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EFFECT OF LONG STORAGE PERIOD ON THE STABILITY OF ANTIBACTERIAL ACTIVITY OF *MYRTUS COMMUNIS* EXTRACT ON MUTI-DRUG RESISTANT *ACINETOBACTER BAUMANNII*

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ABSTRACT

Introduction: Study of the antibacterial activity and the stability of phytoconstituents of medicinal plants is an important field to be studied. **Aim:** This study aimed to test and compare the antibacterial activity of fresh and old methanol extract of leaves of *Myrtus communis* plant against multi-drug resistant clinical *Acinetobacter baumannii* isolate. **Method:** Disc agar diffusion assay was used to assess the antibacterial activity. Absolute Methanol was used as a solvent, and Soxhlet Apparatus and Rotary Evaporator were used for extraction and evaporating purposes. **Results:** This study proved that both fresh and old extract have near close good activity against tested multi-drug resistant *Acinetobacter baumannii* with a mean diameter of inhibition zones of 22.85± 5, and 23mm ± 5, respectively, and suggested that *Myrtus communis* comprise stable compounds that can withstand the long storage at room temperature and retained its biological activity/s. **Conclusion:** This study concludes that *Myrtus communis* contains active phytochemical compounds of good stability and have good activity against multi-drug resistant *Acinetobacter baumannii*, the result making it a promising resource for a new antibacterial agent that may help in saving a patient's life.

KEYWORDS: *Myrtus communis*, Stability on storage, Multi-drug resistant clinical *Acinetobacter baumannii*.

INTRODUCTION

In developing countries people are more tend for use of traditional medicinal plant than the synthetic ones, as they are more accessible and cheaper and are known to have fewer side effects compared to the synthetic antibacterial agents (Akhavan *et al*., 2016; Al-Zahrani *et al.,* 2017; Mbuni *et al*., 2020).). Also, there is insufficient awareness in developing countries of the dangers of misuse of antibiotics, which is a major reason for the emergence and rapid spread of microbial resistance to antimicrobials. Recently, *Acintobacter baumannii* bacterium is mainly documented as multi-drug resistant pathogen that is resisted three and some times more than three classes of antibiotics. This bacterium has been reported as on the top of the major causes of hospital acquired infections where it forms a very risk factor threats patients' lives where this bacterium is known to boosts hospital infection and epidemic spread, through its ability to survive on the inanimate surfaces inside the intensive care units and transmit to the patients and the ICU health workers via direct contact with these contaminated surfaces (Landelle *et al.,* 2013; Munier *et al*., 2019). Many studies conclude that plants' bioactive compounds have many good therapeutic impacts on many ailment types, by which these plants can promote health and wellbeing (Hannan *et al*., 2008). Recently, the search for effective antibacterial agents from natural sources is increased especially with the increasing rate of resistance of bacteria to the most common used antibiotics. *Myrtus communis*, also named as Myrtle is an important aromatic plant species belong to the Myrtaceae family. This family comprises more than 5500 species, and Myrtle in specific comprises about 16 species that are native to Asia and the Middle East, and in medicine is used traditionally as hypoglycemic, hypotensive and reported to used traditionally in some infectious ailments (Akhavan et al., 2016). Understanding how the biological compounds of medicinal plants can offer defenses and treatments for many diseases is an important point for most researchers, but studying the effect of long storage of these compounds on their stability and maintenance of the same level of proven bioactivity with the use of fresh extract. Accordingly, this study aimed to assess the difference between the antibacterial activity of both fresh and old methanol extracts of leaves of *Myrtus communis* plant.

AIM OF THE STUDY

In a thesis study done by Abdulla-Eltawaty, 2019, a fresh methanol extract was tested for its antibacterial activity against a clinical multi-drug resistant bacterium and compared with the effectiveness of six standard antibiotics against the same isolate, and the study proved that the

fresh extract, showed active inhibition zone against the clinical *Acinetobacter baumannii* 22.85± 5 compared to that of 2.4 ± 3, and 2.00 ± 0.0 revealed from Ciprofloxacin 30ug and Gentamicin 10 ug, respectively, where the tested *Acinetobacter baumannii* was resisted all other antibiotics included in the study; Augmentin 30 ug, Ceftazidime 30 ug, Ciprofloxacine 30 ug, and Vancomycin 30 ug. This study aimed to continue the research and find out whether the tested extract retained its activity against the same isolate after a storage period of about three years at room temperature or whether the prolonged storage period and room temperature affected the and decreased activity, since, this plant is widely used by common people and stored it in their houses at room temperatures.

MATERIALS AND METHODS

Plant Material and plant sample preparation

A Methanol extract of *Myrtus communis* leaves was included in this study. The included extract was obtained via previous extraction done before about three years ago from time of this study and was tested at that time as fresh extract for its antibacterial activity, and then kept in a well closed colored bottle at room temperature. The plant sample was prepared as 100 gm of the tested plant leaves has been size minimized and extracted with use of Soxhlet apparatus and the Methanol solvent was evaporated with use of Rotary evaporator. In the day of antibacterial assay 0.2gm of the extract was dissolved in 2ml of sterile distilled water for final concentration of 100mg/ml.

