https://doi.org/10.54361/ljmr.12.1.06

Assessment of safe dose of Libyan Thymus Capitatus essential oil its use in the manufacture of soft drinks.

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Abstract:

In the present work, the Libyan wild-growing Thymus capitatus essential oil (EO) was evaluated for its manufacturing properties. In the organic food industry, no chemical additives can be used to prevent microbial spoilage. As a consequence, the essential oils (EOs) obtained from organic aromatic herbs and spices are gaining interest for their potential as preservatives. The organic Thymus capitatus, which are common in Libya and widely used in the meat industry, could be used as antibacterial agents in food preservation. The aims of this study were to determine or evaluate the good safe dose can be added to soft drinks.

Thyme plant is one of the natural plants that have Aromatic Perfumes liked by human being, and one of the natural resources that sought by lot of researcher sand scientists to use it in scientific research and studies of perfumes and pharmaceuticals and used as flavour in making a lot of industrial products. Components of thyme oil sold as a spice in the Libyan market extracted by steam distillation, the ratio of aromatic volatile of thyme oil was 2.548% and specific gravity up to 0.9609, while the optical refractive index 1. 5111 where thyme oil extracted will be used as input in some food products for human consumption, the study required conduct some laboratory experiments with respect to toxicity to find the dose that kills 50% of the experimental animals, and then to prove that it has no damage when used repeatedly, in this study, the dose that kills 50% of mice and minimal dose lethal to all mice and maximum non-lethal dose was reached, the use of Libyan volatile thyme oil because of its great and direct relationship, especially its use in the manufacture of soft drinks, we are confident now that the use of 0.1 ml of thyme oil to prepare 1 liter of soft drink for human consumption is safe even if used repeatedly by man every day, and the dose reached was 3.84 mg, which killed 50% of mice, weighing 25 grams. This experiments was conducted at faculty of Medicine in Libya.

Keywords: essential oil; Thymus; antibacterial; antioxidant; Steam distillation;

Introduction:

In Libya, Thyme (local name Zahter) is one of many edible plants that grow in mountainous region (e.g. Al kala). It is often used to flavor meats, soups and stews. The edible parts of the plant are the young stem and leaves. It is one of many plants that are used as raw material without any preliminary preparation. Furthermore, thyme is used as salad ingredients and utilized for garnishes or seasonings. The condiment zahtar contains thyme as vital ingredient (Tukan *et al*., 1998). Thyme (Thymus) is a genus containing about 350 species of aromatic perennial herbs and sub-shrubs to 40 cm tall, belong to the family Lamiaceae. This family is distributed throughout the arid, temperate and cold regions including Europe, North Africa and Asia. It is in leaf all year, flowering from July to September (Gruenwald *et al*., 2004). In the recent years there is a renewed interest in natural products and then on plants as source of drugs, perfumery products pharmaceuticals, , cosmetics and aroma compounds used in fragrances and food flavors. by extraction of essential oils from officinal plants, the most of the natural aromas are obtained Essential oils are natural, volatile and complex compounds characterized by a strong odour, produced by plants as secondary metabolites. The essential oils composition is strongly influenced by intrinsic factors such as, clone, species ecotype, cultivar and ecological factors as climatic conditions, geographical origin soil, technological factors, biotic and cultivation techniques, storage conditions of raw materials ,types of collection processes, , processing technologies. Thus, wild plants of the same species but from different backgrounds can express chemical composition and different characters (Russo et al., 2012). The quality of an aromatic plant and especially its biological properties are correlated to the chemical composition of its essential oils. Furthermore, antimicrobial properties of plants (Antibacterial) have been recognized and used since ancient times in medicine and for food preservation. (Conner, 1993). In this work, the attention was focused on plants of the genus *Thymus*, which is one of the most important used as flavour in making a lot of industrial products. e.g. soft drink. The plants of genus *Thymus* are widely used in food flavouring and culinary preparations, as well as in perfumery, folk medicine and in pharmacological sector as spasmolytic, antiseptic and expectorant (Rasooli & Mimostarfa, 2002). Many works have focused on the chemical characterization of the essential oils of different species of *Thymus* with the aim of verifying the presence of chemotypes either for taxonomic that for biological properties: antimicrobial (Bhaskara et al., 1998, Bournatirou et al., 2007, Amarti et al., 2008), antifungal (Daferera et al., 2000; Arras & Usai, 2001; Omidbeygi et al., 2007; Marandi et al., 2011, Silva et al., 2012) antioxidant properties (Miguel et al., 2004; Sacchetti et al., 2005; Mkaddem et al., 2010). Thyme is Herbaceous plant of the platoon species, grows in mountainous areas, used as a beverage instead of or with tea, added to some food to give it an acceptable flavor, the plant is used in folk medicine frequently where it is prescribed to treat mouth infections, stomach,

