

Essential oils biological components



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Essential oils are complex mixtures of many components, about 20-60 can be present at different concentrations depending on the oil. According to Bakkali et al(2008) they are characterized by components of the highest concentrations, there are usually 2 or 3 main components in each oil compared to some components in trace amounts. Major components of the oils determine the biological activity of the oils however the components in smaller amounts produce an additive effect(Bakkali et al, 2007). Chemical analysis of lemongrass and cinnamon oil distinguished several compounds. Terpenes are able to form structurally and functionally different classes. They are made from combinations of several 5 carbon units called isoprenes (Pichersky and Gershenzon). According to Bakkali et al (2007) terpenes constitute 90% of essential oils. Tyagi and Malik (2010) found that phenols and aldehydes exhibited the highest vapour activity followed by alcohols, ketones and esters and lastly hydrocarbons.

Gas Chromatography Mass Spectrometry (GC MS) analysis found cinnamon oil contains high amounts of trans cinnamaldehyde at 86.9% and eugenol at 6.2% as major components. Eugenol has been found to have antifungal activity (Cheng et al, 2008) as has Cinnamyl acetate, 4-terpineol, 1, 8 cineole. The additive effects of the main components have been reported in one study, a mixture of cinnamaldehyde and eugenol inhibited growth of staphylococcus species whereas when they were applied individually they did not inhibit growth (Marei et al, 2007).

Lemongrass exhibited a high concentration of citral, this has two isomers, geranial (trans citral) which presented at 51.3% and neral (cis citral) at 37.4% as major components of this oil. Usually one isomer does not occur

without the other. In addition to the essential oil consists of small quantities of geraniol(alcohol) which often co exists with geranial and neral (Chen and Vilojen, 2010)., This study found strong activity of geraniol against *Candida albicans* (Chen and Vilojen, 2010). Geranyl acetate (Hanaa et al, 2012)and limonene (Gehan et al 2012) have also been found to have antifungal properties. Similar concentration found in this analysis have been previously reported by Inouye et al (2003) A study also found lemongrass oil to show higher anticandidal activity against strains of superficial mycoses than citral suggesting additive effects of minor components (Kumar et al 2012)

4. 2 Vapour phase

Using the vapour phase has been a fairly recent advancement, Tyagi and Malik (2010) found that essential oils in the liquid medium form micelles and suppress the attachment of the essential oils to the organism, however the vapour state allows for free attachment although studies have been carried out there is no standard assay to evaluate inhibition by vapour phase contact (Avila-Sosa et al, 2012). A further study also stated that in liquid medium the diffusability and solubility of the essential oil whilst in vapour depends on the volatility of each compound and in a vapour form can facilitate the solubilisation of lipophilic monoterpenes better by inflicting higher cell damage on the cell membrane(Taweechaisupapong et al, 2012)

Rana (2012) found with *Candida dublienesi* , where the essential oil is placed directly under the fungi the growth is completely inhibited as the vapours are the most volatile here . So growth is fungicidal until a certain point, as the volatile vapours spread out the concentration of the vapours is

not high enough to inhibit growth so a fungistatic effect is produced. Although this effect was not produced with *Candida albicans* it demonstrates how the vapours work. Therefore a dispersal method would be more effective as there would be an even distribution of the oil. If volatile compounds retained less effectively therefore allowing more molecules in the vapour phase with increased antifungal activity promotes fungicidal effect at lower concentrations (Lopez et al, 2005) thought to be due to better penetration and contact (Tyagi and Malik, 2010).

4. 3 Bonding and compounds

Essential oils cannot be used on their own as they are classed as irritants according to health and safety (Look at COSHH form) therefore a method is required where the oil is not as concentrated and can be used in application such as sprays where it is easier to distribute. Essential oils are expensive therefore if mixed with a carrier oil which are usually cheaper and more readily available.

The essential oils blended with carrier oils showed a significant difference in the results for example at 25µl ($p < 0.021$). vegetable oil produced the highest results with lemongrass oil ($p < 0.017$) whereas sunflower oil produced the highest results with cinnamon oil ($p < 0.295$) although no significance is shown there is a small difference between the control and the blend of cinnamon and sunflower oil demonstrating the effectiveness of the oil (85.9% inhibition). Interestingly sunflower oil produced the lowest zone of inhibition when blended with lemongrass oil (30.2% inhibition). Wheatgerm oil produced the lowest zone of inhibition when blended with cinnamon oil

(32.7%) there was significance between the control and the blend of oil ($p < 0.002$) however the difference was too large therefore this would not be an effective carrier oil for use. A varied difference is demonstrated between the cinnamon and lemongrass oil in the order of the results suggesting a difference in the way the carrier oils work with the two oils.

