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A Comparative Study of Maceration and Soxhlet Extraction for Antibacterial Activity of Rosemary (*Rosmarinus officinalis)* leaves.

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Abstract:
The extraction technique is very critical step in studying the biological activity of medicinal plants; it considerably influences the study's final outcome of the experiment. In order to have proper extraction procedure, it is essential to choice an, cheaper, simpler, , efficient ,quicker, and ecologically friendly way and to get the selected bioactive constituents with greater effectiveness. The aim of this study was to compare between maceration and soxhlet procedures in studying the antibacterial effect of Rosemary leaves. Using hexane and methanol respectively as solvents, the data of % of yield extracts showed that soxhlet was have higher yield than maceration, on the other hand, extracts by maceration methods displayed higher antibacterial activity, this could be attributed to that some biological active compounds are heat thermo labile, but still soxhlet consider a good alternative extraction method. In conclusion, maceration extraction is more effective for obtaining extracts with strong antibacterial activity from rosemary leaves. Future research should check Rosemary leaf extracts against a wider range of bacteria, including Gram-positive, Gram-negative, and resistant strains, for a completing understanding of their antibacterial properties.

Keywords: Maceration, Soxhlet, Rosemary, Extraction, Antibacterial.

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دراسة مقارنة بين النقع والاستخلاص بجهاز سوكسلت لتقييم النشاط المضاد للبكتيريا لأوراق نبات إكليل الجبل (*Rosmarinus officinalis*).

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الملخص:

تُعدّ تقنية الاستخلاص خطوة بالغة الأهمية في دراسة النشاط البيولوجي للنباتات الطبية، إذ تؤثر بشكل كبير على النتائج النهائية للدراسة. ولضمان إجراء استخلاص فعال، من الضروري اختيار طريقة أرخص وأبسط وأكثر كفاءة وسرعة وصديقة للبيئة، والحصول على المكونات النشطة بيولوجيًا المختارة بفعالية أكبر. هدفت هذه الدراسة إلى المقارنة بين طريقتي النقع والاستخلاص بجهاز سوكسلت في دراسة التأثير المضاد للبكتيريا لأوراق نبات إكليل الجبل. وباستخدام الهكسان والميثانول كمذيبات على التوالي، أظهرت بيانات نسبة استخلاص المستخلصات أن طريقة سوكسلت حققت إنتاجية أعلى من طريقة النقع. ومن ناحية أخرى، أظهرت المستخلصات المُحضّرة بطريقة النقع نشاطًا مضادًا للبكتيريا أعلى، وقد يُعزى ذلك إلى أن بعض المركبات النشطة بيولوجيًا حساسة للحرارة، ومع ذلك، تُعتبر طريقة سوكسلت بديلًا جيدًا للاستخلاص. في الختام، يُعدّ الاستخلاص بالنقع أكثر فعالية في الحصول على مستخلصات ذات نشاط مضاد للبكتيريا قوي من أوراق إكليل الجبل. ينبغي أن تختبر الأبحاث المستقبلية مستخلصات أوراق إكليل الجبل ضد نطاق أوسع من البكتيريا، بما في ذلك البكتيريا موجبة الغرام وسالبة الغرام والسلالات المقاومة، وذلك لفهم خصائصها المضادة للبكتيريا فهمًا أشمل.

الكلمات المفتاحية: النقع، جهاز سوكسلت، إكليل الجبل، الاستخلاص، مضاد للبكتيريا.

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Introduction:

Medicinal plants are consumed as alternative medicines and prepared for research purposes by using different extraction processes. The concept of preparation of medicinal plants for experimental purposes involves the suitable collection time of the plant, authentication by an expert, sufficient drying, and grinding. (Abubakar, A. R., & Haque, M. 2020). Then, applying suitable extraction, fractionation, and isolation for the bioactive compound (lngle KP 2017, Azwanida NN 2015). Moreover, it includes the quantification and qualitative valuation of bioactive components. Recently, medicinal plants have gained global importance due to their natural origin, affordability, ease of use, and potentially less side effects. Furthermore, herbal medicine may be useful alternative treatment in case of frequent side effects and drug resistance (Azwanida NN 2015). The plant material's nature, solvent characteristics , temperature, and ratio of solvent-sample all effect on selection of the most suitable extraction method. It also depends on the intended use of the final products (lngle KP 2017). As extraction is the first phase of scientific study, it has a significant effect on the final result of the study. As a result of choosing the most suitable one among the extraction methods, it will be easier to obtain the best data. By time, diverse methods have been developed for the extraction of the desired components in foods and plants. In this study, the plant used for extraction by maceration and soxhlet methods is Rosemary (*Rosmarinus officinalis* L.), which belongs to *lameaceae* family, is a common dense, evergreen, aromatic shrub grown in many parts of the world (Moss et al., 2003). The fresh and dried leaves are frequently used in traditional Mediterranean cuisine as an additive. They have a bitter, astringent taste, which complements a wide variety of foods. They are extensively used in cooking, and distinct mustard smell gives off while they are burned(Lietzow, Julika. 2021). A proper extraction method is crucial for standardizing herbal products since it removes soluble components that are needed while leaving others that are not. There is evidence that the solvents used in the extraction process have an effect on the kind and amount of secondary metabolites recovered from the plants. Therefore, important to select a suitable extraction solvent as well as extraction method in order to achieve the required biological activity from these extracts. Furthermore, for purposes of