intestine and airways, coughing and gastroenteritis and expel intestinal worms, Thyme oil can be separated easily from the plant, and this oil contains phenolic substances such as thymol, as well as Carvacrol substance and impurities such as resins, in addition to the substances listed, the plant contains tannin and many other materials. (El-Gadi A, et al, 1989).

Experiments has shown that thyme has an antibacterial effect (Juven BJ, et al, 1994). has loosening effect on stomach muscles (Rieter M, et al, 1980), trachea (VanDen Broucke Co, et al, 1983). Strengthening the heart (Kulieva ZT, et al, ,1980). Lowers blood pressure and analgesic (Simonian AV, et al, 1975). lowering blood cholesterol (Von Ardenne M, 1981). Anti-oxidant (Baardseth P. 1989). And is useful for the treatment of certain skin diseases (Hagedorn, et al, 1989) it was found that the muscles loosening substances in thyme is a flavonoid. (Rieter M, et al, 1980).

Generally, the ability of *Thymus* to inhibit the growth of pathogenic bacteria has been numerous studies (Alves *et al*., 2000; Al-Tarawneh, 2004; Bounatirou *et al*., 2007). Thyme extracts have shown broad antibacterial activity by inhibiting the growth of both gram-positive and gramnegative bacteria (Al-Tarawneh, 2004; Bounatirou *et al*., 2007).

Becsuse, Many reports have shown that *Thymus* possesses biological properties: antimicrobial activity (Alves *et al*., 2000; Al-Tarawneh, 2004; Bounatirou *et al*., 2007;

Ebrahimi *et al*., 2008), antifungal activity (Grayer and Harborne, 1994; Kalemba and

as well as to strengthen the heart

Kunicka, 2003; Ricci *et al*., 2005) and antioxidant activity (Ricci *et al*., 2005; Bounatirou *et al*., 2007; Al-mustafa and Al-thunibat, 2008).

extracts of thyme, thyme essential oil, thymol and carvacrol were found to have strong inhibition activity against *S. aureus, Bacillus subtilis, Shigella sonne*

and *E. coli* (Fan and Chen, 2001; Ebrahimi *et al*., 2008). Aqueous extracts of thyme significantly inhibited the growth of *Helicobacter pylori* (Tabak *et al*., 1996). Nimri and his co-workers (1999) have screened 15 of ethanol

plant extracts for antibacterial activity. These plants are commonly used in traditional medicine of Libya and other Middle East countries. *Thymus* had shown the least effective antimicrobial activity against *P. aeruginosa* and *E. coli*.

Experiments conducted on laboratory rabbits using aromatic oils extracted from some plant species have proved that 1 mg / kg of solution at a concentration 0.5 -5% is safe and led to heart activation and lower blood pressure. (Guscinov Dla, et al, 1987).

As the thyme oil has an acceptable taste, it can be added to soft drinks, and although thyme is used frequently as a beverage, yet, it is necessary to conduct some experiments to find the dose of thyme oil added to the drinks, which kills 50% of the experimental animals, and then to prove that it has no damage when frequently used.

