured in millimeters using a micrometer. To keep consistency in the measuring technique, one person measured all 450 trials.

The above procedure was the second trial of this experiment. A month prior, the first trial was made, however the lack of consistency in the results made it impossible to draw any conclusions. Several significant errors in the initial trial were made and will be outlined here. The agar amount was not measured and therefore each plate varied possibly by as much as 10ml of agar. The agar was poured out of a beaker so that it covered the bottom of the petri dish. This may not have made much in the bacterial growth, however it could have possibly affected the distance an oil could possibly diffuse through the agar. A second major error was that the blank disc was placed on the inoculated petri dish before the oil was dropped on the disc. This gave the blank disc time to soak up liquid from the inoculated agar, thus preventing the disc from soaking up the entire volume of oil used. As a result of this mistake in the procedure, the oil was more likely to spread through the agar, rather than stay isolated on the disc. The only other difference between the two trials was that 10 µl of oil was used the first time, whereas 12 µl was used the second time.

RESULTS

Contrary to expectations, there was no correlation between the price of the oil and bacterial growth inhibition. In nearly every case, with the exception of a few cases with the eucalyptus trials, the standard deviation was under 1.5.

Table I. Trial 1, Growth Inhibitions (in millimeters)

Company	Oregano	Clove Bud	Cinnamon Leaf	Eucalyptus	Tea Tree	Overall Average
Essential Oil University	52.1	28.1	26.1	8.7	14.3	25.9
Anatolian Treasures	49.8	26.2	28.5	18.0	17.0	27.9
Nature's Gift	47.7	26.8	25.3	16.5	15.4	26.3
Aura Cacia	50.9	28.2	27.1	7.2	17.2	26.1
Liberty Natural	49.9	32.1	25.2	11.1	19.7	27.6
Mountain Rose	66.5	41.2	26.2	11.3	18.8	32.8
Young Living	54.0	37.9	43.6	8.0	18.1	29.0
Uncle Harry's	55.9	32.1	23.5	8.8	17.8	27.6
Original Swiss Aromatics	39.1	31.2	25.0	15.8	17.1	25.6
Elizabeth Van Buren	58.9	31.1	27.7	12.9	17.1	29.5

Table II. Trial 2, Growth Inhibitions (in millimeters)

Company	Oregano	Clove Bud	Cinnamon	Eucalyptus	Tea Tree	Overall

			Leaf			Average
Essential Oil University	44.1	21.0	20.6	8.3	14.2	21.6
Anatolian Treasures	35.3	21.0	18.7	18.4	20.0	22.7
Nature's Gift	38.9	21.4	18.4	15.8	20.4	23
Aura Cacia	42.6	19.3	20.8	8.7	19.4	22.2
Liberty Natural	43.0	20.7	20.4	12.9	21.6	23.7
Mountain Rose	43.3	21.9	20.1	11.2	22.0	23.7
Young Living	37.7	22.0	46 (bark)	9.2	20.0	21.6
Uncle Harry's	40.2	20.9	20.0	7.3	20.6	21.8
Original Swiss Aromatics	28.9	21.4	19.7	14.3	17.2	20.3
Elizabeth Van Buren	39.4	20.9	19.7	13.8	20.2	22.8

Table III. Average price/ounce.

Company	Price/Ounce
Essential Oil University	$4.56
Anatolian Treasures	$15.60
Nature's Gift	$20.11
Aura Cacia	$17.33
Liberty Natural	$7.49
Mountain Rose Herbs	$10.50
Young Living	$33.89[1]
Uncle Harry's	$16.00
Original Swiss Aromatics	$31.86
Elizabeth Van Buren	$28.76

DISCUSSION

There are numerous methods for determining the quality of essential oils. While many may seek to extract specific alkaloids to determine the concentration of the constituents, we chose to measure the quality in a more practical and cost-effective manner. In theory, an oil could be more "concentrated", but still prove to have lesser effects when used practically. To the consumer, one would be more concerned with how well an oil performed from a practical standpoint as opposed to the laboratory analysis of the oil (such as with IR spectroscopy).

