Recent advances in oregano (*Lippia graveolens* and *Poliomintha longiflora*): Extraction methods, component determination, and their potential applications.

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Abstract

The work presented here describes the most significant advances in concerning the chemistry of oregano (*Lippia graveolens* and *Poliomintha longiflora*). We present new, green methods for essential oil extraction as well as the potential medicinal properties of said oil. This review also focuses on the determination of its main components, such as carvacrol and thymol. We describe their synthesis, reactivity, and potential applications of both compounds in various areas.

Keywords: oregano, extraction methods, thymol, carvacrol, potential applications.

We can consider plants as small, secondary metabolite factories. These metabolites often have specific functions; they can give the plant its characteristic odor, color, they can offer protection, and a host of other functions. Their production and concentration are functions of many variables which range from the plant's genetics, to climactic conditions. Some metabolites have interesting biological functions and properties, for instance: antibacterial, antifungal and anti-tumor activity, to name a few. These properties make investigating oregano a worthy cause. Metabolite extraction with scientific goals started over two centuries ago with the isolation of morphine (fig. 1) by the German pharmaceutical chemist, Friedrich Wilhelm Adam Sertürmer. Plants play a pivotal role in society, giving us green areas, oxygen, flavor, odor and medical treatment (Sell 2006). For example, the compounds responsible for the burning sensation in chili peppers and cinnamon are known respectively as capsaicin and α-methyl-cinnamaldehyde (fig. 1). The main components in oregano's essential oil are thymol and carvacrol. Thymol gives oregano its characteristic flavor. However, both these compounds present useful biological activity. Based on this, oregano is an extremely attractive subject for scientific inquiry (Arcila *et al*., 2004). Therefore, in this section we outline the main advances in oregano's chemistry of the last two decades.

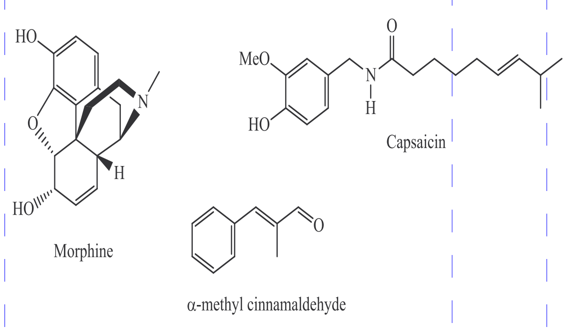


Fig. 1. Secondary metabolites, morphine, capsaicin and α-methyl-cinnamaldehyde.

The word "oregano" derives itself from the Latin word *origanum*, which is itself a derivation of the Greek *origanon*, where *oros* means 'mountain' and *ganousthai* which is roughly translated to 'delight in', delight of the mountains. Europeans started producing essential oils in the XIIth century. Greek mythology recounts Afrodita, goddess of love, as the first to plant oregano, granting the plant an intense fragrance, according to the story, she called the forest nymphs the name of Oreads/Orestiads. Both, Greeks and Romans used oregano with culinary means and for treating neurological disorders. In fact, Theofrast, Aristoteles and Hippocrates regarded it as an effective treatment for respiratory diseases, burns and indigestion. During the middle Ages, it was used to treat liver problems and as a cleanser during epidemics by brazing and mixing it with mint and thyme, nowadays it's used as an acaricidal (Da Silva *et al*., 2011). There are many varieties of this aromatic herb, many of which are found in the wild throughout Europe and Western Asia. America's discovery in the XVth century brought the herb to the new world, where it quickly adapted to the climactic and soil conditions. So much so, that it quickly made its way into traditional recipes, where it became a staple of many a cuisine. In the modern age, the largest markets for this herb are located in the Mediterranean, California and Mexico, though countries such as Peru and Argentina dedicate large swathes of landmass to its cultivation for exportation.

DESCRIPTION OF *LIPPIA GREVEOLENS* H.B.K. (VERBANACEAE).

A polycarpic (various reproductive cycles in a year) bush species with deciduous leaves (loses them seasonally), that can grow to over two meters tall and have up to a meter squared of foliage cover. It branches out profusely from its trunk's base. Its development is relatively quick in high humidity conditions, though this quick growth brings with it a short life span, typically of around five to ten years depending on climactic conditions. Observational studies suggest that branching occurs shortly after it rains, ending around six weeks later. Oregano is very dependent on soil humidity, as it has been observed that with reduced soil humidity its leaves slowly start turning yellow and brown, until they fall off approximately six weeks later. The plant's flowers are small, around four millimeters in diameter, white, sessile and zygomorphic. Its chalyx is gamosepal with four lobes and its corolla is gamopetal with five unequal cream colored lobes. The flowers average lifespan is of 13 days. The inflorescences are undetermined spigots, auxiliaries and generally found in groups of four, which can attract polonaises. These flowers start appearing after the surrounding leaves are completely developed, which is around five to six weeks after the start of the rain season. Oregano's flowers are hermaphrodites, can autopollinate (Ocampo-Velázquez *et al*., 2009) and the fruits are dry capsules whose development begins two weeks after flowers start growing, around the time when leaves start turning yellow and start falling. It's worth noting that these periods vary in accordance to geographic zone. The seeds have an oval shape with diameter smaller than 0.5 mm, brown in color and generally each fruit only contains one, though a small percentage of fruits can have two seeds. Not all flowers in the inflorescence produce a fruit, it's been found that on average only one out of eight flowers happen to produce one.

Fig. 2. *Lippia graveolens* at the Padron Juárez village, Jaumave, Tamaulipas.

DESCRIPTION OF *POLIOMINTHA LONGIFLORA* GRAY (LAMIACEAE).

