



MACERATION TECHNIQUES EXTRACTION OF *THYMUS VULGARIS* AND LAUREL (*LAURUS NOBILIS*) LEAVES WITH ANTIBACTERIAL STUDY

Semma H. Shalaal¹, Afrah T. Halail², Fadil M. Hamed³ and Bassam A. Hassan^{4*}

^{1*, 3 & 4}Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Thi-Qar University, Iraq.

²Department of Clinical Science, Faculty of Pharmacy, Thi-Qar University, Iraq.

Abstract

Thymus vulgaris and Laurel (*Laurus nobilis*) leaves belong to Lamiaceae and Lauraceae family respectively. Both are popular plant in Iraq and main vegetable used in Iraqis food and cooking. Both leaves mentioned above possess antibacterial, antiviral and antifungal functions and in commercial medicine as an essential component.

Thymus vulgaris and Laurel (*Laurus nobilis*) leaves were extracted through cold method of extraction by acetone which called maceration. Then Phytochemical tests applied where showed present and absent some chemical compound as showed in table 1 and 2.

In vitro antibacterial activity results were summarized in table 2, of *Thymus vulgaris* and Laurel leaves against some pathogenic bacterial strains *Klebsiella*, *E. coli* which separated from patients urine.

Key words : Thymus, Laurel, Phytochemical, Antibacterial activity and maceration.

Introduction

Extraction is the separation and isolation of active medicinally constituents of a plant using selective solvents through standard procedures. The purpose of extraction is to separate and isolate the soluble plant metabolites, leaving behind the insoluble (residue). The initial crude extracts using these methods contain a complex mixture of many plant metabolites, such as alkaloids, resins, glycosides, sterols, phenolics, tannic acids, terpenoids, coumarins and flavonoids. Some of the initially obtained extracts may be ready for use as medicinal agents in the form of tinctures and fluid extracts but some need further processing.

Maceration one of the most important methods of involved soaking plant materials (leaves or powdered) in a container with a cold solvent and allowed to stand at room temperature for a period of minimum 3 days with frequent shaking agitation. The processed intended to soften and break the plant's cell wall to release the soluble phytochemicals. After 3 days, the mixture is pressed or

strained by the filtration process. In this conventional method, heat is transferred through convection and conduction and the choice of solvents will determine the type of compound extracted from the samples (Azwanida N.N. *et al.*, 2015).

Many natural product occurring compounds found in herbal of plants and spices have various activity compounds (Fadil M. Hamed *et al.*, 2019). In the past 150 years, chemists and pharmacists have been extracted, isolated and phytochemically identified the active portions from plants as try to produce vital pharmaceutical drugs. For example, morphine from opium poppy and Digoxine from *Digitalis purpurea* plant, reserpine (snakeroot of Indian) *Rauwolfia serpentina* (Firas F. Alyaseen *et al.*, 2018).

Medicinal plants have played an essential role in the development of human culture. Medicinal plants are a source of traditional medicine. Among different species of Thymus, (*Thymus vulgaris* L.) is used more than other species in therapeutic dosage forms. In Traditional

*Author for correspondence : E-mail: bassam-abd@utq.edu.iq , bassam_org@yahoo.com

medicine, *Thymus vulgaris* L. is cultivated in many countries by most people especially in rural areas depend on herbal medicines to treat many diseases including inflammation-related ailments such as rheumatism, muscle swelling, insect bites and pains. Also the modern medicine in essential oil of thyme has demonstrated the compounds have shown antioxidant, antibacterial and antifungal properties. (Eqbal M.A. Dauqan *et al.*, 2017).

Thyme is one herb of Lamiaceae family is a group of about 210 genera and some 3500 species. Many of them are commonly used as culinary herbs. They are often cultivated because of their aromatic qualities and also of their easy cultivation. Many species of the family are reported with high phenolic contents and antioxidant capacities (Hayrapetyan and Vardanyan *et al.*, 2013, A. Hassan, 2016).

