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Investigation of the Antimicrobial Activity of Some Species Belonging to Pinaceae Family

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ABSTRACT
The aim of this study was to investigation of the antimicrobial activity of some species belonging to pinaceae family collected from turkey, Duration of study one year 2016.
In this research, the purpose to examine the inhibitory effects by using an oil that extracted of Pinaceae family plants in a way that steam distealation by Soxhlet apparatus were tested against eighteen microorganisms (Srains Gram-positive, Gram-negative and Candida albicanis). By Minimum Inhibition Concentrations (MIC) and MBC as shown, Noted in P. burtia have affected against Gram-positive bacteria: Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis DSMZ 20044, E.durans E. faecium and Bacillus subtilis ADSMZ 1971, L. monocytogenes and L.innoeua where were 9µ/ml,3 µ/ml,1 µ/ml,2 µ/ml,1 µ/ml,5 µ/ml and 2 µ/ml respectively. While has no affected against E. facium. And Gram-native bacteria Salmonella typhimurium, Salmonella kentucky, Salmonella infantis, Salmonella enterlidis SL 1344, E.coli ATCC 25922 Enterobacter aerogenes ATCC 13048, Pseudomonas aeruginosa, Pseudomonas fluorescens DSMZ 50071, Klebsiella pneumonia ATCC 7544 where were 1 µ/ml,2 µ/ml,1 µ/ml,2 µ/ml,1 µ/ml,1 µ/ml,6 µ/ml,2 µ/ml and 1 µ/ml respectively. On the other hand have no affect against C. albicans.

Keywords: *antimicrobial activity, pinaceae family, E.durans, E.faecium, bacillus subtilis*

I. INTRODUCTION

Since traditional medicine has always been the most budget-friendly and easily accessible treatment type, it has been the most commonly preferred primary health care system, especially among poor communities. Throughout the history, traditional plants were used by the local people for therapeutic purposes. As we can understand from the writings, the therapeutic use of medicinal plants, which was firstly used by the Chinese who use the plants as the natural herbal medicines, dates back to ancient times, circa 4000 - 5000 B.C. (1) However, the Rig-Veda, which is known to be written between 1600 - 3500 B.C., states that plants were firstly used for medicinal purposes in India(2). The ancient physicians focusing on ancient medical treatments in India as a local medicine system studied the characteristics and remedial usage of medicinal plants in detail and recorded the empirical data related to their studies. Throughout the world, medicinal plants are an integral component of endemic medical systems. The ethno botany stands out as a rich reference guide in terms of the research and development activities with respect to the natural drugs. The significant historical use is meant by "Traditional" use of phytomedicines (herbal medicines), and this definitely applies to many products that are accessible as "traditional herbal medicines".(3) A great number of the people in many developing countries mostly depend on traditional practitioners and their treatment techniques using plants therapeutically to deal with their health care needs(8). Modern medicine can be applied along with such traditional treatment procedures; however, use of plants for medicinal purposes has not fallen from grace thanks to cultural and historical reasons(4). Natural products have been instruments in treating and preventing human diseases all around the world(7). The production of natural medicines highly depends on Earthbound plants, earthbound microorganisms, marine living beings, and earthly vertebrates and creatures are different origin substances. Various reviews and reports elaborated on the importance of natural products in modern medicine. Within this context(5), it is possible to understand the value of natural products by looking at the following criteria: 1) the rate of the newly introduced chemical substances that have wide structural diversity and serve as a template for semi synthetic and total synthetic modification, 2) the number of the diseases prevented or cured by these chemical entities, and 3) the frequency of these substances used to treat diseases(6).

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II. MATERIAL AND METHOD

Plant Material

Plants material used as wood and leaves as (Table1).