Aromatic bush species with small, deciduous leaves whose shape varies from linear to oblong, with a full margin, glandular-pointed with a short or non-existent petiole. Its max height is slightly over a meter, although in sites where it's intensively harvested, these plants don't reach over half a meter tall, which incentivizes ramification and they present greyish secondary trunks from the base in tetragonal fashion. It presents a short life cycle which can vary anywhere from 3 to 8 depending on the climate. It's found in sites that surpass 2000 meters above sea level and forms difficult to access patches generally on north-eastern cliff faces where they're protected from excessive solar radiation. The species' flowers are solitary or found in pairs with bracts similar to leaves in shape and form. Flowers are sessile, zymomorphic and bilipped with five subequal lobes and conical superior lip, while the inferior lip is shorter and narrower. The corolla's tube has a hairy ring with anywhere between 13 to 15 curved veins. Direct observation at Palmillas, Tamaulipas suggests that the species reacts to light rains, generating more branches and leaves immediately after the rain ceases. There has been an increased tally of floral visitors, predominantly humming birds, which suggests that its sexual reproduction depends on this group of birds. Nevertheless, it remains imperative to further investigate this.

Fig. 3. *Poliomintha longiflora* Gray in its reproductive stage at the Sierra Picachos, south of Nuevo León (Image: Carlos Velazco).

Oregano in Mexico

Oregano is comprised of various species utilized for culinary means, the most common being *Origanum vulgare*, native to Europe, and *Lippia graveolens*, which originated in Mexico. Nowadays there exists approximately 40 species of oregano in Mexico. Mexico is the world leader in *Lippia graveolens* (Mexican oregano), export, around 35 to 40% of all *Lippia graveolens* exports come from Mexican soil. The average lifespan of oregano is 2 years. These plants grow in practically all climates and terrains depending on the species; some grow in warm zones, while others grow in frigid ones. Oregano has a large tolerance for altitudes and temperatures. However, the greatest percentage of essential oils is obtained from cold areas. Even though they are resistant to cold, if the temperature drops below 5 °C their growth is slowed and the leaves' edges get damaged. This crop adapts itself to any soil type that isn't alkaline, best yields are obtained in soils comprised mainly of sand, silt and clay. Wild strains are found in arid and semiarid regions of at least 24 states in Mexico. Different families are found in the states of Chihuahua, Durango, Jalisco and Oaxaca. Their main habitats are the rocky soils of mesas, mountainsides and cliffs between 400 and 2000 meters above sea level, though they are mostly found between 1400 and 1800 meters. Thanks to this, season, climactic and soil conditions affect the essential oil's composition. In the state of Nuevo León, oregano it is reported that oregano grows in the municipalities of Allende, Linares, Monterrey and in particular Higueras, where the author (Castillo, 1986) reports the presence of the genus *Poliomintha Longiflora* Gray. In Higueras, farmers claim its habitat to be the following parts: Las Rucias, Los Pinos, El Camaján, La Caja Pinta, Infiernillo, Cerritos and Los Picos.

Uses and applications of oregano and its essential oil.

Oregano is a very useful plant due to its wide variety of uses and applications; which range from medicinal to cosmetic. The greatest demand for oregano in Mexico is for culinary purposes. After the discovery of its antioxidant capabilities in 1998 (Baratta *et al*., 1998), this plant is used to preserve raw or cooked food, after treatment the food is frozen. This ensures that flavor and chemical properties are maintained after storing, thawing and cooking. Oregano is also used as an extract, infusion, essential oil and even as a colorant. In the cosmetic industry, it's used to slow skin aging due to its antioxidant properties. The essential oil is used in fragrance making together with musk, which result in high quality extracts. On the medicinal side, oregano helps in digestion and stimulates bile production. The infusion is sour, but stimulates development of the intestinal flora and helps combat certain bowel conditions such as intestinal spasms and other abdominal pains. It's a discrete antiseptic, which contributes in the treatment of respiratory ailments, such as coughing, bronchitis, tonsillitis and can even be used as an asthma treatment. Habitual consumption of the plant is recommended in order to treat severe headaches and neurological afflictions such as anxiety and insomnia.

Medicinal

In Europe, it is used in anti-rheumatic, analgesic and anti-inflammatory creams commonly prescribed with other drugs. It's also used in the preparation of antiseptics and syrups. It is also used in tablets and capsules which present anti-flatulent properties. It's also used as a bactericide, anti-septing, analgesic, and in anti-parasite treatments. It's also used to treat cellulitis, rheumatic, muscular and articular conditions as well as pediculosis. Oregano is highly utilized due to its biological activity in many areas of medicine; more specifically, its antioxidant, antimicrobial, antiparasitic, oestrogenic, antigenotoxic activity, as well as functioning as a competent insecticide. All these characteristics make it an interesting subject for investigation and as a food additive to improve shelf life and healthiness. On the other hand, several plants are considered as excellent sources of antioxidant agents. They include mainly simple phenols, phenolic acids, coumarins, tannins and flavonoids (Proestos *et al*., 2006; Cetkovic *et al*., 2004). The antioxidant potential of oregano extracts has been observed due to its capacity to inhibit lipidic peroxidation, protecting DNA damage from hydroxyl radicals. These extracts have been proven to be very effective, in some cases more so than propyl galate, BHT and BHA, which are currently used in packaged foodstuffs to prevent their rapid oxidation. Also, oregano's essential oils have been found to harbor antimicrobial activity on gram negative bacteria such as Salmonella typhimurium, Escherichia coli, Klebsiella pneumoniae, Yersinia enterocolitica and Enterobacter cloacae; and gram positive bacteria such as Staphylococcus aureus, Staphylococcus epidermidis, Listeria monocytogenes and Bacillus subtilis; they also present antifungal activity against Cándida albicans, C.tropicalis, Torulopsis glabrata, Aspergillus Níger, Geotrichum and Rhodotorula, but not against *Pseudonoma aeruginosa*. This presents an enormous opportunity, if we can figure out how to efficiently oregano to replace artificial antibiotics and antimycotics, with the right breeding and genetic engineering programs, we may be able to attain better results according to some studies. As a antiparasitic, the essential oil of *L. multiflora* is considered an effective agent against lice and Sarcopetes scaniei; surprisingly, to a greater degree that benzyl benzoate, the most commonly used pharmaceutical to combat these parasites. The essential oil also presents antimalarial activity, which makes it a very desirable alternative treatment due to its low toxicity in the human body. Extracts from *L. beriandieri* possess elevated antigiardia activity with a trofozoite mortality rate of 90%, much greater than the commonly used drug, timidazol, whose trofozoite mortality rate is of 79%. The oestrogenic action, oregano has the ability to reduce the probability of developing osteoporosis and cardiovascular afflictions. This capacity is attributed to endogenous oestrogens such as 17-β-estradiol.