Bacterial isolate

A multi-drug resistant clinical *Acinetobacter baumannii* isolate is included in this study and was obtained from Al-Akeed labroratory, Benghazi, Libya; which was isolated from a blood sample of patient admitted to Benghazi Medical Center, Libya (2018), and was kept for about three years at 4 $^{\circ}$C in a closed tube contain nutrient agar slant covered with paraffin oil.

Antimicrobial screening assay

Disc diffusion method was used for the determination of the antibacterial activity of the old extract under the same conditions previously used for testing the fresh extract. The Mueller-Hinton agar petri dish surface was seeded with 100µl of the tested *Acinetobacter baumannii* suspension which was freshly prepared and adjusted to 0.5 MacFarland solution. Sterile Discs, 6 mm in diameter (Wattman paper N°1) were placed in four replicate on the seeded Mueller-Hinton agar surface, then 20 µl of tested solution of *Myrtus communis* extract was

added on each disc. The Petri dish was then incubated at 37°C for 20 hours. The diameters of the inhibition zone were measured in mm and the average mean was calculated.

Statistical Analysis: Data were expressed as mean ± SD. The statistical examination was performed utilizing SPSS version 20; A one-way analysis of variance (ANOVA) followed by the LSD Post Hoc test.

RESULTS AND DISCUSSION

This study was carried out to investigate if the long storage period will affect the stability of the antibacterial activity of methanol extract of *Myrtus communis* leaves on Muti-Drug Resistant *Acinetobacter baumannii* compared the antibacterial activity of the plant fresh methanol leaves extract. The results of the study revealed that the tested old stored methanol extract of *Myrtus communis* leaves showed active growth inhibition performance against the tested clinical multi-drug resistant *Acinetobacter baumannii* with a mean of diameter of inhibition zone of 23mm ± 5. This result is near close to 22.85mm± 5 inhibition zone that revealed from the fresh extract of the same plant's part with the same solvent tested previously by Abdulla-Eltawaty, 2019. The Post Hoc test showed no significant difference (P-value ≥ 0.05) in the growth inhibition performance between the fresh and old extracts. This result is in an agreement with Akhavan *et al*., 2016, in that *Myrtus communis* plant in general have an antibacterial activity against *Acinetobacter baumanni* bacterium. Since Akhavan and his team were used different solvents in their study including the Methanol and their study's results conclude that the ethyl acetate extracted fraction appeared as the highest in activity against *Acinetobacter baumanni* at 400 mg/ml compared with Methanol and Ethanol extracts. In specific, from side of concentration view, Akhavan and his team result is in disagreement with this study in that, the concentration of the Ethyl acetate which showed the powerful activity against *Acinetobacter baumanni* in Akhavan study, is three fold higher than the effective concentration of the Methanol extract used in this study; 100mg/ml. The results which highlighted that the Libyan *Myrtus communis* contains bioactive constituent/s of same polarity of Methanol that can diminish the growth of *Acinetobacter baumanni* at lower concentration compare to the one collected by Akhavan team form southern Iran and showed higher antibacterial activity. Since this study proved the good effects of the Libyan *Myrtus communis*, it suggests that this plant may have the ability to inhibit biofilm formation, the mechanism by which this bacterium can survive inside the intensive care units and then be transmitted to the patients and the ICU health workers. This suggestion is in agreement

with that of Alyousef at al., 2021, who said that "methanol extract of *Myrtus communis* could interfere with the formation of biofilm", and also inhibit the swarming motility of *Acinetobacter baumannii*. Alyousef in his study referred their suggestion to the presence of Linalool compound as one of the major bioactive constituents constituting *Myrtus communis* since this compound is well documented as having anti-Quorum sensing and anti-biofilm potential activities. In addition, with the promising anti *Acinetobacter baumannii* activity shown by both the fresh extract (in Abdulla-Eltawaty study, 2019), and the old extract (tested in this study) with insignificant difference, this study suggests that *Myrtus communis* plant contains a stable phenolic compound/s can withstand the condition of long storage at room temperature and retained its activity against *Acinetobacter baumannii*. It has been reported that the phenolic compounds are considered as the major in the plant secondary metabolites that responsible directly for the antimicrobial activities shown by the medicinal plants (Laher *et al*., 2013). Kapcum, in his study observed a high decrease in some bioactive compounds while others bioactive compounds were increased during storage, especially at 30 °C, and with other studies reported that phytochemical compounds stability and their pharmacological activity were different between plant parts and species upon storage (Kapcum and Uriyapongson, 2018; Laher *et al*., 2013).

CONCLUSION

This study proved the leaves of the Libyan *Myrtus communis* contain phytochemical compounds of good stability upon long storage at room temperature and have promising growth inhibition activity against multi-drug resistant *Acinetobacter baumannii*, which suggests this plant considered a promising resource for a new antibacterial agent may help in control the risk of this bacterium and save patients life.

Recommendation

Continued research must be carried out to identify the active compound(s) presented in this plant and to study and understand the mechanism by which this plant acts to destroy this risky bacterium.

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