Table:1. Plants material

Plant name	Sampling site	GPS	Plant parts	Collection date (2016)
Pinus nigra subsp. *pallasina*	KASTAMONU	41.422128° 33.769934°	Leaves	3\8\2016
Abies nordmanniana subsp. *equi-trojani*	KASTAMONU	41.067900° 33.733164°	Leaves	30\8\2016
Pinus sylvestris	KASTAMONU	41.422964° 33.769494°	Leaves	22\9\2016
Picea orientalis	KASTAMONU	41.425113° 33.772207°	Leaves	5\10\2016
Pinus brutia	KASTAMONU	41.627530° 34.517599°	Leaves	12\10\2016
Cedrus libani	KASTAMONU	41.358305° 33.759586°	Wood	14\10\2016

III. PREPARATİON OF CRUDE EXTRACTS

Extract essential oils from leaves and wood: Steam distillation by used Clevenger apparatus oil (photo :1) to separate oil of plants.

Photo 2: *pinus nigra*

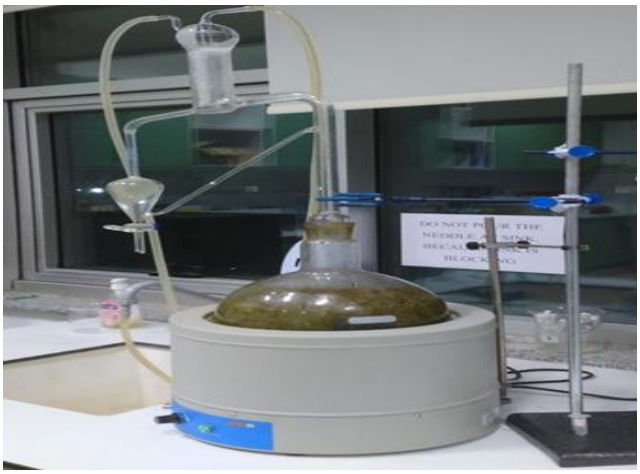


Photo 1: Clevenger apparatus oil

One Kg from *pinus nigra* leaves gave oil about 1.65ml as (Photo :2).
One Kg from *Abies equi-trojani* gave oil about 2ml as (Photo :3), One Kg from *Pinus sylvestriys* gave oil about1.0ml as (Photo :4).

Photo 4: *Pinus sylvestris*

Photo 3: *Abies equi-trojani*

600g from *Picea orientalis* gave oil about 1ml as (Photo :5), One Kg from *Pinus brutia* gave oil about 4ml as (Photo :6).

Photo 6: *Pinus brutia*

Photo 5: *Picea orientalis*

One Kg from *Cedrus libani* gave oil about1.3ml.

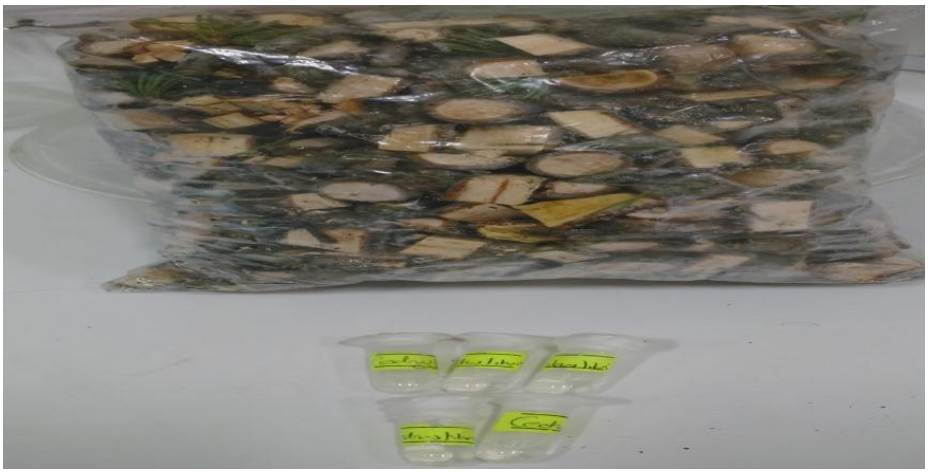


Photo 7: *Cedrus libani*

3.2. Microbial Material

Strains of fungi and bacteria obtained from the biology Laboratory in Kastamonu University:

i. Srains Gram-positive bacteria: *Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis DSMZ 20044, Enterococcus faecium, Enterococcus faecalis ATCC 29212, Bacillus subtilisA DSMZ 1971* (Table 3.2).
ii. Strains Gram-negative bacteria: *Salmonella typhimurium, Salmonella kentucky, Salmonella infantis, Salmonella enterlidis SL 1344, E.coli ATCC 25922, Enterobacter aerogenes ATCC 13048, Pseudomonas aeruginosa, Pseudomonas fluorescens DSMZ 50071, Klebsiella pneumonia ATCC 7544* (Table 3.3).