On the other hand, other components have been found to have antioestrogenic activity, because they have been shown to prevent the formation of breast cancer tumors. Some essential oils and their components possess a wide sectrum of activity against insects, acari, fungi and nematodes, such as Rhyzopertha dominica, *Tribolium castaneum*, and *Sitophilus oryzae*. These organisms attack stored grain and spoil it, so it's imperative they are prevented from growing on it, whilst keeping human toxicity to a minimum. As antigenotoxic, it has been reported that some monoterpenes, which are part of oregano's essential oil have the capacity to be effective inhibitors of carcinogenesis. The oil has the capacity to induce an increase in the detoxifying glutation S-transferase (GST) enzymatic activity, when taken orally, which suggests a potential anticarcenogenic effect. However, other studies have found that *Oregano spp* is an allergen, so excessive consumption of *O vulgare* and *O. majorama* must be prevented during pregnancy, it's worth noting that they also have abortive properties. These are only a few known and reported properties of said plant. Given the fact that it's been used throughout history, it's probable that new applications may be discovered, in fact, many of the essential oil's main components can act as precursors for many useful chemical compounds.

Essential oil extraction methods.

Plant essential oils are widely used in the cosmetic, medicine and agricultural industries (Boelens 1985). Due to the properties of oregano's essential oils, diverse extraction methods have been devised, they include: vapour assisted extraction, hydrodistillation, Soxlet (Fauta Kawuase *et al.,* 2013), microwave (Cardoso-Ugarte *et al.,* 2013;), organic solvents, supercritical CO_2 (Craviero *et al.,* 1989), ultrasound (Roldán-Gutierrez *et al.*, 2008;), solvent-free microwave (Bayramoglu *et al.,* 2008), supercritical H_2O(Herreo *et al.,* 2006), with the last two being considered green methods. Solvent-free microwave extraction presents the highest percentage yield (0.054 mL/g). However, very few extraction methods are scalable to industrial levels, currently vapour assisted extraction and hydrodistillation are the two most used methods for industrial extraction of essential oils. Essential oil extraction from oregano (*Lippia graveolens*) has been reported using vapor assisted extraction in a distillation apparatus. The extraction process is based on the different volatilities of its components, which also allows for a coarse separation of components (Kuklisky 1993). Numerous investigations report the oil as having an intense yellow color. Different oregano species have different essential oil yields. *Poliomintha longiflora* has a yield of 0.7% (Aranda *et al*., 2009), while *Lippia graveolens* has a yield of 2.5 to 3% (Baez *et al*., 2005). However, conventional methods present various disadvantages, examples being the loss of volatile compounds, low extraction efficiency and the degradation of unsaturated compounds and esters. Based on these reasons, finding a better method would be highly beneficial (Ferhat *et al*., 2007).

Chemical composition of *Poliomintha longiflora*'s essential oil.

The oil is comprised by many compounds including: thymol, terpineol, carvacrol, limonene, α- and β-pinene, piminem, sesquiterpenes (β-cariofilene, bisabolene). It also contains phenolic acids such as caffeic, rosmarinic, ascorbic and ursolic acids; flavonoids such as: kampferol, apigenin, diosmentin, tannins, resins, souring agents, etc. The oil also contains potassium, calcium, sodium, iron, phosphorus, vitamin A, vitamin B and carbohydrates. The sour taste is due to tannins, which are polyphenols. The number and proportion of components varies from species to species, geographic location among other factors (Burt 2004). The main components of the also vary between both species, for *Poliomintha longiflora* the main constituent is carvacrol, whereas for *Lippia graveolens* they are both thymol and carvacrol (fig 4). Pinene and terpinene have also been found in the species' essential oil. It has also been reported that *Poliomintha longiflora*'s oil also contains vanillic and caffeic acids as well as luteolin (Zen *et al*., 2001) (fig. 5).

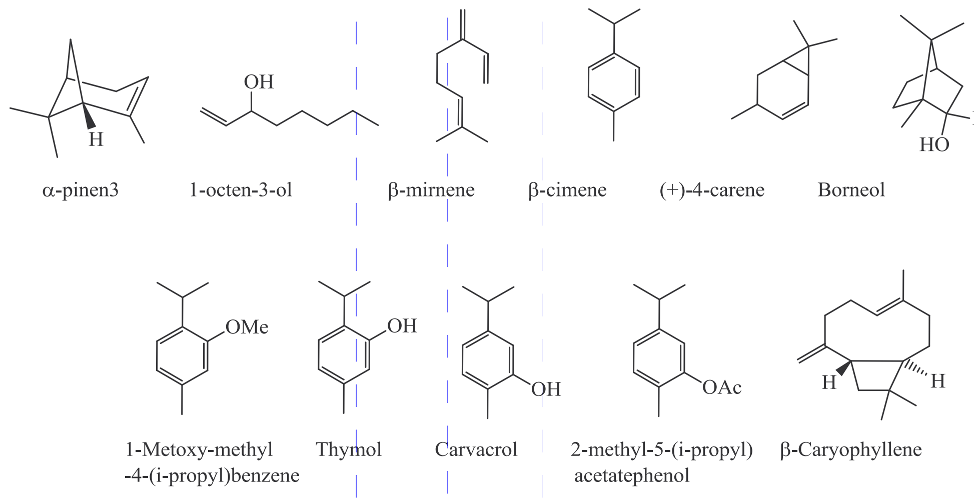


Fig. 4. Major components of *Poliomintha longiflora*'s essential oil in accordance to vapour assisted extraction.