Carbon and oxygen form a polar bond (carbonyl group $C=O$) therefore if this bond is part of a molecule it affects how the molecule behaves making it more reactive. The greater the difference in electronegativity of molecules the greater the polarity of the bond. Polar molecules are hydrophilic (water loving) whilst most essential oils are generally insoluble if a part of it has a polar molecule it will be slightly soluble to a certain extent. Polar bonds have different electronegativities and therefore unequal sharing of the bonding electron pair. This results in a bond where the electron pair is displaced towards the more electronegative atom. The atom obtains a partial negative charge while the less electronegative has a partial positive charge. Non-polar molecules are hydrophobic (water hating) non-polar compounds share the same electronegativity therefore have equal sharing of the electron bonding pair. Because non-polar molecules do not have a partially negative region it cannot take part in bonding therefore cannot mix with polar molecules. Essential oils are of mixed polarity, with some polar and some non-polar compounds in the oils (Pitman, 2004).

Carrier oils are non-polar molecules (Pitman, 2004) and have electrons equally distributed therefore will not be attracted to polar molecules. In carrier oils there is a mixture of long carbon structures made up of a fatty acid and glycerol which are non-polar. The longer the carbon chain the compound

becomes less soluble in water the molecule becomes more like a hydrocarbon chain (Bruice, 2006). Therefore depending on the structures in the carrier oils affects how they interact with the essential oils. The length of the hydrocarbon varies according to the type of fatty acid this is hydrophobic whereas the carboxylic acid group is hydrophilic. The structure of the hydrocarbon is different for different fatty acids by length as well as the type of carbon to carbon bonding. Saturated chains contain single bonds whereas unsaturated ones contain one double bond known as monounsaturated or more than one double bond polyunsaturated (Pitman, 2004).

The suggested theory is the polar molecules in the essential oil are likely to have an attraction for the polar molecules in the carrier oil for example palmitic acid(see table 7) contains a smaller hydrocarbon chain, as the chain size decreases hydrogen bonding becomes more important and Van der Waals less so. Hydrogen bonds are formed when hydrogen is bonded to the lone pair of electrons on the oxygen. This is the strongest type of interaction (Bruice, 2007). However the longer hydrocarbon chain will be non polar such as Arachidic acid (see table 7) as it has a longer chain therefore will not have an attraction for the polar molecules but will do for the non polar components forming weak Van der Waals. It is essential to find the structure of carrier oils as it may depend on how long the hydrocarbon chains are in working out which oil works best as a carrier oil. As the solubility of the carrier oils could be linked to the lipophilicity. Cinnamaldehyde contains a benzene ring which is non polar however the aldehyde is polar, overall the molecule is non polar. As the benzene ring is flat, Van der Waals forces can occur between the longer acids from the carrier oils with cinnamaldehyde.

Citral is also non polar it also contains double bonds this cause less attraction which means fewer Van der Waals forces can occur between molecules. This molecule also contains an aldehyde group at the end. However it is less non polar than cinnamaldehyde as it contains fewer double bonds therefore is able to create more Van der Waals, so components more likely to be held and not released as a vapour. The shorter molecules of the carrier oil are likely to vaporise as normal as are the other polar molecules of the essential oil.

The tables in order of how well they worked with lemongrass and cinnamon oil produced no clear link in terms of lipohilicity . However the composition of the oils was obtained from previous literature analysis. Consequently the oils tested against the species may not have the same composition as those tested in the literature. GC MS analysis was carried out on the carrier oils however they were not volatile enough to produce any results. To produce results esterification will be required.