And fungi: *Candida albicans DSMZ 1386*.

3.2.1. Prepare Microorganisms

A bacteria culture (which has been adjusted to 0.5 McFarland standards (1.0 x 108 CFU/ml) deliberated using the Turbidometer (Oxoid, UK)). Took swab of the bacterial and fungal suspensions by sterile swab then mixed (saline solution) in the test tube (Photo :8). After that, wrote the bacteria and fungi names on the tubes then mixed by the shaker befor used.(9)

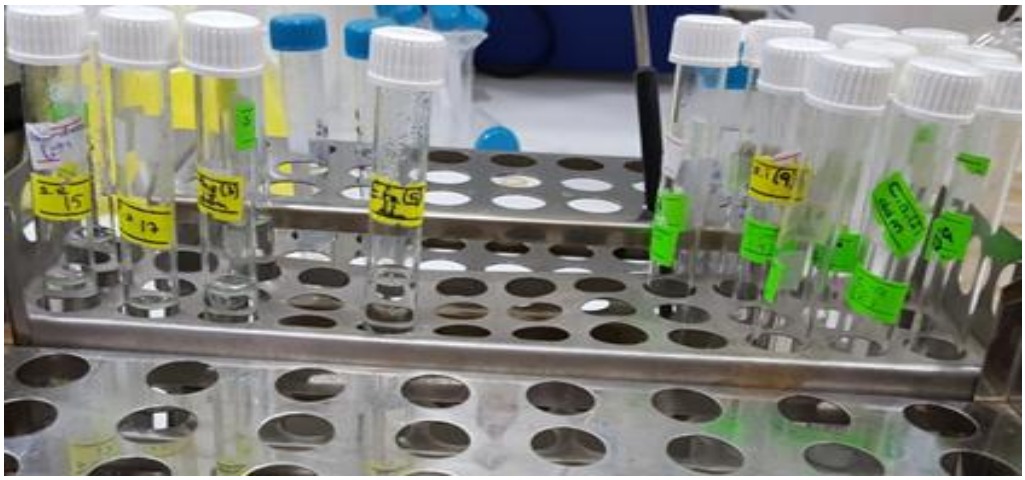


Photo 8: Prepare microbs in test tube

Antimicrobial Activity of Extract Oil

Minimum Inhibition Concentrations (MIC's)

The negligible inhibitory focus (MIC) of an antimicrobial operator is the most minimal (i.e. negligible). We decide the focus in the research facility by hatching a known amount of bacteria with indicated weakenings of the antimicrobial operator this method has just used with samples have a doubt with their results. Have to follow the instructions to get results valid and reliable of any susceptibility test for detecting antimicrobial-resistant bacteria. (10).

Had included the concentrates into the Mueller-Hinton agar that arranged from a dehybasdrated form, the pH of the agar must be in the vicinity of 7.2 and 7.4 at room temperature which every plate containing an alternate grouping of the concentrate (Photo 2.13). Inside 15 minutes of modifying the inoculum to the 0.5McFarland turbidity norms, blended the suspension and weaken it so that the last focus in every well is 5×10^5 CFU/ml. Convey 2.0 mL of the first suspension into 38 mL of water (1:20 weakening) [11].