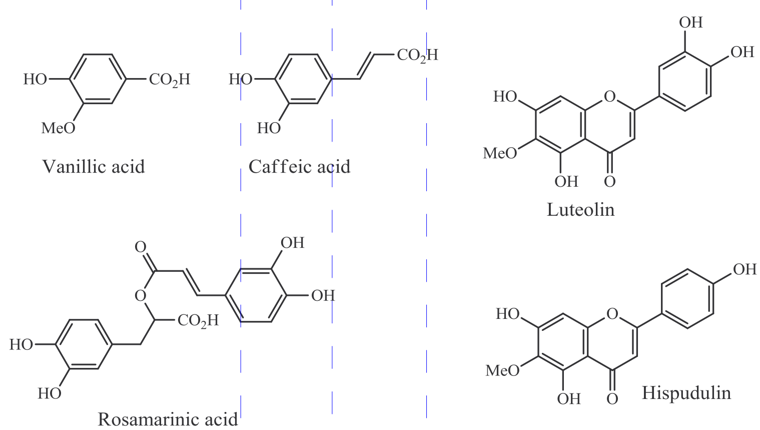


Fig. 5. Major components of *Poliomintha longiflora*'s essential oil in accordance to extraction by phosphoric acid solution.

Chemical composition of *Lippia graveolens*' essential oil.

Lippia graveolens is utilized to treat gastrointestinal and respiratory ailments; it's used as an antiseptic, digestion aid, anti-inflammatory (Compadre *et al*., 1987; Domíguez *et al*., 1989) and more recently, antioxidant and antimutagenic characteristics have been reported (Martínez-Rocha *et al*., 2008). Due to this, it is imperative to know the composition of the plant's essential oil. The apparent number of components and apparent percentage abundance is highly dependent on the extraction method, solvents used and the specific part of the plant used. The analysis of the leaves' essential oil by methanolic extraction and gas chromatography coupled to mass spectroscopy reveal that the main components are cavacrol, α-terpinilacetate, m-cimene, thymol and β-pinene (Hernández *et al*., 2009). In addition, it has been reported that it also contains iridions (Rastrelli *et al*., 1998) and glucosidic flavonoids (Lin *et al*., 2007). More recently, extraction with methanol and acetone has revealed new compounds along the lines of triterpenes with δ-lactones on the E ring. X-ray structures of these new compounds have been obtained (fig 6.) (Maldonado *et al*., 2009).

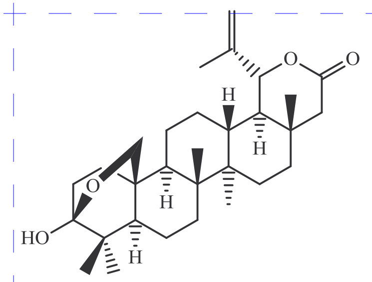
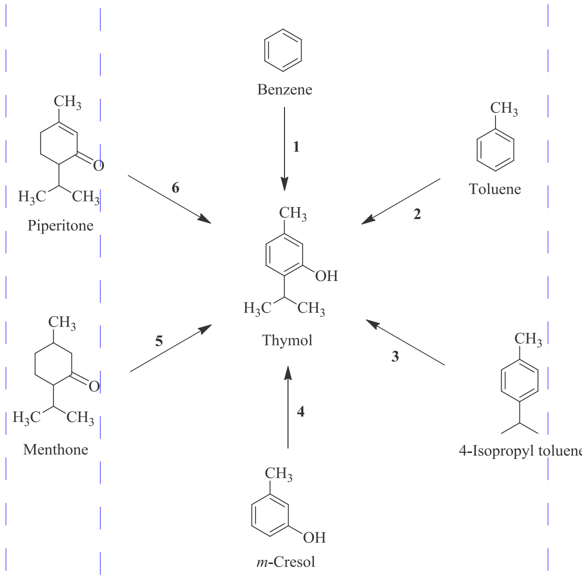


Fig. 6. Triterpene with δ-lactone on the E ring, component of *Lippia graveolens*'s essential oil.

Synthesis, reactivity and applications of thymol and carvacrol.

Thymol synthesis

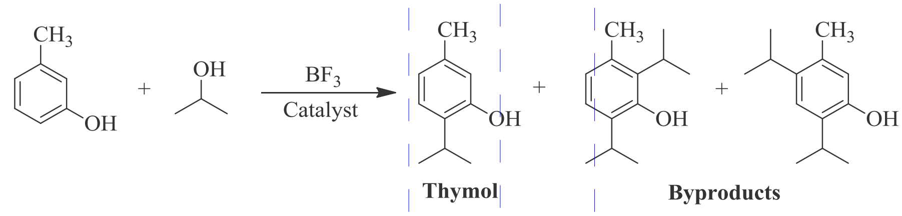
Thymol, (2-isopropyl-5-methylphenol) is a crystalline, colorless substance with a characteristic odor. It is found in many essential oils from many different plants from which it can be extracted. Nevertheless, it can be obtained through artificial means from many different starting points, which include but are not limited to benzene, toluene, 4-isopropyl toluene, *m*-cresol, menthone, and piperitone to name but a few (Biedermann *et al.,* 1978; Velu *et al.,* 1998; Grabowska *et al*., 2001; Umamaheswari *et al*., 2002; Selvaraj *et al.,* 2008; Yadav *et al.,* 2005; Grabowska *et al*., 2001; Nitta *et al*., 1976; Grabowskaa *et al*., 2004; Wimmer *et al*., 1991; Nitta *et al*., 1974; Yamanka, 1976) (scheme 1).



Scheme 1. Thymol synthesis from various starting points.