Thyme dates back to 3500 BC by Sumerians and Egyptians (Hayrapetyan *et al.*, 2013). Its spread to Europe was due to Romans, as they used it to give aromatic flavors to liqueurs and cheese. Thyme is the general name for the many herb varieties of the *Thymus* species, all of which are native to Europe and Asia. Common or garden thyme is considered the principal type and is utilized commercially for flowering and ornamental purposes (Malik N.R. *et al.*, 2017).

Table 1: Phytochemical screening (where, - absent and + present).

Code No	Chemical structure	Chemical test	Test result of Thyme leaves	Chemical note	Test result of Laurel leaves	Chemical note
1	Alkaloids	Dragendroff reagent Wagner reagent	-ve-ve	No formation Orange No Brown ppt	-ve-ve	No formation Orange No Brown ppt
2	Flavonoids	Shinoda test	+ve	Formation of pinkish violet color	-ve	formation of pinkish violet color
3	Carbohydrate	Molish test	+ve	Violet color ring formation	+ve	Violet color ring formation
4	Glycoside	Fehling's test	+ve	Blue color formation	+ve	Blue color formation
5	Tannin	FeCl ₃	+ve	Bluish black color Formation	+ve	Bluish black color Formation
6	Saponin	Shaken of the extraction	-ve	No Formation of foam	-ve	No Formation of foam
7	Sterols	Liebermann burchard	-ve	Formation green-blue color	+ve	Formation green-blue color
8	Coumarine	Filter paper soaked by diluted NaOH	+ve	formation of yellowish green color on filter paper	-ve	No formation of yellowish green color on filter paper
9	Terpenoids	Salkowski reaction	-ve	No Formation of reddish brown color	-ve	No Formation of reddish brown color
10	Resins	Ethanol 95% + boiling + 4% hcl	-ve	Noformation of turbidity	-ve	No formation of turbidity
11	Anthraquinons	Borntrager's test	-ve	NoFormation of red rose color	-ve	No Formation of red rose color

Laurus nobilis L. (Lauraceae), is an evergreen tree or shrub which is native to the Mediterranean region and Turkey. Its leaves, which have been used as a spice since antiquity primarily because of its essential oil content, laurel leaves are widely used as flavor enhancers for foods such as sauces, meats, soups, fish in the food industry as a food preservative.

Additionally, it has antimicrobial and antioxidant activities and may also exhibit antifungal, insecticidal, antiparasitic and antiviral (Beste Bayramoglu *et al.*, 2009).

The present study revealed maceration extracted, phytochemical screened and *in vitro* antibacterial studied of *Thymus vulgaris* and Laurel against some pathogenic bacterial strains (*E. coli*, *Klebsiella*) separated from urine of patients results were summarized in table 2.

Materials and Methods

Materials

Thymus vulgaris and Laurel leaves were collected from the local. Garden of Nasiriya and dried then grounded to a powder then kept in dry container. All chemicals obtained from the college laboratory. The work was performed at the organic chemistry laboratory of Pharmaceutical Chemistry Science department.

Preliminary phytochemical screening

Preparation of acetone extract by macerating 60 grams of *Thymus vulgaris* and Laurel leaves powder in 300 mL of acetone for Two week. The extract solution was filtered and acetone was evaporated on a rotator evaporator under vacuum at a temperature of 450°C one to fifth. The filtrate was used for phytochemical screening to confirm the phytochemicals present by the following test (Fadil M. Hamed, 2019), (F. Alyaseen1 *et al.*, 2018), (Bassam A. Hassan *et al.*, 2018).

Alkaloids (dragendorff's tests), flavonoids (shinoda), glycosides (molish tests), tannis acid (10% fec l3 test), saponins (foam tests), sterols (liberman-burchard test), coumarin (test of filter paper soaked by diluted naoh), anthraquinons (borntreger's test) were carried out As showed in table 1 (Bassam A. Hassan *et al.*, 2019).