The inoculator of the prongs will exchange 0.01 mL (1:10 weakening) into every well. Immunize MIC board precisely to abstain from sprinkling starting with one well then onto the next (Fig 2.1) [36]. After hatched 18-24 hours at 37˚C temperature, Read the MIC endpoint as the least grouping of antimicrobial specialist that totally represses the development of the living being as identified by the helpless eye.(12)

3.3.2. MBC

For the distinguishing proof of chemical segments, every example was dissected by GCMS QP 2010 Ultra (Shimadzu) furnished with Rtx-5MS narrow section (30m·0.25 mm; covering thickness 0.25 ?m). Systematic conditions were injector temperature, 250 ∘C; transporter gas Helium at 1 mL/min; infusion mode: split, split proportion 1:10; volume infused: 1 L of an answer in hexane of the oil; and stove temperature modified from 40∘C to 240˚C at 4∘C/min, pressure:100kPa, cleanse flow:3 ml/min. The MS examine conditions utilized incorporated an exchange line temperature of 250˚C, an interface temperature of 250˚C, a particle source temperature of 200˚C. Recognizable proof of the constituents depended on examination of the maintenance times and on PC coordinating against Wiley Data library. At the point when conceivable reference mixes were cochromatographed to affirm GC maintenance times.(13)

IV. RESULTS

In this research, the purpose to examine the inhibitory effects by using an oil that extracted of *Pinaceae* family plants in a way that steam distealation by Soxhlet apparatus were tested against eighteen microorganisms (Srains Gram-positive, Gram-negative and *Candida albicanis*). By Minimum Inhibition Concentrations (MIC) and MBC as shown (Table :2, 3).

Table 2: *MIC results µ/ml.*

Microorganisms	***P. b***	***P. n***	***P. s***	***C. l***	***P.o***	***Abies***
S. enteritidis	2	1	1	2	9	4
C. albicans	-	-	-	-	-	-
S. aureus	9	8	9	9	8	10
E. faecium	2	6	2	1	6	6
E.s faecalis	-	4	2	-	-	-
L. monogtogenies	5	1	6	6	6	8
S. typhimirim	1	1	2	2	7	1
E. aerogens	1	2	1	2	6	3
S. infantis	1	3	2	5	7	4
S.a kentucky	2	1	1	1	7	4
L.innoeua	2	1	2	2	5	2
P. fluorescence	2	2	2	2	6	2
K. pneumoniae	1	1	1	2	5	3
Bacillus subtilis	1	2	1	3	5	2
S. epidermidis	3	4	3	10	8	8
Escherichia coli	1	1	1	2	5	5
P.ariginosa	6	7	4	5	6	4
E. durans	1	1	2	3	5	4

"-''= No affact

Table 3: *MBC results µ/ml.*

Microorganisms	***P. b***	***P. n***	***P.s***	***C. l***	***P. o***	***Abies***
S. enteritidis	1	1	1	-	6	4
C. albicans	-	-	-	-	-	-
S. aureus	2	-	4	2	2	4
E. faecium	2	1	2	-	5	3
E.s faecalis	-	2	2	-	-	-
L. monogtogenies	2	-	4	2	1	4
S. typhimirim	1	-	1	-	6	-
E. aerogens	-	-	1	-	5	1
S. infantis	1	-	1	1	7	1
S.a kentucky	2	-	1	-	6	4
L.innoeua	1	-	1	-	4	1
P. fluorescence	-	-	2	-	4	2
K. pneumoniae	1	-	1	-	5	2
Bacillus subtilis	-	-	1	-	5	2
S. epidermidis	2	2	3	-	4	5
Escherichia coli	1	-	-	-	3	5
P.ariginosa	-	-	1	-	2	2
E. durans	1	1	2	-	5	2

"-''= No affact

Noted in *P. burtia* have affected against Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* DSMZ 20044, *E.durans E. faecium* and *Bacillus subtilis* ADSMZ 1971, *L. monocytogenes* and *L.innoeua* where were 9µ/ml,3 µ/ml,1 µ/ml,2 µ/ml,1 µ/ml,5 µ/ml and 2 µ/ml respectively. While has no affected against *E. facium.* And Gram-native *bacteria Salmonella typhimurium, Salmonella kentucky, Salmonella infantis, Salmonella enterlidis* SL 1344, E.coli ATCC 25922 *Enterobacter aerogenes* ATCC 13048, *Pseudomonas aeruginosa, Pseudomonas fluorescens* DSMZ 50071, *Klebsiella pneumonia* ATCC 7544 where were 1 µ/ml,2 µ/ml,1 µ/ml,2 µ/ml,1 µ/ml,1 µ/ml,6 µ/ml,2 µ/ml and 1 µ/ml respectively. On the other hand have no affect against C. albicans. As shown in (Graphic 8).