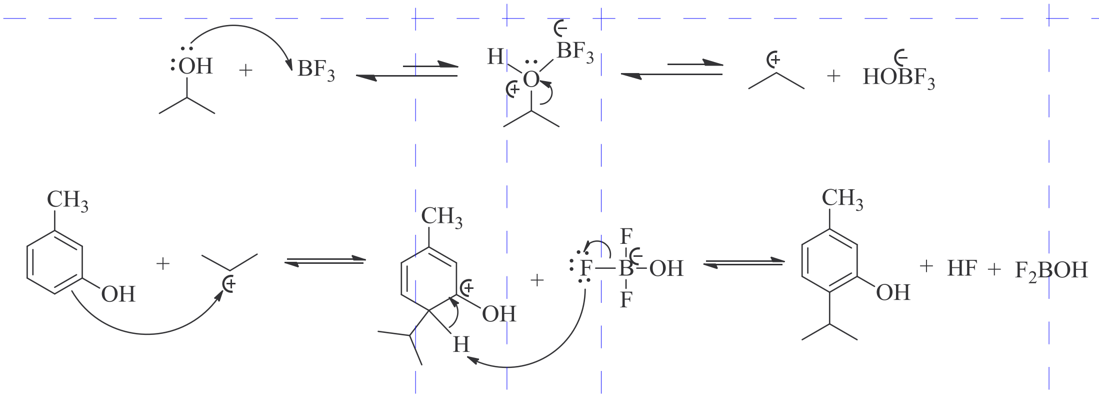
Thymol synthesis utilizing *m*-cresol and 2-propanol.

Friedel-Crafts reactions are a type of aromatic electrophilic substitutions where a hydrogen atom is substituted by an alkyl group. In this sense, alkylation of m-cresol with iso-propanol catalyzed by a Lewis acid gives thymol as a main final product, however, unwanted subproducts are also formed thanks to the effect that the hydroxyl and methyl group have as activators, which orient substitution in the *ortho* and *para* positions (scheme 2).



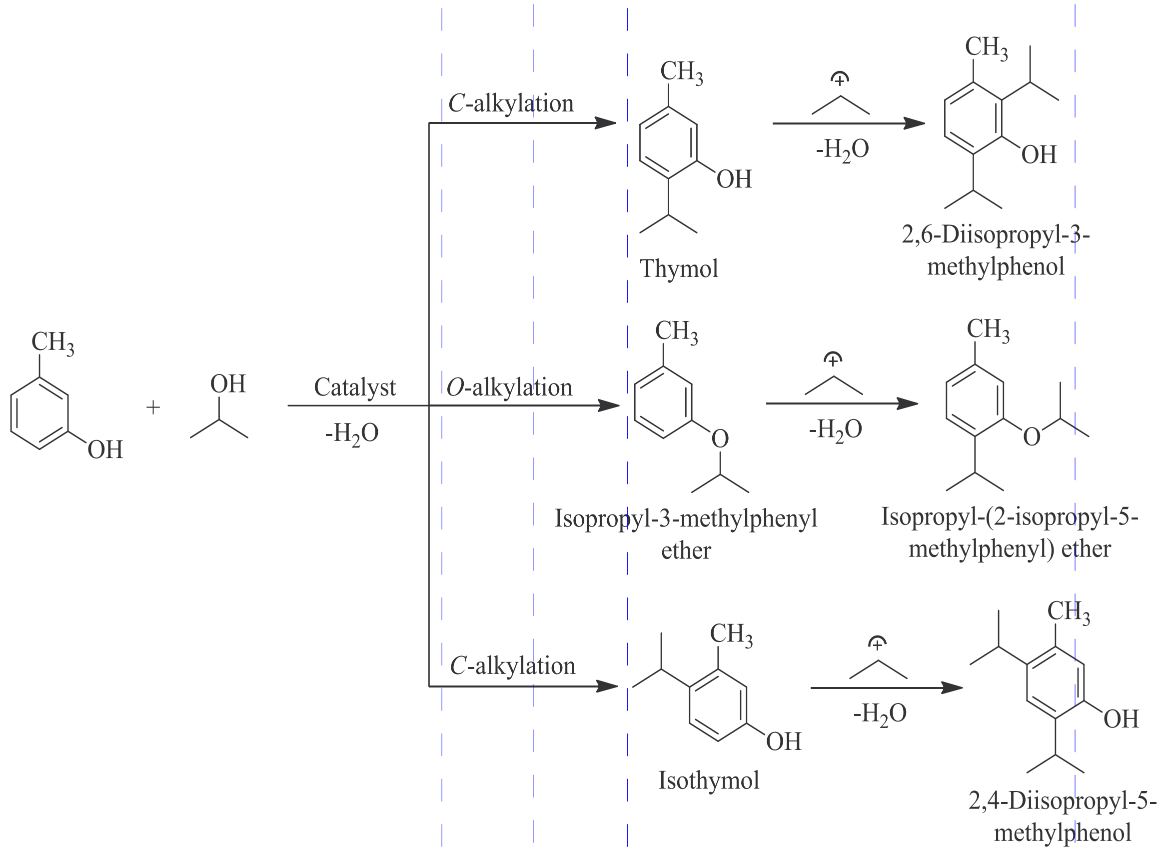
Scheme 2. Friedel-Crafts alkylation.

The reaction mechanism which explains the formation of thymol as the main product involves the formation of a secondary carbocation, which is created when iso-propil alcohol is reacted with BF_3 or any other Lewis acid. The carbocation then reacts with the electron rich aromatic ring, which donates an electron pair to the carbocation, which generates a non-aromatic secondary carbocation on the ring, which expels a proton and forms a double bond which reconstitutes the ring's aromaticity and yields a neutral product. It's important to note that even though all three possible positions have roughly the same electron density, and the deciding factor on which product is formed in the largest quantity, is steric hindrance, which is lowest on the *ortho* position to the hydroxyl and *para* to the methyl, giving thymol as a final product (scheme 3).



Scheme 3. Reaction mechanism of the alkylation of *m*-cresol.

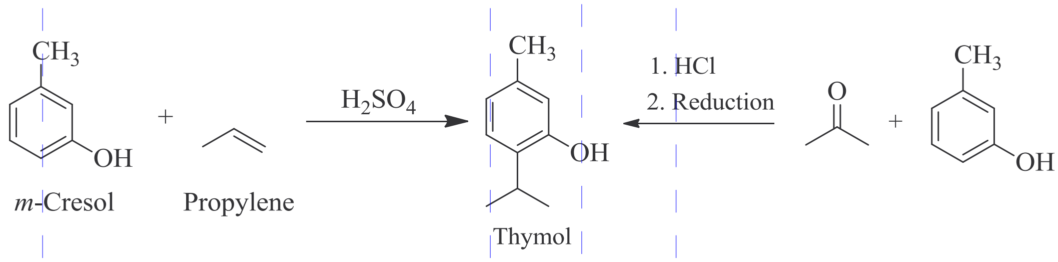
Recently, different investigation groups have been focusing their efforts in the synthesis of thymol by modifying the reaction conditions. For example, In 2008 Selvaraj and Kawi reported a thymol synthesis utilizing different solid acid catalysts using gas phase conditions, which demonstrates that thymol synthesis is still a worthy topic for in-depth study (scheme 4).