Materials and methods:

In the initial study, the simplified method was used (Smith, et al, 1960). Where 2 mice were taken (weighing between 22 to 25 grams) that is for each group, were injected with a volume of solution of 0.1 ml per mouse, exponentially concentrations of dilutions in the Peritoneal cavity to reach the dose that kills all the rats and emphasize a higher dose, then immediately mice were observed in terms of behavior and return to normal state when placed on its back and the presence of any thrills or shakes and breathing for a period of 5 hours then every hour for 12 hours and then at 16 hours and then at 24 hours and registration of death if occurred.

from doses given, the dose that killed 50% of the mice and a dose lower than the dose larger ones were selected and give these doses into three groups of mice each composed of a number 8 mice, and thus determine the dose that kills 50% of the mice in a group larger in number than the previous.

Since the thyme oil does not dissolve well in water, a drop of TWEEN 80 was added to 1 ml of solution Which is non-toxic and has no effect of any kind, knowing that thyme oil was tested by dissolving in alcohol and water is added to dilute, but found that ethanol at the added concentrations to a solution of the oil in itself has a detrimental effect on mice and it was replaced with TWEEN 80.

Essential oils extraction:

Thyme oil can be separated easily from the plant, and this oil contains phenolic substances such as thymol, as well as Carvacrol substance and impurities such as resins, in addition to the substances listed, the plant contains tannin and many other materials. [1]

The essential oils and extracts of many Thymus species (Lamiaceae family) native to Mediterranean basin are widely used in pharmaceutical, cosmetic and perfume industry, and for flavouring and preservation of several food products (Bauer et al., 1997). The essential oils of *Thymus* species are rich sources of phenolic monoterpenes such as thymol and carvacrol (Karousou et al., 2005; Miceli et al., 2006). Several studies have been published on biological properties of *Thymus capitatus* (L.) Hoffmanns and Link as antibacterial (Akrout et al., 2010; Cosentino et al., 1999; Usai et al., 2010), antifungal (Goren et al., 2003), antioxidant (Biondi et al., 2006; Bounatirou et al., 2007; Dorman et al., 2004;

Harmandar et al., 2006; Mkaddem et al., 2010) and antiviral activities (Salah-Fatnassi et al., 2010). The essential oils of *capitata* L. and *Thymbra capitata* (L.) Cav) have been investigated by many researchers who reported the chemical variability of essential oils from this species (Akrout et al., 2010; Bentes et al., 2009; Biondi et al., 2006; Bounatirou et al., 2007, 2010; Cosentino et al., 1999; Goren et al., 2003; Karouso et al., 2005; Miceli et al., 2006; Mkaddem et al., 2010; Napoli et

T. capitatus (Syn. *Coridothymus capitatus* (L.) Rchb.f., *Satureja*

al., 2010; Ibraliu et al., 2011). The objective of the present investigation was to get a knowledge on chemical composition and antioxidant activity of *T. capitatus* from Libya by detect Pharmacological dose of thyme oil that used safely with soft drink and for a possible valorization of essential oil and solvent extracts.

Results and Discussion:

1- Solution diluted 30 times: walking was somehow abnormal; no death at 24 hours or even after a week, the experiment was repeated with 8 mice, this was the maximal concentration tolerated by mice with no death.

2- Solution diluted 25 times: this experiment was made with 8 mice, 4 of which died before 16 hours and 4 remained alive even after 24 hours and even at one week, this was the dose that killed 50% of mice.

3- Solution diluted 20 times: lead to poor movement and death before 9 hours, when repeated with 8 mice, all died before 16 hours, this was the minimal concentration killed all mice.

4- Solution diluted 10 times: lead to poor movement before half an hour followed by head falling on both sides during walking then death before 8 hours.

5- Solution diluted 5 times: lead to loss of coordination and consciousness in less than 15 minutes and death before 5 hours.

Conclusion:

1- Maximum non-lethal dose is the solution diluted 30 times.

2- Dose that kill 50% of mice is the solution diluted 25 times.

3- The least lethal dose is the solution diluted 20 times.

4- As oil density was 961 g/l this means that 1000 ml of oil contains 961g, if the solution diluted 25 times, then 25000 ml contains96100 mg

i.e. 25 ml contains 961 mg.

5- if 0.1 ml contains 961 X 0.1/25 = 3.84 mg this is the dose that killed 50% of mice weighing 25 g.

Therefore : dose for every 1g is 3.84 X1000/25 =153.6mg/kg.

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