ANTIMICROBIAL ACTIVITY OF THUJA OCCIDENTALIS EXTRACTS

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Abstract. *The spread of multidrug-resistant bacteria has become a significant cause for concern. A real and topical issue is the control and treatment of bacterial diseases, primarily caused by these bacterial mutants resistant to most available antibiotics, which is a real and pressing concern. Numerous studies concentrate on alternative or complementary antimicrobial strategies due to these facts. Antimicrobial compounds derived from natural resources, such as plant extracts, are garnering increasing interest for their activity against various microorganisms in the hope that, unlike antibiotics, they will be effective without inducing resistance. The purpose of this work is to test the antimicrobial efficacy of Thuja occidentalis (TO) extracts against Gram-negative bacteria and fungi represented by the following reference strains: Shigella flexneri (ATCC 12022), Pseudomonas aeruginosa (ATCC 27853), Escherichia coli (ATCC 25922), Salmonella typhimurium (ATCC 14028), Haemophilus influenzae type B (ATCC 10211), Candida parapsilopsis (ATCC 22019) and Candida albicans (ATCC 10231), respectively. The evaluation was conducted in accordance with ISO 20776-1:2019 by measuring the loss of microbial mass using spectrophotometry to ascertain the optical density (OD). The best effect was for TO2, and it recommends it as a potential future candidate in natural products with antimicrobial activity. Our findings could allow TO2 usage in several areas, such as products for antimicrobial dermal treatments, the area being strongly affected by the increase in antimicrobial resistance to commercial products. The statistical analysis indicates that TO is highly effective against Gram-negative bacteria and Candida spp., making it a promising candidate for future research.*

Keywords*: Thuja occidentalis, extract, Gram-negative, Candida, antimicrobial efficacy.*

INTRODUCTION

Plants have been employed as traditional medicine since the dawn of human civilization. Many ancient writings revealed that plants were utilized for therapeutic purposes long before the Christian era in China, India, Egypt, and Greece. Plants were employed as antimicrobials long before microbiological research began. (SHIV, N.S, 2017). Plant-derived bioactive compounds are regarded as a very good and inexpensive source of pharmaceuticals that play an important part in human health improvement and are used to combat various types of microbial diseases. (KUMAR, P.V, 2006)

Plants are extremely useful in medicine because illnesses produced by drug-resistant bacteria have become a serious therapeutic issue in recent years (OBISTIOIU, D, 2021). Furthermore, plant extracts and phytochemicals are gaining popularity as possible microbial and viral inhibitor sources. As a result, thousands of researchers have focused their attention on the phytochemical elements of plants for human health (JASUJA, N, D, 2012).

Thuja occidentalis (Cupressaceae) is a coniferous tree native to Canada and North America that is planted as an ornamental tree throughout Europe, including Romania (CHOI, H A, 2017). Thuja occidentalis was first grown in North America. It is a native European tree that can grow to a height of 15-20 m. It exhibits pyramidal coniferous traits, with flattened branches and twigs in one plane and small-scale-like leaves. The leaves remain green all year, with a brighter green on the lower side where the resin glands are located. The seeds are found in little, 1-2 cm long green to brown coniferous pins. ()

Thuja has been used in folk medicine to treat disorders of the respiratory system (bronchial catarrh), the urinary and reproductive systems (enuresis, cystitis, amenorrhea), and rheumatic and autoimmune diseases (psoriasis)(ALVES, L.D.S, 2014).

MATERIAL AND METHODS

Plant material and extraction method

TO plants were taken from Liebling, Timis County, Romania (45°34′00″N 21°19′54″E). The extraction was carried out within the Physico-chemical Analysis Laboratory of the Interdisciplinary Research Platform of the University of Life Sciences Timisoara. 10 grams of Thuja occidentalis smaragd (TO 1), Thuja occidentalis golden smaragd (TO 2), and Thuja occidentalis fastigiata (TO 3) were ground and extracted in the 1:10 ratio in an alcoholic mixture 70% (ethyl alcohol 96% Chimreactiv S.R.L, Romania). The extract was left to be stirred using the Hot Plate Stirrer magnetic agitator (IDL LMS-1003, IDL GMBH&CO, England) for 24 hours. Subsequently, the extract was filtered with filter paper, and the resulting mixture was again filtered using a syringe filter Whatman Uniflo 25mm 0.2 μm (Thermo Fisher Scientific Inc., France).