Scheme 4. Gas phase thymol synthesis utilizing a solid state acid catalyst, *m*-cresol and isopropanol.

Thymol synthesis using *m*-cresol and propylene.

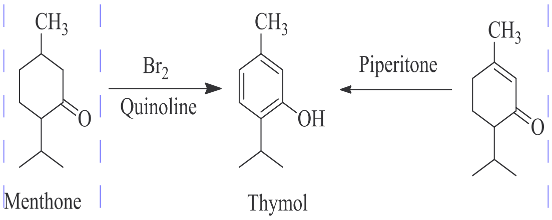
Thymol can also be synthesized with *m*-cresol and propylene with sulfuric acid as a catalyst. This is a very common and straight forward way for synthesizing thymol (scheme 5). Another method is condensation of acetone with m-cresol with hydrochloric acid, followed by a reduction reaction.



Scheme 5. Synthesis utilizing 1-propylene or using acetone.

Thymol synthesis from menthone.

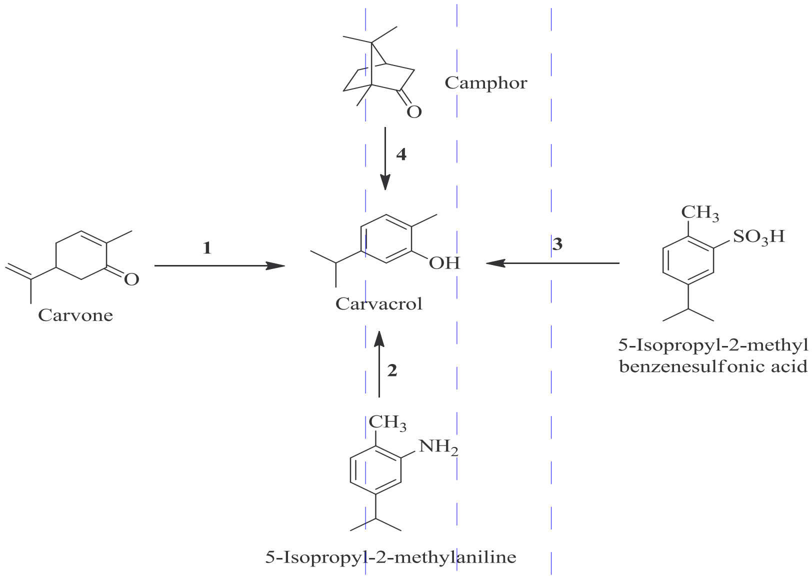
Menthone is reacted with bromine, followed by treatment with quinolone. Also, can be synthesized from piperitone, which is generally obtained from australian eucalipti, poperitone is treated with ferric chloride in order to give thymol (Scheme 6).



Scheme 6. Synthesis utilising menthone.

Carvacrol synthesis

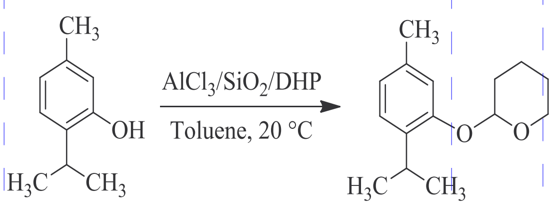
Carvacrol (5-isopropyl-2-methylphenol) is an isomer of thymol, which can be synthesized by heating carvone with aqueous sulphuric acid, by reacting1-methyl-2-amino-4-isopropylbenzene with nitrous acid going through the formation of a diazonium salt as an intermediary, by reacting 5-isopropyl-2-methylbencensulphonic acid with potassium hydroxide, by prolonged heating of calcanfor with iodine, heating carvol with glacial phosphoric acid or the dehydrogenation of carvone with a Pd/C catalyst (Scheme 7).



Scheme 7. Different synthetic routes to obtain carvacrol.

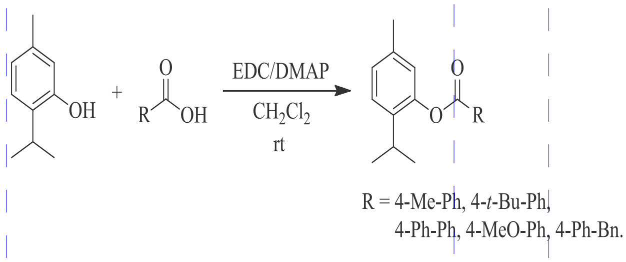
Reactivity of thymol and carvacrol

Based on their chemical structure, thymol and carvacrol, it can be said that they present two reaction sites, the first is based on the reactivity of the aromatic ring, and the second is based on the reactivity of the hydroxyl group. These characteristics allow both compounds to be used as intermediates in the preparation of other biologically important compounds. For instance, in 2008 Camera *et al*. carried out a catalytic study of the O-alkylation of thymol with DHP using various catalysts based on $AlCl_3$ on different silicon supports (Scheme 8).



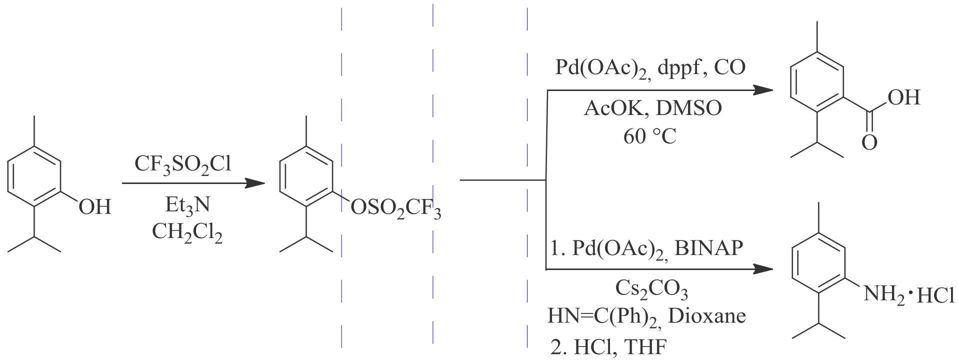
Scheme 8. Tetrahydropiranilation of thymol aided by $AlCl_3/SiO_2$.