One would expect consistency when comparing oil potencies with different bacterium. For example, if company A had the strongest bactericidal effects against S. aureus, then it would be expected that company A would also prove to be the best against the other S. pneumoniae and E. coli . Our experiment proves that this is not the case. This leaves only two possibilities: (1) the micropipette lacked precision or (2) each company's version of the same oil contains a slightly different array of alkaloids, such that one oil might have alkaloids that inhibit the growth of staphylococcus. while that oil has fewer of the alkaloids that are more likely to inhibit the growth of streptococcus. The first option is a very real possibility, especially when considering the tiny volume of oil that was used. When dropping the oil using the micropipette tips, oil could collect on the outside or inside of the tip, thus causing each drop to be slightly different in its volume.

It can be concluded that if the three measurements were within just a few millimeters, then there was excellent consistency with the laboratory procedures. However, if the standard deviation was above 2, then likely those trials lacked consistency and thus carries much less weight.

When analyzing the results, it would first be expected that the most potent oil would be the most effective against all three bacteria; however this was not the case. With both cinnamon and clove trials, all the oils were approximately the same in their bacterial growth inhibition. Since Young Living does not offer cinnamon leaf, we decided to buy their cinnamon bark, which far outperformed any other oil. Unfortunately, most of the other companies do not offer cinnamon bark; therefore,

[1] Cinnamon is not included in this calculation companies that provide, since the cinnamon bark is about five times as much as cinnamon leaf.

Young Living's cinnamon cannot be used in the comparison. Eucalyptus oil was the most problematic. In many cases, the oil had two inhibition rings, an inner ring that inhibited nearly 100% of growth and an outer ring that inhibited around 50% of the growth. And frequently, the inner ring was not more than a millimeter away from the disc. It was more difficult to get precise results. Because of the variability in the eucalyptus measurements, it should not really be taken into consideration.

Overall, there is not one company that stands above the other companies, with respect to either the worst performance or the best performance. As a result of the effective oil potencies being very similar, when buying essential oil, one should not take "quality" into consideration. Rather, price should be the primary concern. Therefore, companies offering expensive oils, do so without having superior quality oils. Therefore, the three companies that should be avoided would include Young Living, Original Swiss Aromatics, and Elizabeth Van Buren. Whereas, Essential Oil University, Liberty Natural, and Mountain Rose Herbs are the three the cheapest oils price/ounce. Interestingly, in the overall averages of growth inhibition, the two top companies are Mountain Rose Herbs and Liberty Natural. It does appear worthwhile to note that although the initial trial was problematic in its procedure, Mountain Rose Herbs still performed the best in both trials.

Sources of potential error

The largest error was made in the first trial, when the cotton discs were placed on the agar well before (sometimes up to 10 minutes) the oil was dropped on the disc. Then when dropping the oil on the disc, the disc had more trouble soaking up the oil because it was already partially saturated from condensation that the disc was sitting on. As a result some of the oils then oversaturated the disc and seeped into the agar. In these cases, not only could the viscous properties of the oils themselves potentially have some impact on the outcome, but also the thickness of the agar would likely affect the distance of penetration of the oils into the media.

The micropipette could potentially be a source of error. The same tip was used nine times for each consecutive trial of the same oil. Using such small amounts of oil (only 12 microliters) makes it impossible to get all the oil out of the pipette tip, usually leaving a tiny amount in the tip (or on the outside of the tip). Therefore, with each consecutive trial, up to 10% more oil is used on the disc. Even if only each tip were used once and then discarded, the accuracy of dealing with such small quantities of oil might still around +/- 10%, since it remains difficult to get all 12 microliters of oil out of the tip.

Stronger antibacterial oils (mainly oregano) inhibit the growth so much such that the zone of inhibition is sometimes difficult to determine. With such cases, the dish had to be held up to the light and pen marks were then made where this zone could be found.

Weaker antibacterial oils (mainly eucalyptus) often did not even have a clear zone of inhibition, but rather there existed a zone of reduced growth. Because of the variability and inconsistency with eucalyptus, it would be better to not consider this oil in the final company comparison.

Since the oils are all very aromatic, the airborne oil particles could have an inhibitory effect on the growth of the bacteria, which is likely what took place in the oregano dishes.

Our method of testing the antibiotic properties of oils could possibly be skewed. The oil can diffuse into the agar, and the inhibition of bacterial growth could possibly be from the physical properties of the oil (i.e. the viscosity) rather than antibiotic properties. For example, the oils in our skin create a physical environment that is not suitable for growing many bacteria, however, these oils are not necessarily "antibiotic." A better experiment to actually test the quality of the antibiotic properties of the oils would be to culture the bacteria in a test tube, with a drop of oil; then the colonies could be counted a day later.