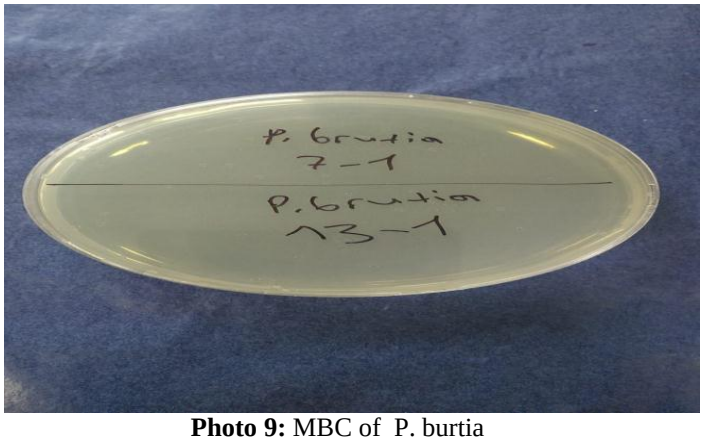


Photo 9: MBC of P. burtia

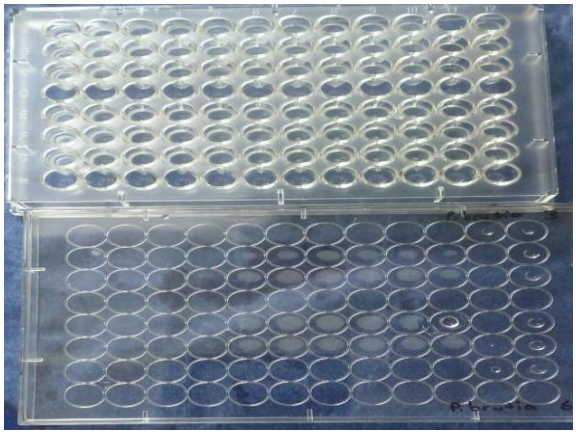
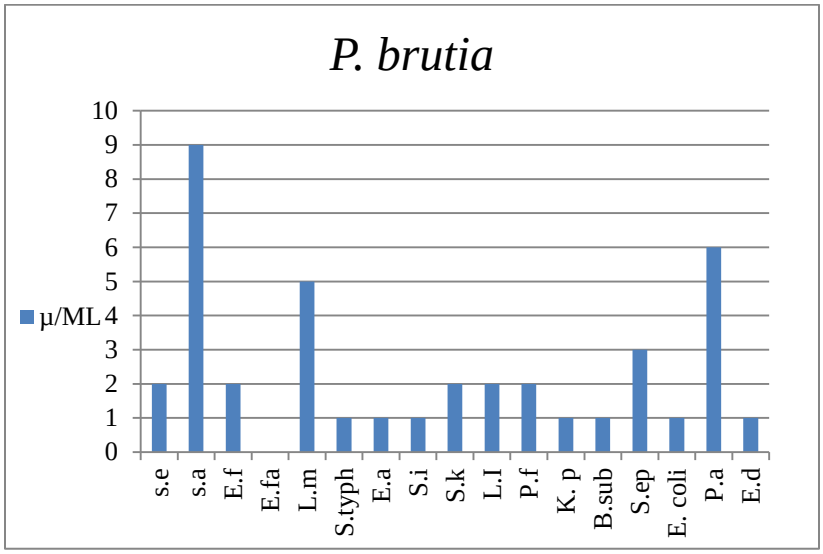


Photo 8: MIC of *P. burtia*



Graphic 1: Antimicrobial Activity of *P. brutia*

P. nigra have affected against Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, Staphylococcus epidermidis DSMZ 20044, *E.durans E. faecium*, E. facalis and *Bacillus subtilis* ADSMZ 1971, *L. monocytogenes* and *L.innoeua* where were 8 µ/ml, 4µ/ml, 1µ/ml, 6 µ/ml, 4 µ/ml, 2 µ/ml, 4 µ/ml and 1 respectively. On the other hand have no affect against C. albicans. And Gram-native bacteria *Salmonella typhimurium, Salmonella kentucky, Salmonella infantis, S. enterlidis, E.coli, E. aerogenes, P.aeruginosa, P. fluorescens and K. pneumonia* where were 1µ/ml, 1µ/ml, 3µ/ml, 1µ/ml, 1µ/ml, 2µ/ml, 7µ/ml, 2µ/ml and 1µ/ml. As shown (Graphic 1).