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up scaling, such as from bench stage to pilot plant level, selecting the proper extraction method and optimizing various parameters are crucial. (wood-Black,F.,2014)

Therefore, the main aim of this study is comparing the efficiency of maceration and soxhlet extraction methods in extracting bioactive compounds from rosemary (*Rosmarinus officinalis*).

Materials and Methods:

CHEMICALS AND REAGENTS:

The Hexane and Methanol used for extraction were from Sigma, Mueller-Hinton Agar (MHA),Nutrient Broth,Dimethyl sulfoxide (DMSO),McFarland standard.

Plant material

The rosemary plant was collected in July (2024) and documented botanically at a flowerless stage without wild flowers. It was harvested manually from a home farm garden. The plant was washed, spread out and dried in the shade for 3 days. It was crushed using and electric grinder into a fine powder. It was weighed and its weight was 630 grams.

Extraction methods

Maceration method

Eighty grams (80g) from the plant sample were weighted and macerated in hexane (750ml) and methanol (750ml) respectively based on different in polarity for three days. Then, the extracts were filtered with filter paper, the filtrates were evaporated to dryness using a rotary evaporator to get a good extracts. The two extracts were stored in dark bottles and stored in fridge till the time of experiments.

Soxhlet method:

Another Eighty grams (80g) from the plant sample was weighed and then put inside the Soxhlet device. The solvents used were n-hexane and methanol respectively ,the extracts were collected then filtered and evaporated in the rotary device, then stored in dark bottles and stored in fridge till the time of experiments.

Antibacterial Activity Testing

Test bacteria including *Staphylococcus aureus* (ATCC 25923), *Salmonella* (ATCC14028) *Shigella* (ATCC12022),*Escherichia coli* (ATCC 25922) were cultured in MHA plates and incubated at 37°C for 24 hours. The activity of extracts were studied using disk

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diffusion method. The effect of different extracts were measured by calculating the zone of inhibition in millimeter.

Statistical Analysis:

The average zone of inhibition for each concentration of rosemary extracts and for each bacterial strain was measured and then two-way ANOVA analysis was used to compare the antibacterial activity of different concentrations of rosemary extracts for each bacterial strain using Graph pad prism8 software. All data were resulting from at least three independent experiments, with statistical significance set at p- value < 0.05.

Results:

1. The Effect of type of extraction technique on the % of extracts yield:

For Rosemary leaves extracted by both maceration and Soxhlet extraction procedures, the extract yield (expressed as grams per 100 grams of dried leaves) was calculated through the following formula:

% Yield = (Weight of Dry Extract / Weight of Dry Plant Material) x 100%

Table 1: Extracts % yield by maceration and soxhlet methods

METHODS OF EXTRACTION	SOLVENT USED	PERCENTAGE OF YIELD %
Maceration	**Hexane**	**3.5**
	Methanol	**15**
Soxhlet	**Hexane**	**5.85**
	Methanol	**20.38**

2. Comparisons between the antibacterial activity for all different Rosemary plant extracts:

The data (Figure1) show that all plant extract concentrations have different effects on all bacterial strains , the most clear that the effect was concentration dependent, there is significant different between 25% and 50,100% concentrations ,whereas, no significant difference between 50and100% concentrations for most samples. The interesting finding was in Rosemary sample which extracted by methanol using soxhlet method which give antibacterial effect only

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at 100% concentration while lower concentrations had not any effect. Statistical analysis show that there is significant difference among all concentrations as P value was ≤0.0001.