Theoretical sources of variability in essential oil potency

The most obvious source of variability would originate from the quality and potency of the herb source that the production company makes their oils from. One batch or harvest of plants could have been injured and the company could have suffered from a temporary reduction in quality of the final product. However, if this were really the case, then the entire production of that specific herb would be reduced for the time period it took to use up that herb which could be months or longer, depending on how much the company made/sold.

The location the herbs are obtained could be another variable. While the location is likely to be fixed, it could be that the source of the herbs may be from various locations throughout the world in a single year, depending on the growth seasons of each herb.

The amount of time between production/manufacturing and shipping of the product to the customer may change the potency of the oil.

A company (or distributor) could easily cut or dilute the essential oil with a cheaper and less aromatic oil, such as almond oil or olive oil. If this were the case, then the price should go down accordingly.

The problem with these uncontrollable variables, is that they could lead one to believe that the specific product is not worth the money, when that same product might be far superior several months down the road.

Potential future experiments

There are numerous possible considerations for future experiments. The first suggestion would be to invest in a much more expensive micropipette that focuses in on the volume range between 5 and 20 microliters. Then one could perform three trials, one with 5 microliters, one with 10, and the third with 15 microliters. If the measurements were truly precise then all three trials should show consistency in their comparisons of different oils.

Also, one could compare dropping the oil on to a disc versus just dropping the oil onto the agar itself. If the latter experiment were done, special care would be needed to ensure that the exact same quantities of agar were poured into each plate.

To measure the true antibacterial properties of the oils a drop of oil should be added to a test tube bacterial culture and then the number of colonies should be counted after a set time period.

To further confirm our findings that all companies produce approximately the same quality of essential oil, the best experiment to perform would be to cut an oil with different amounts of almond oil (or some other oil) and compare the growth inhibitions of the cut oil. Then, for example, if 100% oregano produced the same growth of inhibition as the 50% (cut) oregano, then our conclusion would be faulty. However, if the cut oregano only inhibited the growth half as much (assume a linear relationship), then it would further confirm the findings of this experiment.

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About the oils

Cinnamomum Zeylanicum (cinnamon leaf), *Eugenia aromatical* (clove bud), *Eucalyptus globulus* (eucalyptus), *Origanum vulgare* (oregano), and *Melaleuca alternifolia* (tea tree).

Cinnamon

Botanical Name: Cinnamomum Zeylanicum
Common Method Of Extraction: Steam distillation
Parts Used: Leaves and Twigs
Note Classification:
Aroma: warm, spicy, musky
Largest Producing Countries: Sri Lanka, India, Madagascar

Clove Bud Essential Oil

Botanical Name: *Eugenia aromatica*
Common Method Of Extraction: Steam distilled
Parts Used: Unopened dry flower buds
Note Classification: Middle

Aroma: Warm, spicy, and woody odor with a subnote of leather
Largest Producing Countries: Sri Lanka, Indonesia, and Madagascar

Eucalyptus Essential Oil

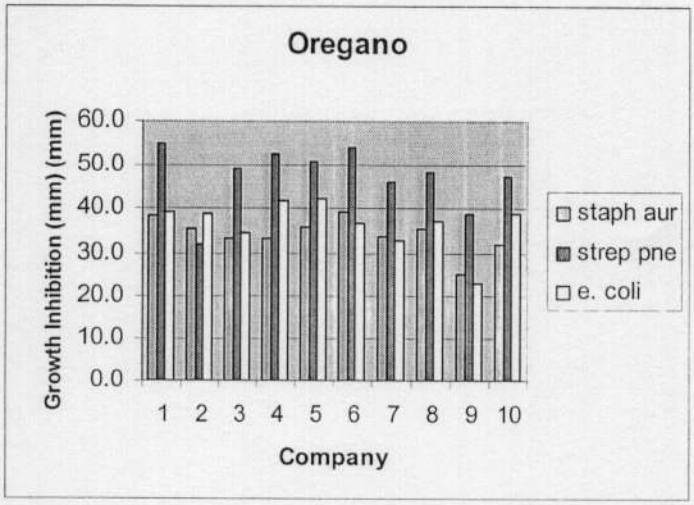
Botanical Name: *Eucalyptus globulus*
Common Method Of Extraction: Steam distilled
Parts Used: Leaves and twigs
Note Classification: Top
Aroma: Strong, woody camphory
Largest Producing Countries: Australia, Spain, Portugal, Brazil, Russia, USA, and China