Microbiological method

The Gram-positive reference microbial strains (ATCC) used in this study were obtained from the culture collection of the Microbiology Laboratory of the Interdisciplinary Research Platform of the University of Life Sciences Timisoara.

TO samples were tested on the following reference strains: *Shigella flexneri (ATCC 12022), Pseudomonas aeruginosa (ATCC 27853), Escherichia coli (ATCC 25922), Salmonella typhimurium (ATCC 14028), Haemophilus influenzae type B (ATCC 10211), Candida parapsilopsis (ATCC 22019)* and *Candida albicans (ATCC 10231),* respectively. MIC is defined as the lowest test concentration, which does not cause any visible, detectable growth of microorganisms. Our previous research has described the method as a microbial mass loss by OD measurement by spectrophotometry according to ISO 20776-1:2019.

Microbiological method

The method used is described by ALEXA, E. 2018 and COCAN, I, 2018. A dilution of $^{10-3}$ of strain was used to perform the test, an inoculum equivalent to a standard of 0.5 McFarland. The bacterial strains were revived overnight in the Brain Heart Infusion (BHI) broth (Oxoid, CM1135) at 37 °C and subsequently switched to BHI Agar (Oxoid, CM1136) for 24 hours at 37 °C. The cultures were then diluted to an optical density (OD) of 0.5 McFarland standard (1.5×10^8CFU×mL) using BHI broth and evaluated with a McFarland densimeter (Grand-Bio, England). The dilutions were spotted at a volume of 100 μL in each well of the 96-well microdilution plate using a Calibra 852 digital multichannel pipette. The tested TO was added in the amount of 25 μL, 50 μL, 75 μL and 100 μL. Plates were covered and left for 24 hours at 37 °C. After 24 hours, the DO was measured at 540 nm using an ELISA reader (BIORAD PR 1100, Hercules, CA, USA). Triplicate tests were performed for all samples. Strain suspensions in BHI were used as a positive control.

The method of microdilution in broth is one of the most basic methods of testing antimicrobial susceptibility (CLSI, 2017). The technique involves testing double dilutions of

the antimicrobial agent analyzed in a liquid growth medium distributed in microtitre plates with 96 wells. (CHOUHAN, S, 2017)

MIC is the lowest concentration of antimicrobial agent that inhibits the growth of the body. CLSI has standardized the broth microdilution method to test aerobically growing bacteria, yeasts and filamentous fungi. The EUCAST broth microdilution method is similar to that of CLSI (CLSI, 2017), with changes that typically refer to some test parameters, such as inoculum preparation, inoculum size and MIC reading.

The results are presented as the bacterial growth rate (BGR%)/ mycelial growth rate (MGR%) and bacterial inhibition rate (BIR%)/ mycelial inhibition rate (MIR%), calculated rates using the formulas (1), (2):

$$\text{BGR/MGR\%} = \frac{OD_{SAMPLE}}{OD_{CONTROL}} \times 100\ (\%) \quad (1)$$

$$\text{BIR/MIR\%} = 100 - \text{BGR/MGR}\ (\%) \quad (2)$$

RESULTS AND DISCUSSIONS

The results are presented as values of the growth rate respectively of the inhibition rate and those obtained using the formulae given under the microbiological analysis method.

All the results obtained are presented as follows as graphical representations.

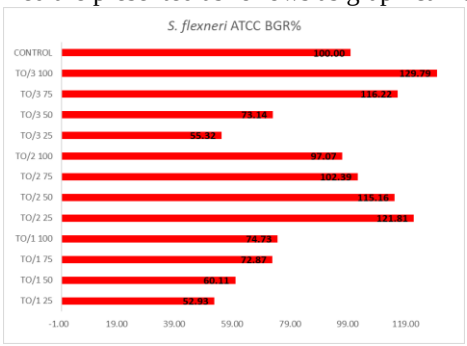

Figure 1. Graphic representation of the activity of TO extracts against *S. flexneri* expressed as BGR%

Figure 1 shows the graphical representation of the antibacterial activity of TO extracts against a Shigella ATCC strain, with value expressed in the form of bacterial growth rate. The potentiation activity of bacterial growth by extract one with values of BGR% ranging from 55,32 % to 129,79 % is evident.