Photo 11: MBC of P. nigra

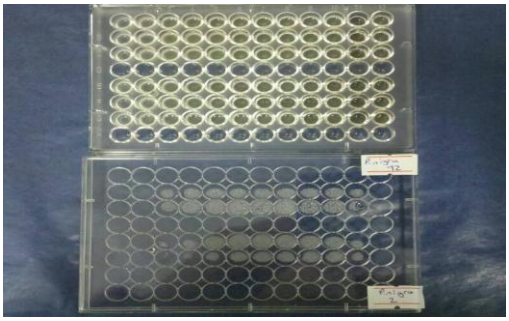
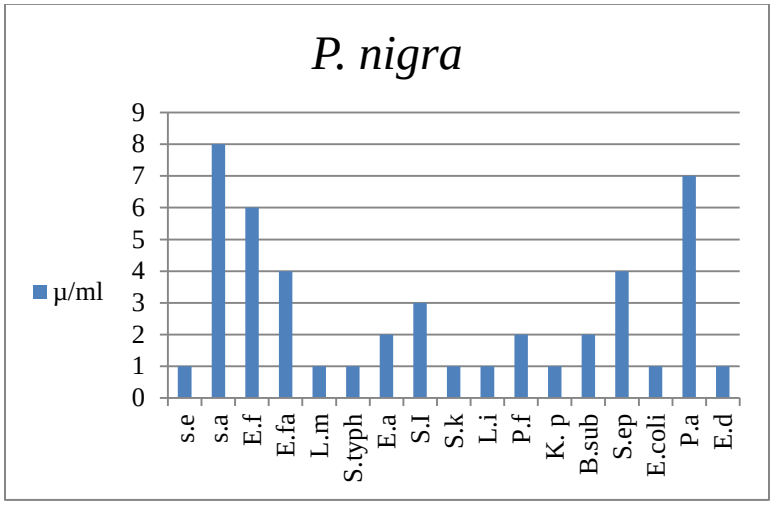


Photo 10: MIC of *P. nigra*



Graphic 2: Antimicrobial Activity of *P. nigra*

P. sylvestris have affected against Gram-positive bacteria*: S. aureus, S. epidermidis*, *E.durans E. faecium,* E. facalis, *B. subtilis* L.*monocytogenes* and *L.innoeua where were 9* µ/ml, 3 µ/ml, 2 µ/ml, 2 µ/ml, 1µ/ml, 6 µ/ml and 2µ/ml respectively. On the other hand have no affect against *C. albicans. And Gram-native bacteria Salmonella typhimurium, Salmonella kentucky, Salmonella infantis, S. enterlidis, E.coli, E. aerogenes, P.aeruginosa, P. fluorescens and K. pneumonia* where were 2µ/ml, 1µ/ml, 2µ/ml, 1µ/ml, 1µ/ml, 1µ/ml, 4µ/ml, 2µ/ml and 1µ/ml.