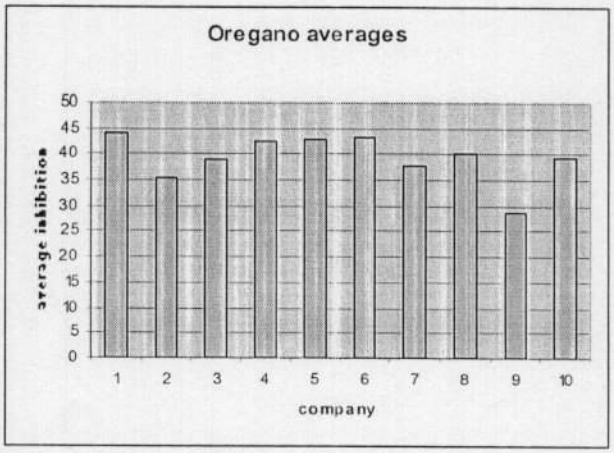
Oregano Essential Oil

Botanical Name: *Origanum vulgare*
Common Method Of Extraction: Steam distilled
Parts Used: Dried flowering herb
Note Classification: Middle
Aroma: Warm, spicy-herbaceous, and camphoraceous
Largest Producing Countries: USA, Bulgaria, Turkey, Spain and Italy

Tea Tree Essential Oil

Botanical Name: *Melaleuca alternifolia*
Common Method Of Extraction: Steam distilled
Parts Used: Leaves and twigs
Note Classification: Middle
Aroma: Warm, Fresh, spicy-camphoraceous
Largest Producing Countries: Australia


Oregano
Growth Inhibition (mm) (mm)
60.0
50.0
40.0
30.0
20.0
10.0
0.0
1 2 3 4 5 6 7 8 9 10
Company
staph aur
strep pne
e. coli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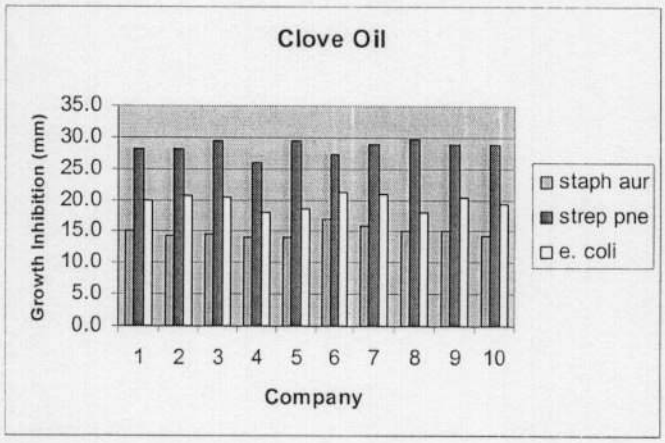
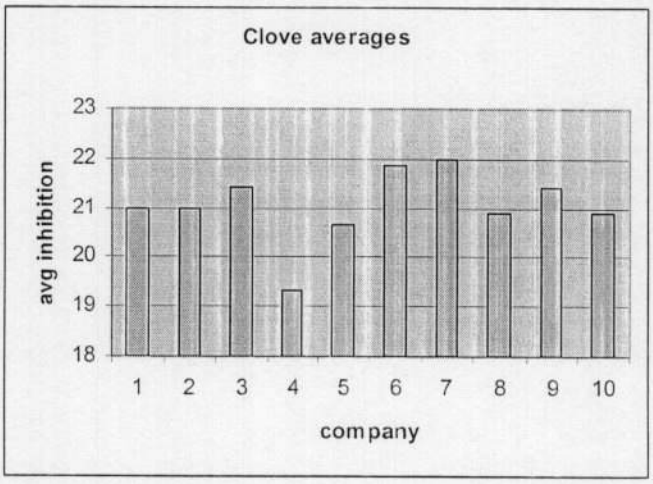
45
40
35
30
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10
company


Clove Oil
Growth Inhibition (mm)
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
1 2 3 4 5 6 7 8 9 10
Company
staph aur
strep pne
e. coli