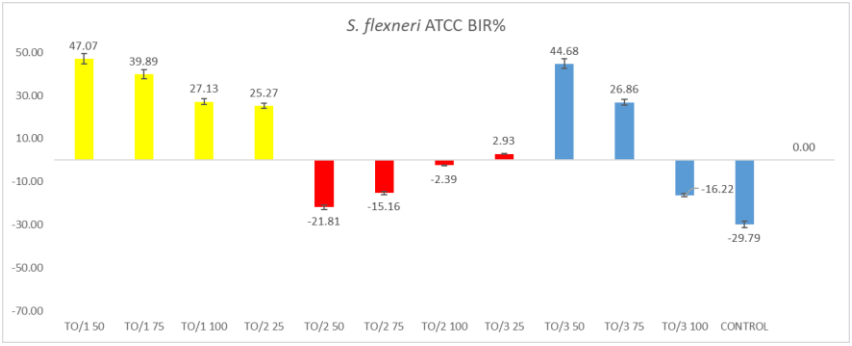

Figure 2. Graphic representation of the activity of TO extracts against *S. flexneri* expressed as BIR%

TO extracts showed a relatively moderate bacterial inhibition activity. TO 1 extract shows high inhibition values, which are, however, in negative correlation with the increase in concentration, so the extract's effectiveness decreases with increasing the amount tested. Extract TO 2 positively correlates with the increase in concentration, so the first 3 quantities tested demonstrated only negative values. Extract 3 shows positive values at the first 2 concentrations tested, the effectiveness decreasing with the increase in the amount tested, resulting in negative values in the last 2 tests. Our findings concerning the effectiveness of TO extracts against *S. flexneri* are similar to the data presented by ALVES, L D. S, 2014 AND NASER, B, 2005.

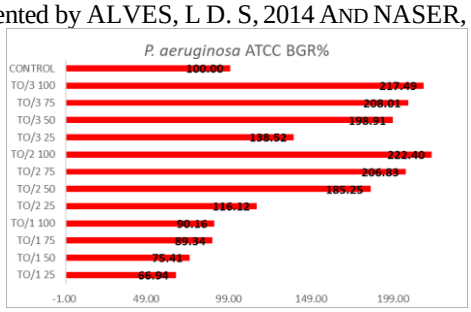

Figure 3. Graphic representation of the activity of TO extracts against *P. aeruginosa* expressed as BGR%

Figure 3 shows the activity of TO extracts reported as the growth rate, the values much higher than those obtained at Shigella. Thus, the TO 3 extract's values vary from 138,52 % to 217,49 %. TO 1 extract has the smallest potentiator effect, ranging from 66,94 % to 116,13 %.

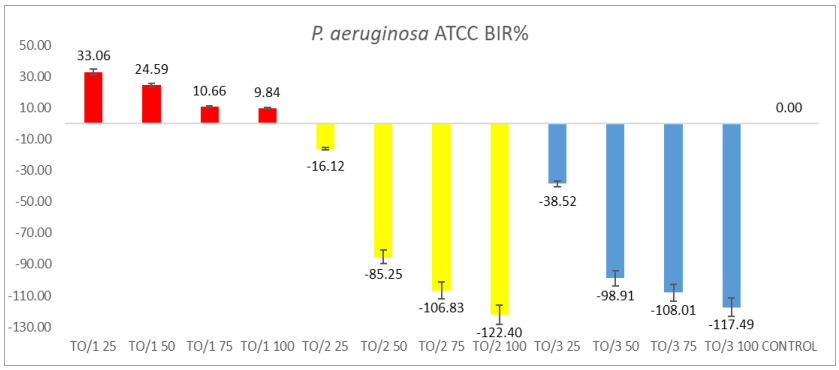

Figure 4. Graphic representation of the activity of TO extracts against *P. aeruginosa* expressed as BIR%

The antibacterial activity of TO extracts against the strain of *Pseudomonas* is very low. In the case of extract TO 1, the evolution is negatively correlated with the increase in concentration and the values of effectiveness decrease. The same evolution occurs in the case of the TO 2 extract and TO 3 extract, and in the case of these 2 extracts, the BIR% values are negative. CARUNTU, S. 2020 presented similar findings to our results.