4. 6 Bandages

http://www.tis-gdv.de/tis_e/ware/fasern/baumwoll/baumwoll.htm

Chemical Composition Of Flax Fiber | Linen Fiber Composition

Bandage

Composition

Tubular

85% cotton, 10% latex, 5% polyester

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Non woven bandage

100% viscose(medical textiles paper)

Stretch bandage

36%cotton, 36%viscose, 28%polyamide

Crepe

Cotton mixed with wool

Cotton crepe

100% high twist cotton and rayon yarns

Gauze

100% hydrophilic cotton gauze

Cotton

Cotton 98% waxes 0. 60%, fatty substances 0. 40%

mineral salts 0. 20%

non adherent

Porous polyethylene film and absorbent cotton pad.

Lint

92% cellulose 2% hemicellulose lignin 4% others 2%

In natural textiles the essential oils can be fixed by Van der Waals forces (physical bonding or Covalent bonds(chemical bonding). As already established the essential oils are of mixed polarity therefore the affinity between the textile and the essential oil is mixed depending on the composition of the textile as each textile contains a different proportion of material although they may contain similar components. Textiles may be classified as two types. Non polar, synthetic polymers and polar natural textiles. This can be further divided into polymers from amino acids such as silk and wool or from cellulose such as cotton.

All fibres are made of polymers . Polymers are formed when hundreds and thousands of small molecules are covalently bonded into a linear chain. The backbone of most polymers for textile fibres contain covalently bonded carbon atoms.

4. 7 Cinnamon oil and bandages

Lint produced the highest inhibition at 80%. Absorbent lint is composed of cotton. Cotton is the most common cellulosic fibre. Hydroxyl groups are mainly present in the structure of cellulose. They are hydrophilic, therefore any non polar(hydrophobic) molecules will not bind to the surface and will not be absorbed and released as a vapour due to its volatility . Gauze is also composed of 100% hydrophilic cotton gauze therefore this produced similar results to lint at 76. 8% as it has the same effect.

However this is not shown by cotton which produced a low result of 33% although at 1µl it gave a result of 32. 9% therefore not much difference between 25 and 1 ul however cotton contains a small amount of water which

is highly polar it may retain components even more so components which may be slightly soluble will also form hydrogen bonds. Cotton crepe produced only a slightly better result than crepe at 25µl however it produced better results at all concentrations therefore reiterating the polarity effect. Cotton crepe contains 100% high twist cotton and rayon yarns. Rayon is viscose it has the same polymer structure as cellulosic fibres which is the same as cotton. Interestingly Viscose is a regenerated fibre from natural polymers therefore is not classed as a synthetic fibre although it is heavily processed so nor is it a natural product. it produced 51.6% inhibition in between the highest and lowest result in line with the theory

The results of the mixed synthetic and natural fibres show a significant reduction in inhibition ($p < .$ Non woven fabric has a wide range of use in the medical field. They are disposable, sterile and cheap and are beneficial in prevention of cross infection. The material is 100% viscose highly porous allow air and water permeability unlike cotton crepe which comprises half viscose although inhibition was only slightly higher at 49.3%, however inhibition was lower at 10µl and 5µl.

Tubular bandages are composed of cellulose and elastic fibre such as latex blends in a knit structure. Cellulose is hydrophilic whereas the polyester is hydrophobic. Although there is a small amount of polyester the majority of the fibre is hydrophobic therefore the hydrophilic components attract the polar molecules in the essential oil whereas the non polar molecules in the bandage will cause some of the components to be released.

Stretch bandage contains 36% cotton therefore cellulosic and Polyamide usually present in bandages is nylon this is hydrophobic although it has the highest absorption capacity among synthetic fibres because of the polar amide group present. Viscose is hydrophilic So in this fibre there are more hydrophilic groups than hydrophobic therefore inhibition 45.5%.

non adherent: porous polyethylene film and absorbent cotton pad.

Polyethylene is non polar therefore the terpenes will bind to this Usually used for burns. Hydrophobic polyethylene film with absorbent cotton pad.

4.8 Lemongrass oil and natural textiles

With lemongrass oil Crepe produced a 100% inhibition. Crepe has high polarity as it is mix of wool and cotton. Wool has the highest capacity of water absorption and contains hydrophilic functional groups primary amine NH_2 and carboxyl these structural features contribute to its ability to hold onto water molecules tightly. Therefore higher results produced because of the presence of the groups.

Cotton 90.5%. Cotton produced a much higher result with lemongrass oil than with cinnamon oil. This may be because of the polarity of the molecules or the difference in polarity of the molecules. Citral is less non polar than cinnamaldehyde because of the presence of the double bonds in the molecule. It maybe the effects of other components in the oil. Gauze and lint produced similar results with cinnamon oil producing zones of inhibition between 70 and 90%.