Clove averages
avg inhibition
23
22
21
20
19
18
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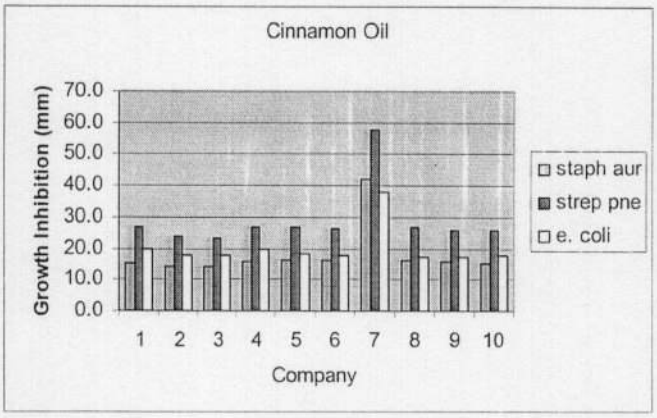
company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Growth Inhibition (mm)
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60.0
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20.0
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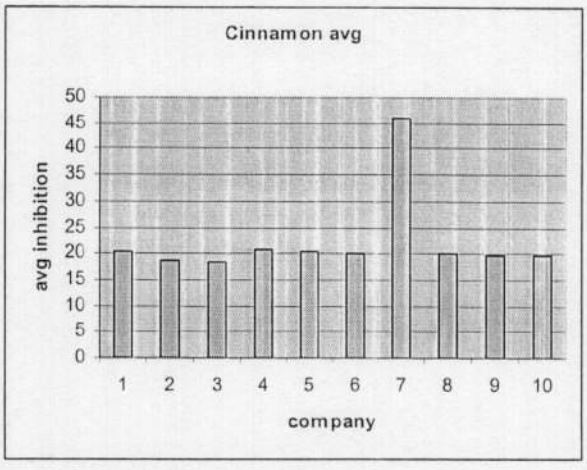
Company
staph aur
strep pne
e. coli


Cinnamon avg
avg inhibition
50
45
40
35
30
25
20
15
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5
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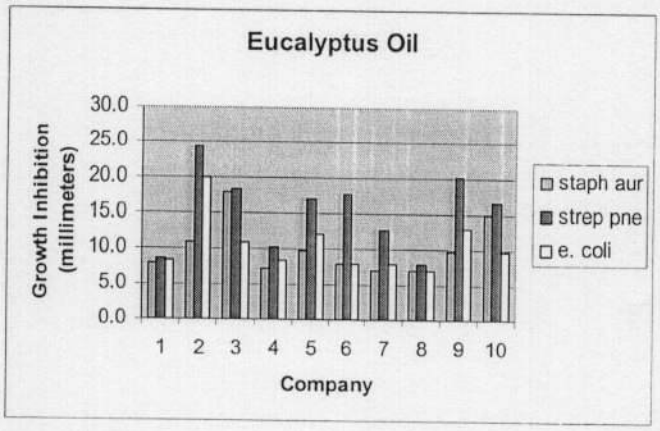
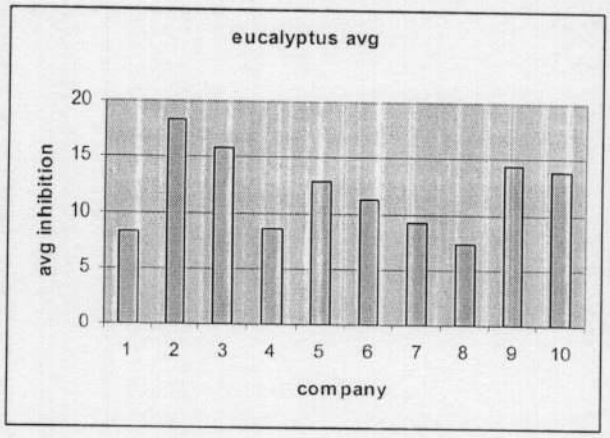
company