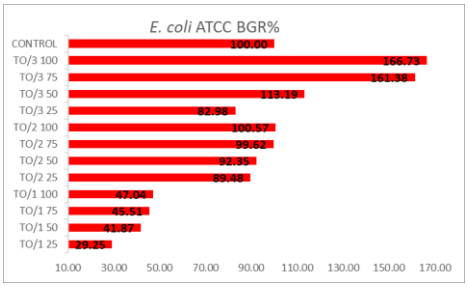

Figure 5. Graphic representation of the activity of TO extracts against *E. coli* expressed as BGR%

The activity of extracts on the *E. coli* strain in terms of growth potentiation is lower than in other strains tested, the highest value being recorded in the case of extract 3 with a value of 161.38%.

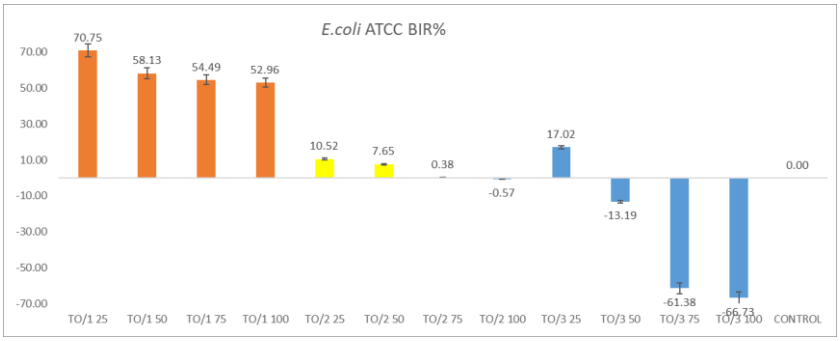

Figure 6. Graphic representation of the activity of TO extracts against *E. coli* expressed as BIR%

Figure 6 shows a graphical representation of the activity of the TO extracts tested against *the strain of E. coli* expressed as the rate of bacterial inhibition. The effectiveness of the extracts is largely supported by the results obtained in the case of the TO 1 extract, which, although it shows a negative correlation with the concentration, all values obtained are positive, ranging from 70,75 % to 52,96 % good. TO 2 extract and TO 3 extract had negative correlations with the increase in concentration. Their efficacy is supported in the case of TO 2 extract by the first 3 concentrations tested. In the case of the TO 3 extract, only the first value obtained at the first test concentration is positive, with a value of 17.02%.

IKRAM, M., E, 2017 and his collaborators demonstrated a good antibacterial effectiveness of the TO seeds against *E.coli*, with values of 20ul extracts tested.

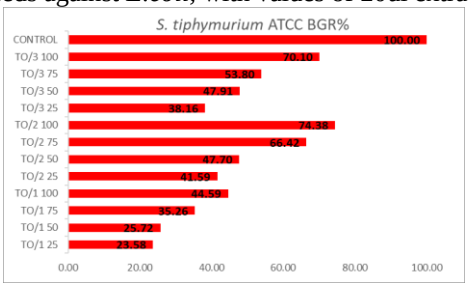

Figure 7. Graphic representation of the activity of TO extracts against *S. tiphymurium* expressed as BGR%

The bacterial growth rate shown in Figure 7 as a consequence of the potentiation activity of the extracts on *the Salmonella* ATCC strain shows an average buff picture with values ranging from 23.58% to 70.1 %.

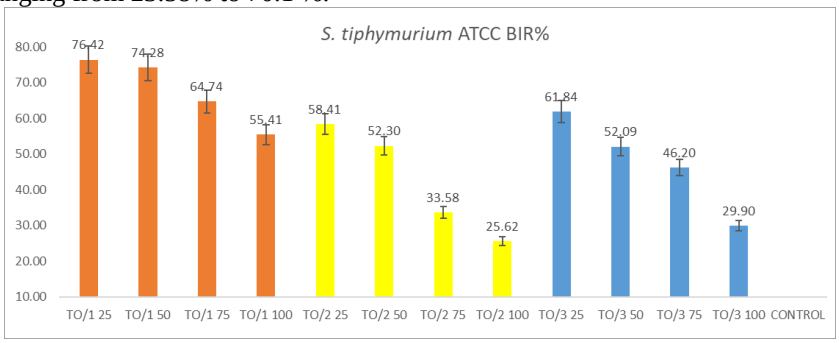

Figure 8. Graphic representation of the activity of TO extracts against *S. tiphymurium* expressed as BIR%