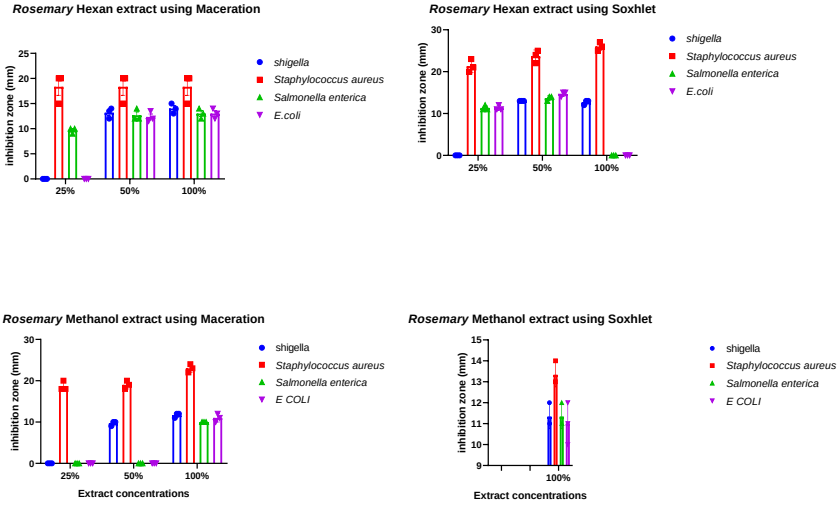

Figure 2: The antibacterial effect of all plant extracts with different concentrations

Discussion:

It is important to select a proper extraction solvent as well as extraction method in order to achieve the required biological activity from these extracts. The main aim of this study was answering the following question: which the best extraction method ,maceration(cold) or soxhlet (hot) for extracting antibacterial active components from Rosemary leaves? To answering this question, four extracts with different two solvents (hexane and methanol) and two different extraction methods (maceration and soxhlet) were prepared as mentioned in materials and methods chapter. After calculating % of yield all extracted were subjected to antibacterial activity assay to assess their antibacterial activity to compare the activities between two extraction methods maceration(cold) and soxhlet (hot). The extract yields of Rosemary leaves (Table1) were comparatively higher when extraction was done by Soxhlet than by maceration, which might be due to the use of heat that makes it easier to dissolve the components with similar polarities into the solvents. The data obtained in this study are

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agreed with (Kim et al. 2019) that also found a relatively higher extract yield in the soxhlet extraction than in the conventional maceration method and temperature was the most vital factor in extracting bioactive compounds from plants. The antibacterial activity of most the extracts are a dose-dependent effect; however, hexane (25%,50 % and 100 %) obtained by maceration were highly effective against S. aureus and Salmonella. hexane extracts of Rosemary leaves showed a comparatively higher zone of inhibitions against S. aureus . Methanol was used in this study to extract the bioactive components by maceration as well, their extracts used in screening the Rosemary activity against mentioned bacterial strains, the results (Figure 3.2) showed that ,only S.aerius bacteria was sensitive to all concentrations of plant extract , whereas other bacterial strains were affected only at 100% plant extracts, the data presented in figure3.1 and figure3.2 are similar to the results Ak genena et.al ,(2008) who indicated that the rosemary extracts showed antibacterial activity, mainly against the Gram-positive bacteria (S. aureus .),on the other hand, data of this study are agreed with Weckesser et al. (2007), how mentioned that effect against the Gram-negative bacteria (E. coli) was less efficient than that presented against the Gram-positive bacteria. By comparing the data of extracted that prepared by maceration method with that by soxhlet apparatus, there is clear indication that maceration method was the most convenient method for extract the antibacterial active component in the Rosemary leaves. The differences in antibacterial effect among extracts with two extractions method used could be a consequence of the extraction temperature resulting in rupture of the plant cell walls, leading to diffusion of the plant constituents into the solvent medium. Moreover, the dose dependent effect of extracts might be due to reduced compound solubility in the solvents, as well as the extraction conditions. However, an increase in temperature causes a decrease in polar dielectric constant, and as a result, fewer polar compounds will be dissolved in it.(Onyebuchi, C., and Kavaz, D,2020).

Conclusion:

The study comparing maceration and Soxhlet extraction methods for antibacterial activity of Rosmarinus officinalis (rosemary) leaves highlights several important findings. Both extraction

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techniques demonstrated antibacterial properties against a range of bacterial strains, indicating that rosemary leaves contain bioactive compounds with potential medicinal benefits. However, significant differences were observed in the efficiency and potency of the extracts. The Soxhlet extraction method, which involves prolonged extraction using a solvent, produced higher yields compared to maceration. This can be attributed to the continuous extraction process, which maximizes the interaction between the solvent and the plant material, allowing for a more thorough extraction of active compounds. In contrast, the maceration method, though simpler and more cost-effective, resulted in higher antibacterial activity compared with soxhlet method, possibly due to active bio compound could be affected by heat.. Based on the results, it can be concluded that maceration extraction is more effective for obtaining extracts with strong antibacterial activity from rosemary leaves. However, soxhlet can still be considered as a viable alternative. Further studies are recommended to identify the specific bioactive compounds responsible for the antibacterial effects and to optimize the extraction methods for even better results.

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