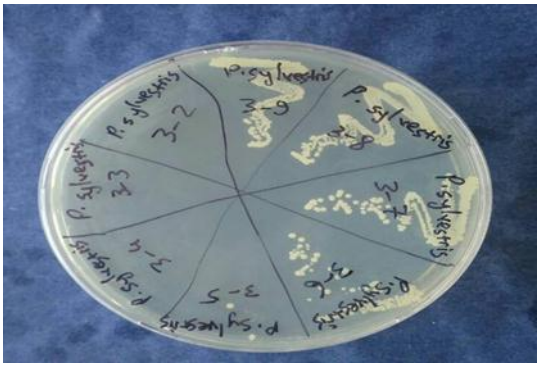
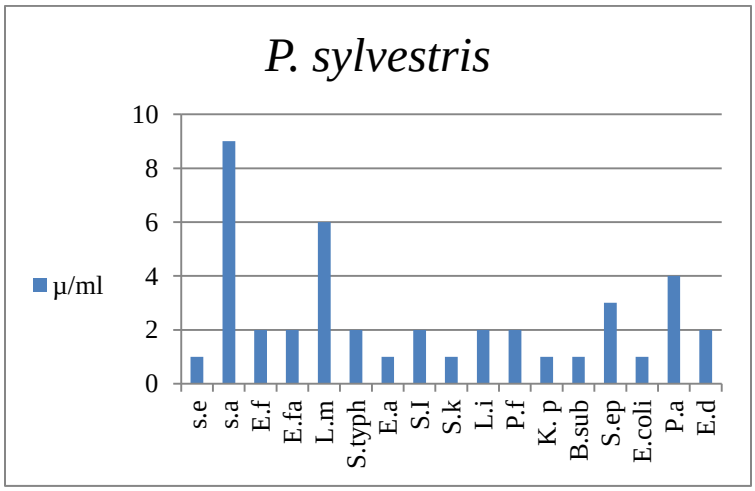


Photo 13: MBC of *P. sylvestris*

Photo 12: MIC of *P. sylvestris*



Graphic 3: Antimicrobial Activity of *P.sylvestris*

C. libani have affected against Gram-positive bacteria*: S. aureus, S. epidermidis, E.durans E. faecium, B. subtilis* L.*monocytogenes* and *L.innoeua* where were 9µ/ml, 10 µ/ml, 3 µ/ml, 1µ/ml, 3µ/ml, 6µ/ml and 2 µ/ml respectively. But have no affect against *E. facalis and fungi of C. albicans. And Gram-native bacteria Salmonella typhimurium, Salmonella kentucky, Salmonella infantis, S. enterlidis, E.coli, E. aerogenes, P.aeruginosa, P. fluorescens and K. pneumonia* where were 2µ/ml, 1µ/ml, 5µ/ml, 2µ/ml, 2µ/ml, 2µ/ml, 5µ/ml, 2µ/ml and 2µ/ml.
As shown (Graphic 3).

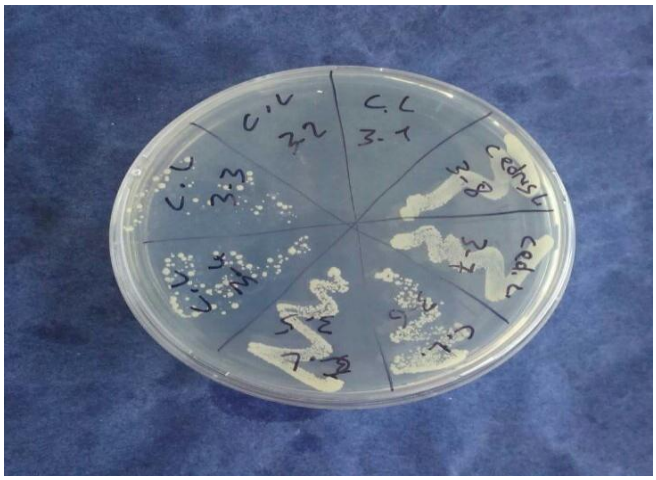
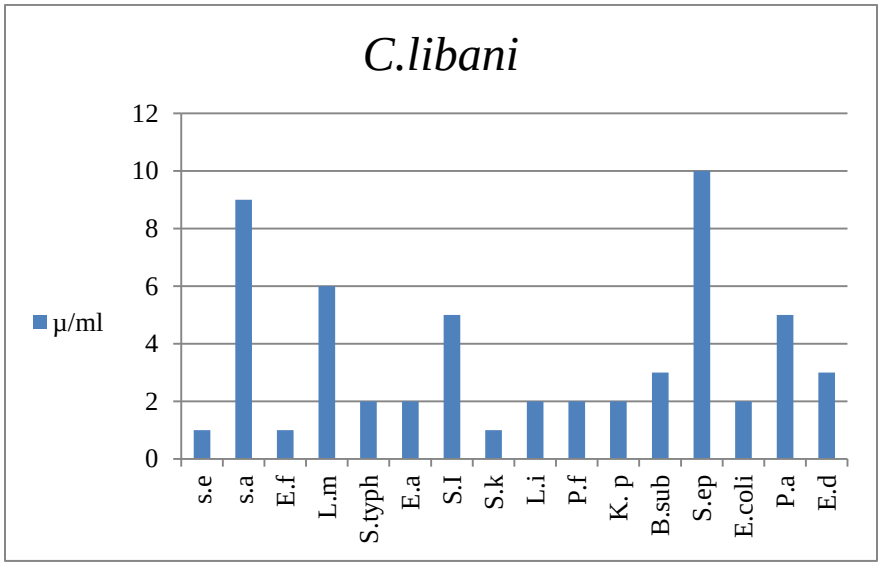