In Figure 8, the rate of bacterial inhibition on the *Salmonella typhimurium* ATCC strain also has average to high values. The results show a similar efficacy between TO2 and TO3 extracts, and in the case of TO1 extract, the values, similar to the other 2 other extracts, are in negative correlation with the increase in concentration. KYOUNG, S. S., 2017 AND SAH, S. N, 2017, both present values of inhibition similar to our findings.

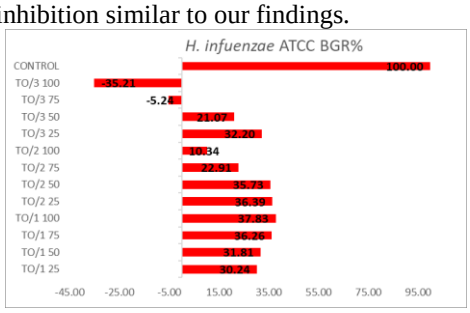

Figure 9. Graphic representation of the activity of TO extracts against *S. pyogenes* expressed as BGR%

The antibacterial activity of TO extracts on the *Haemophilus influenzae* strain is expressed in terms of potentiation effect expressed as BGR%. It demonstrates a weak potentiator effect, with values ranging between -35.21% (a negative value that proves the effectiveness of the TO3 effect) and 37.83%.

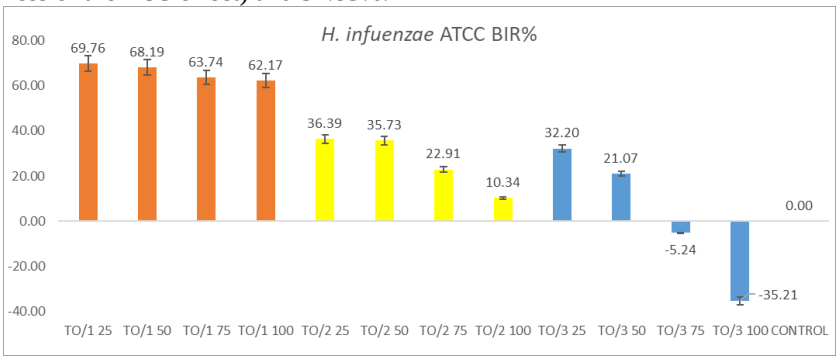

Figure 10. Graphic representation of the activity of TO extracts against *S. pyogenes* expressed as BIR%

Regarding the inhibition rate of TO extracts, BIR% demonstrates an evolution negatively correlated with the increase in concentration for all three extracts. Thus, for TO1 and TO2, all the results are positive but with negative evolution, while, in the case of TO3, only the first 2 concentrations tested inhibited the evolution of the strain, the last two proving negative values, therefore proving a potentiation effect. NAKULESHWAR, D. J, in their research in 2013 demonstrated similar efficient quantities tested.

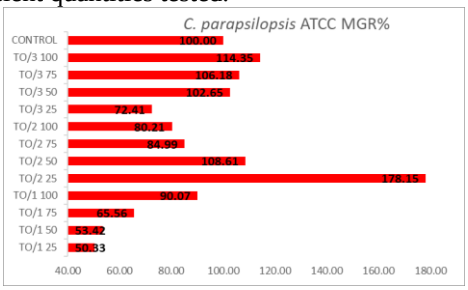

Figure 11. Graphic representation of the activity of TO extracts against *C. parapsilopsis* expressed as BGR%

As antifungal efficacy, the extracts have been tested against two strains of Candida. Thus, in the case of the effect against C. parapsilopsis, the BGR% growth rate was highest in the case of TO2 in the first tested concentration, proving a value of 178.15% BGR%. TO1 presented the lowest value (50.33%) at the first concentration.