Recently, Ortar *et al.* 2012 synthesized a series of esters and amides derived from thymol, which were evaluated as thermo-ionic channel modulators (TRP). TRP channels are involved in a wide variety of physiological functions such as vision, taste, smell, hearing, touch and thermal sensitivity, among others. Some TRP channels act as polimodal sensors which are activated by physical stimuli such as temperature as well as chemical stimuli. The scheme 11 shows how the reaction of thymol with the right carboxylic acid in presence of 1-ethyl-3-(3,3-dimethylaminopropyl)carbodiimine (EDC) as the carboxylic acid's activating agent and 4-dimethylaminopiridine (DMAP) as the nucleophilic catalyst, utilizing dichloromethane as a solvent generate the corresponding esters (scheme 9).



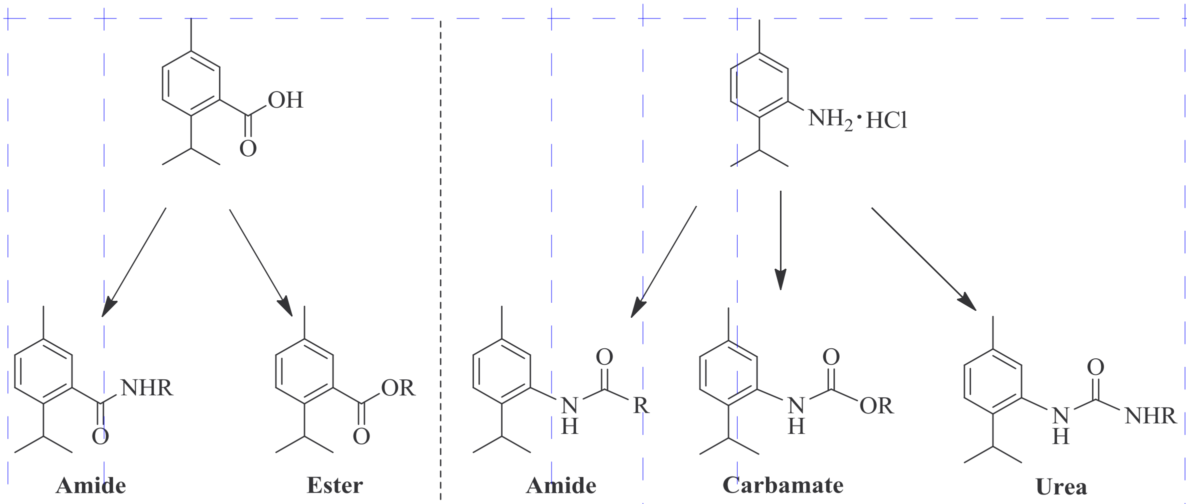
Scheme 9. Ester synthesis using thymol as a reagent.

The same authors describe how thymol can be treated with trifluoromethanesulfonate, which is later transformed into the amine hydrochloride through its coupling with the imine derivef from bezophenone, followed by acid hydrolysis of the resulting imine, and to the carboxylic acid through a hydrocarboxylation reaction catalyzed by palladium acetate (Cacchi *et al.,* 1992; Radivoy *et al.,* 1999; Wolfe *et al.,* 1997) (scheme 10).



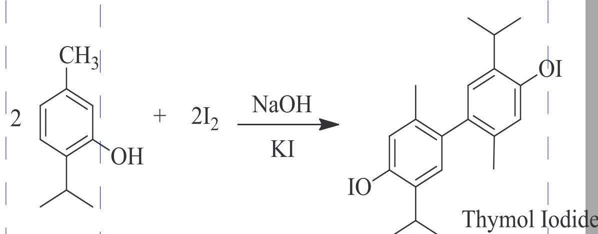
Scheme 10. Carboxylic acid and amine derivatives of thymol.

The amine and carboxylic acid derivatives can be transformed into esters, amides, carbamates and ureas by treating them with appropriate phenols, amines, arylchloroformiates and isocianates (scheme 11).



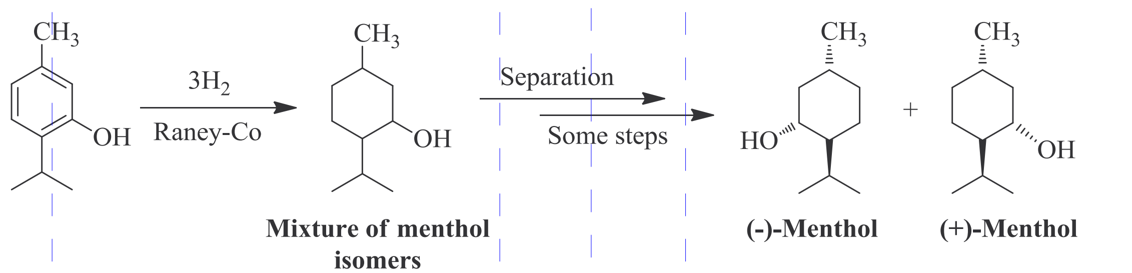
Scheme 11. Thymol derivatives.

When thymol is treated with NaOH in presence of a I_2-KI solution generates thymol iodide, which has antiseptic and antimycotic properties (scheme 12).



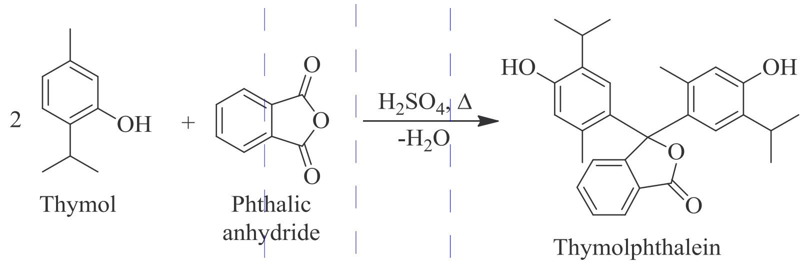
Scheme 12. Synthesis of thymol iodide.