Stock solution preparation

The stock solution *Thymus vulgaris* and Laurel was done by dissolving 0.5 gm of the *Thymus vulgaris* and Laurel extracts and its in 10 ml of acetone to get a 50 mg/ml which was the concentration tested as shown in table 3. Sterilization was done by filtration wares through a Millipore 0.45 mm and 0.22 mm.

Bacterial strains	Inhibition zone diameters in mm (Laurel)	Inhibition zone diameters in mm (Thyme)
<i>S.aureus</i>	18mm(S)	14mm(R)
<i>E.coli</i>	14mm(R)	12mm(R)

Performance standards for antimicrobial susceptibility testing.
Resistances: 0-14, Sensitivity:17.



Fig. 1: *S.aureus* susceptible to Laurel in well number 4 in Mueller-Hinton agar

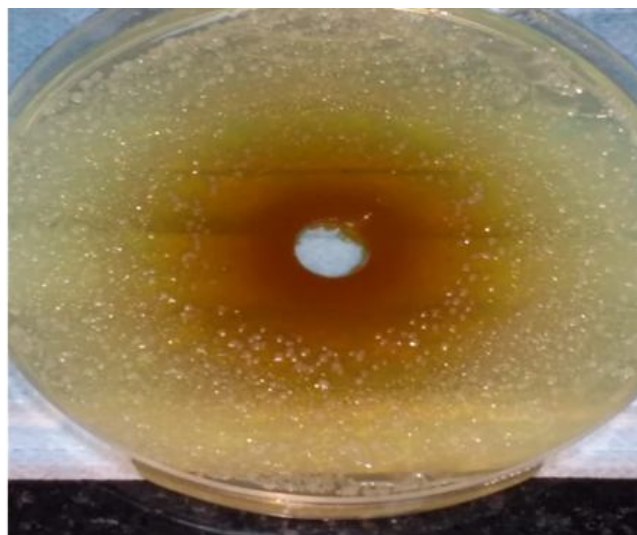


Fig. 2: *S.aureus* susceptible to Laurel in well only in Mueller-Hinton agar.

Biological Activity

After 20 Sample collection, isolation and identification of bacteria to know the biological effect of Thyme extract and Laurel. The extract was tested on two types of bacteria isolated from the urine were tested for antibacterial activity using the diffusion technique on Mueller-Hinton agar, where it was noted that the bacteria (Bassam Abdulhussein Hasan Alsafee *et al.*, 2017), (Maitham M. Abdulridha *et al.*, 2018), (B.N. Berad *et al.*, 2012) and (Bassam A. Hassan *et al.*, 2018).

S.aureus are more sensitive to laurel compared thyme to the resistances while *E. coli* bacteria were resistant to both extract. The analysis were defined as the minimum concentration of an extract with the capacity to inhibit bacterial growth. The result are summarized in table (Uday AbdulReda Hussein *et al.*, 2012) and (Maitham M. Abdulridha *et al.*, 2017).

Conclusion

As discussed previously, Thyme and Laurel leave macerated by acetone, Phytochemical screening of Thyme leaves showed the absence of Alkaloids, Saponin, Resins and Anthraquinons, Terpenoids while presence of flavonoids, tannins, glycosides and Carbohydrate.

Phytochemical screening of Laurel leaves showed the absence of alkaloids, flavonoids, terpenoids, Coumarin and saponins, anthraquinons and steroids while presence of tannins, Carbohydrate, glycosides.

The World Health Organization (WHO) estimates that 80% of the world's inhabitants depend mainly on traditional medicine for their primary health care. It is difficult to speculate the mechanism by which these bioactive compounds act as bactericidal.

The result of biological activity study suggest that the acetone extraction of Luarel (eucalyptol) founded very much effective agonist *Staphylococcus aureus* compare to thymus that resistance to *Staphylococcus aureus* While the *E. coli* show resistance to both extract. The most important in our study the eucalyptol more effective than the thymus. It is difficult to speculate the mechanism by which these bioactive compounds act as bactericidal.

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