Eucalyptus Oil
Growth Inhibition (millimeters)
30.0
25.0
20.0
15.0
10.0
5.0
0.0
1 2 3 4 5 6 7 8 9 10
Company
staph aur
strep pne
e. coli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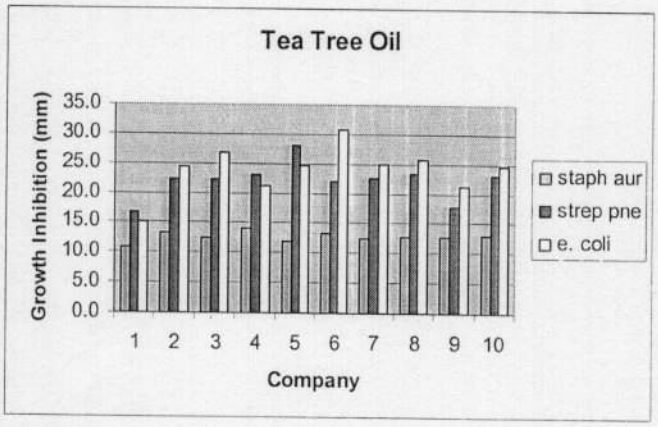
company


Tea Tree Oil
Growth Inhibition (mm)
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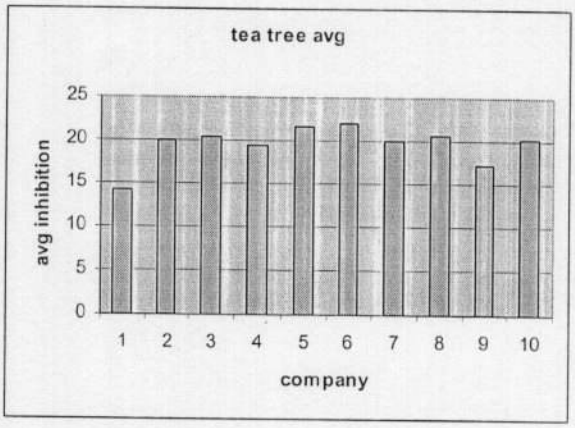
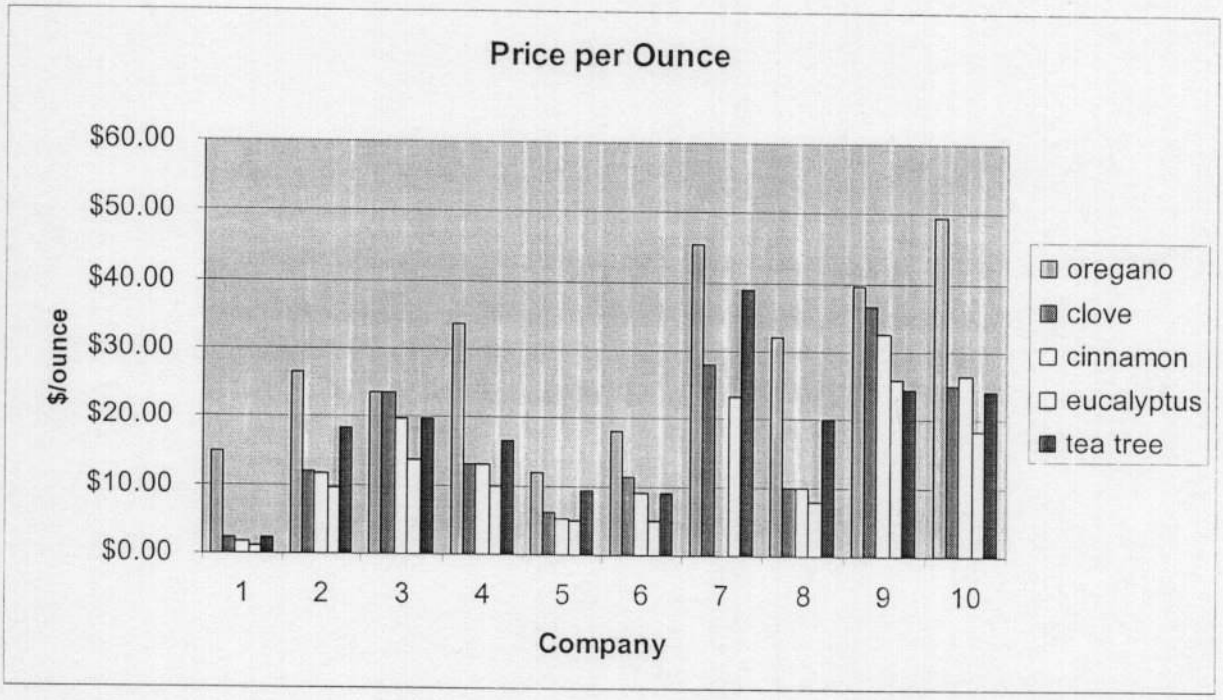
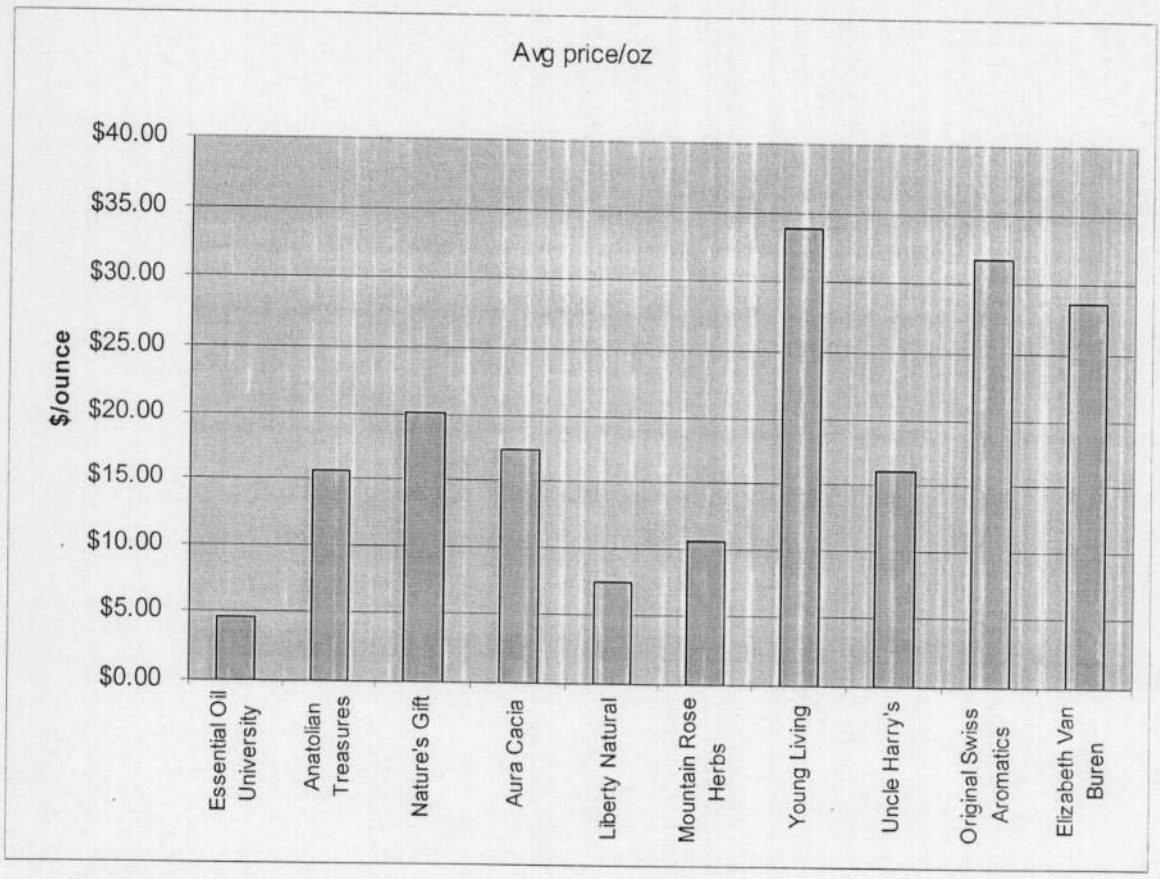
30.0
25.0
20.0
15.0
10.0
5.0
0.0
1 2 3 4 5 6 7 8 9 10
Company
staph aur
strep pne
e. coli


tea tree avg
avg inhibition
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10
company


Price per Ounce
$/ounce
$60.00
$50.00
$40.00
$30.00
$20.00
$10.00
$0.00
1 2 3 4 5 6 7 8 9 10
Company
oregano
clove
cinnamon
eucalyptus
tea tree


Avg price/oz
$/ounce
$40.00
$35.00
$30.00
$25.00
$20.00
$15.00
$10.00
$5.00
$0.00
Essential Oil University
Anatolian Treasures
Nature's Gift
Aura Cacia
Liberty Natural
Mountain Rose Herbs
Young Living
Uncle Harry's
Original Swiss Aromatics
Elizabeth Van Buren