Thymol can also be hydrogenated to obtain menthol (Allakhverdiev, *et al.,* 1995; Allakhverdiev *et al.,* 1994; Krause *et al.,* 1999). The importance of menthol lies in the fact that it is one of the most important terpenoids. After vanilla, menthol is the most widely used aroma in the world. Its use is largely thanks to the cosmetic, fragrance, taste, tobacco, pharmaceutical and oral hygiene industries; furthermore, it is the major component in mint's essential oil (Dudas, *et al.,* 2009). In this sense, hydrogenation of thymol utilizing a Nickel-Raney and/or Copper/Raney catalyst produces a mixture of stereoisomers of menthol, which require a series of steps to separate and purify in order to obtain pure menthol (Barner *et al*., 1994) (scheme 13).

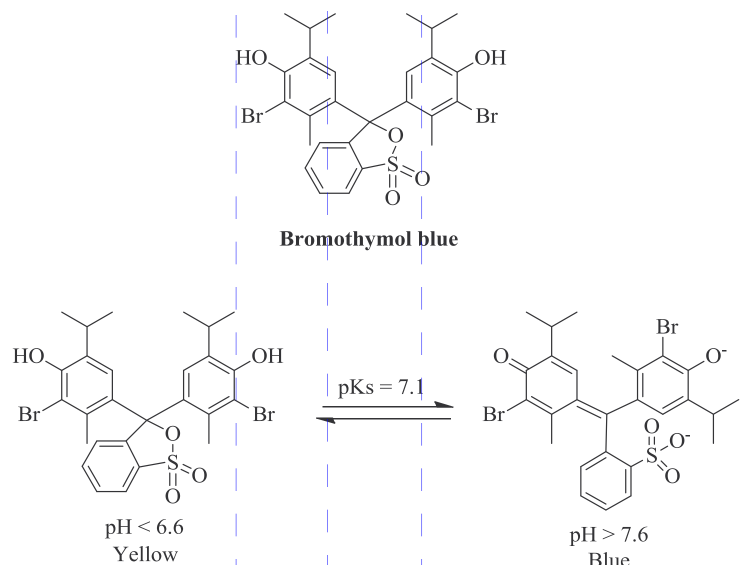


Scheme 13. Menthol synthesis from thymol.

Thymol has also been used in the synthesis of thymolphthaleine, a pH indicator. The reaction consists on reacting two equivalents of thymol with phthalic anhydride catalyzed by sulphuric acid and heating (scheme 14). Additional examples of pH indicators derived from thymol are thymol blue and thymol bromide blue. Thymol bromide blue is an adequate indicator for probing solutions of weak acids and bases, preferably with a solution pH of around 7. One of its typical applications is as an indicator for pH determination in fish tanks and fish farms. It's used to observe photosynthetic activity as well as respiration, because it turns yellow when carbonic acid is formed as a result of the reaction between carbon dioxide (from respiration) with water. It is also used to measure pH in leather processing, among other things (scheme 15).



Scheme 14. Thymolphthalein synthesis utilising thymol and phthalic anhydride.



Scheme 15. Structure of bromothymol blue and derivates

Applications of thymol and carvacrol.

Thymol possesses antiseptic, antimicrobial, antifungal and antioxidant properties. They are attributed to the phenolic hydroxyl group present in its structure. Phenolic compounds are known to be able to react and neutralize free radicals which can negatively affect biomolecules. The use of thymol, along with its isomer, carvacrol is ancient indeed. It was used as far back as the times when the ancient egyptions used it in the mummification process. It is also used as the active ingredient in aromas, perfumes, cosmetics, mouthwash, and innumerable oils and creams formulated to massaging articulations, and even in the treatment of fungal infections of finger and toe nails. According to the Environmental Protection Agency (EPA), there are no known adverse effects of thymol and carvacrol when used in animals and humans (Dorman *et al*., 2000; Aneta *et al*., 2007; Yanishlieva *et al*., 1999; Aeschbach *et al*., 1994). Thymol is generally used as an antimicrobial additive to prevent bacterial food spoilage (Mastromatteo *et al*., 2009).

Carvacrol is a colorless liquid, slightly water soluble and soluble in ethanol and ether. It's used as a food preservative; it's also used by the cosmetic industry, disinfectants and alternative medicine. Its use is also thanks to its antibacterial, antimycotic, analgesic and antioxidant properties. Carvacrol is used in natural and biodegradable disinfectants, frequently alongside other natural compounds such as cyclamen, eucalyptus oil, eugenol and thymol. Also, used as a food preservative thanks to its antimicrobial activity as well as its subtle taste and aroma. It inhibits growth and quantity of many pathogenic bacteria, like Salmonella enteritidis and Escherichia coli. It's usually applied in the gas phase so as to make sure it gets in every nook and cranny. In aromatherapy – a form of alternative medicine which uses essential oils as treatment – oregano's essential oil along with other carvacrol rich extracts are used, due to the chemical's anti-inflammatory, analgesic and bactericidal properties. It's proven effective enough to warrant rigorous scientific investigation ever since the start of the year 2011. A study published in 2005 in the journal "Cytotechnology" reached the conclusion that carvacrol is a strong inhibitor of the carrier cell A549, which is related to lung cancer in humans. The carvacrol found in oregano's essential oil is widely used by the cosmetic industry as a fragrance, as well as a component in soap, creams and perfumes.

CONCLUSION

Oregano's essential oil has multiple applications, many of which are just being discovered, which justifies continuous scientific inquiry and technological development for more efficient, greener extraction techniques. Its components show ample application and potential, which enables prevention and treatment of diverse pathologies, which affect millions of people worldwide. We think everything exposed here warrants enough reason to keep researching the topic, in the struggle to reach a sustainable way of living.

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