Tubular , non adherent , stretch and non woven bandage produced the lowest results . Showing the same general trend with cinnamon oil although in a slightly different order of reactivity. This is caused by the lower polarity of these fibres, as they are made of hydrophobic fibres. Van der Waals forces occur between the molecules and so the volatile molecules are not released as vapours. Although the textiles did produce inhibition with the mixed fibres , the lowest being at 45% with the non woven bandage. This is suggestive of additive effects of other components in the oil. Suggests that the antifungal components are non polar and the major ones of this is citral and cinnamaldehyde. Other terpenes present in cinnamon and lemongrass oil are also non polar such as geraniol and cinnamyl acetate they also which have also been found to have antifungal effects.

4. 9 Biological effects

According to Bakkali et al (2007) essential oils are not thought to have any specific cellular targets as they have many constituents. As lipophiles they pass through the cell wall and cytoplasmic membrane and disturb the structure of their different layers of polysaccharides, fatty acids and phospholipids and make them permeable (Bakkali et al, 2007). This is mainly the effect of terpenes which are the major components of essential oils. They alter cell permeability, by penetrating between the fatty acyl chain making up the membrane lipid bilayers disrupting lipid packing and changing membrane fluidity . This leads to morphological alterations reducing adherence capacity of candida albicans (Taweechaisupapong et al, 2012). Tyagi and Malik(2010)also found the same results with lemongrass oil vapours, components were able to enter and alter the permeability of the

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cell by disrupting the fatty acyl chain making up membrane lipid bilayers therefore properties of the membrane maybe changed. Which may affect the regulation and function of membrane bound enzymes and may alter the synthesis of components of the cell wall polysaccharides such as beta glucan, chitin and mannan and alter cell growth and morphogenesis.

Specifically essential oils can coagulate the cytoplasm (Gustafson et al, 1998) and damage lipids and proteins. Damage to cell wall and cell membrane can lead to the leakage of macromolecules and to lysis. In eukaryotic cells essential oils can aggravate depolarization of the mitochondrial membranes by decreasing the membrane potential affecting ionic Ca^{++} cycling . They change the fluidity of membranes which become abnormally permeable resulting in leakage of radicals, cytochrome C, calcium ions and proteins. Permeabilization of outer and inner mitochondria membranes leads to cell death by apoptosis and necrosis. Bakkali et al (2007) suggested chain reactions from the cell wall or the outer cell membrane invade the whole cell, through the membranes of different organelles like mitochondria and peroxisomes.

If the effects of essential oils are due to synergism or reflect only those of only the highest level which were shown by gas chromatography. The amplitude of their effect, being dependent on their concentration. It is possible the activity of the main components is modulated by minor components of the oils. It is suggested by Cal(2006) that several components of the essential oil play a role in defining the amount of cell penetration, lipophilic or hydrophilic attraction and fixation on cell wall and membranes as well as cellular distribution. Cellular distribution is an important feature

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because the distribution of the oil in the cell determines the different types of radical reactions produced depending on the compartmentation in the cell (Bakkali et al 2007)

Very few studies have analysed enough essential oils and biological endpoints to determine whether there is specificity for different effects according to different oils. It has been demonstrated by Bakkali et al (2007) that there is a difference in the amplitude but not in the mode of action of the biological effects.

However one study specifically investigated the role of cinnamaldehyde by studying its effect on sterol biosynthesis and plasma membrane ATPase activity of fungi. ATPase is an important fungal pump it creates a membrane gradient which is used for transport of nutrient. The pump is activated in the presence of glucose which forces more H⁺ ions out. The *Candida* isolates which demonstrated susceptibility to cinnamaldehyde also showed inhibition of H⁺ ATPase mediated proton pumping the study suggested the two actions are linked. The study also found that cinnamaldehyde may have a similar mode of action to fluconazole as it inhibits ergosterol synthesis in sensitive strains. As the essential oil blocks the synthesis of ergosterol found that cinnamaldehyde acts in a dose dependent manner in decreasing ergosterol. Suggests that these are the primary target of cinnamaldehyde and could be a promising drug (Shreaz et al, 2011)