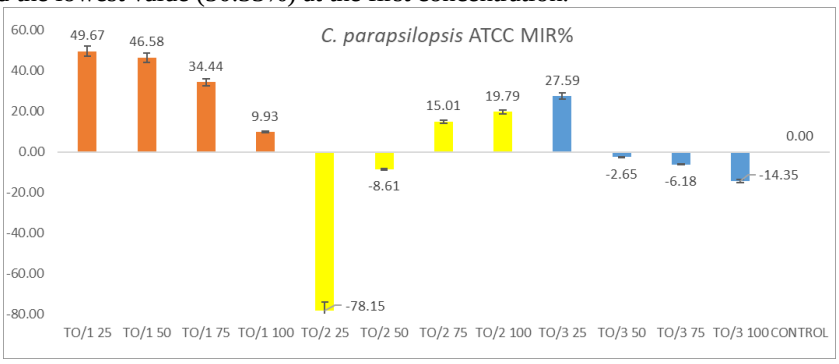

Figure 12. Graphic representation of the activity of TO extracts against *C. parapsilopsis* expressed as BIR%

Figure 12 shows the inhibitory activity of TO extracts against the Candida parapsilopsis ATCC strain. TO1 extract presents positive values but with negative evolution. TO2 is the only extract that shows a positive evolution, but only the last two concentrations tested have positive BIR% values. TO3 showed the weakest antifungal efficacy, the only concentration with a positive result of 25 microlitres. The other three concentrations tested have negative BIR values, the entire evolution being one in negative correlation with the increase in concentration.

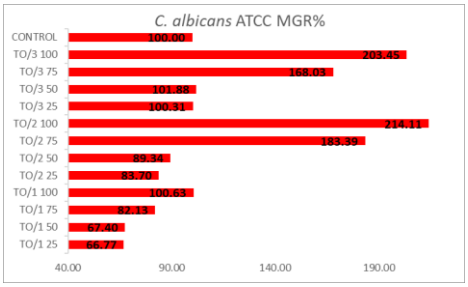

Figure 13. Graphic representation of the activity of TO extracts against *C. albicans* expressed as BGR%

The *C. albicans* strain was moderate to strongly influenced by the TO extracts regarding growth potency. Thus, the values ranged from 66.77% to 203.45%.

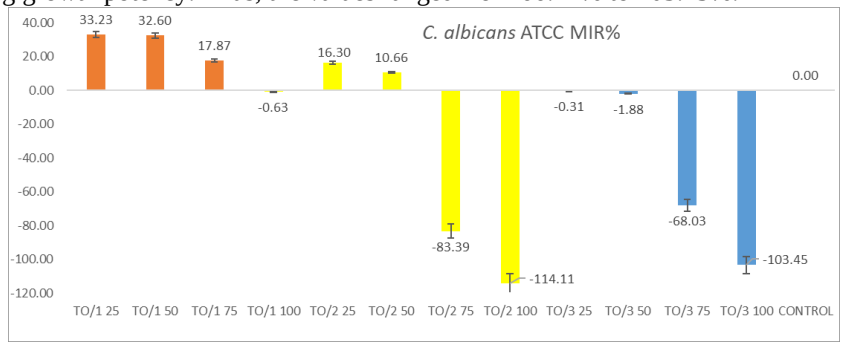

Figure 14. Graphic representation of the activity of TO extracts against *C. albicans* expressed as BIR%

Figure 14 shows the inhibition rate produced by TO extracts on the *C. albicans* strain. TO1 extract is the only one that presents 3 positive values as a value that have a negative evolution in correlation with the increase in concentration. TO2 and TO3 both show a negative evolution with negative values starting either from the third concentration tested (in the case of TO2) or from the first concentration, as was the case with the TO3 extract.

SATISH, K.V., 2010 AND TEKADAY, D., 2020, provided similar findings concerning the sensitivity of Candida strains to T. occidentalis.

CONCLUSIONS

As a result of our research, we can state that all the extracts, especially at the first concentration tested cause an inhibiting effect on Gram-negative bacteria and fungi.

The best effect was for TO2, and it recommends it as a potential future candidate in natural products with antimicrobial activity. Our findings could allow TO2 usage in several areas, such as products for antimicrobial dermal treatments, the area being strongly affected by the increase in antimicrobial resistance to commercial products.

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