Photo 15. ***C. libani.***

Photo 14: MIC of *C. libani*



Graphic 4: Antimicrobial Activity of *C.libani*

P. orientali have affected against Gram-positive bacteria*: S. aureus, S. epidermidis, E.durans E. faecium, B. subtilis,* L.*monocytogenes* and *L.innoeua where were 8µ/ml, 8*µ/ml, 5µ/ml, 6µ/ml, 5µ/ml, 6µ/ml and 5µ/ml respectively. But have no affect against *E. facalis and fungi of C. albicans*. And Gram-native bacteria *Salmonella typhimurium, Salmonella kentucky, Salmonella infantis, S. enterlidis, E.coli, E. aerogenes, P.aeruginosa, P. fluorescens and K. pneumonia* where were 7µ/ml, 7µ/ml, 7µ/ml, 9µ/ml, 5µ/ml, 6µ/ml, 6µ/ml, 6 µ/ml and 5µ/ml. As shown in (Graphic 4).

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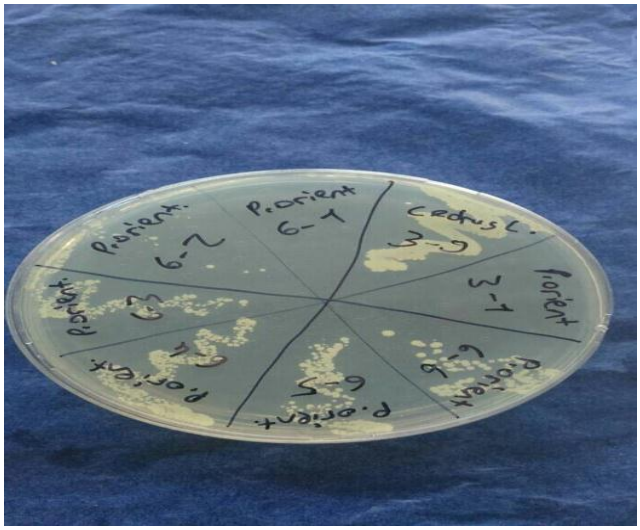


Photo 17: MBC of *P. orientalis*

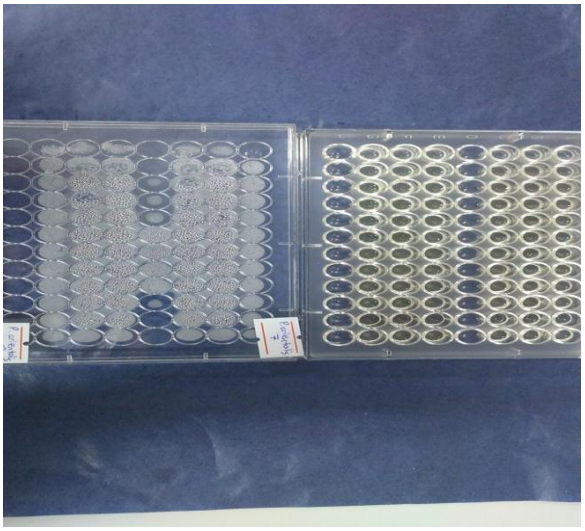
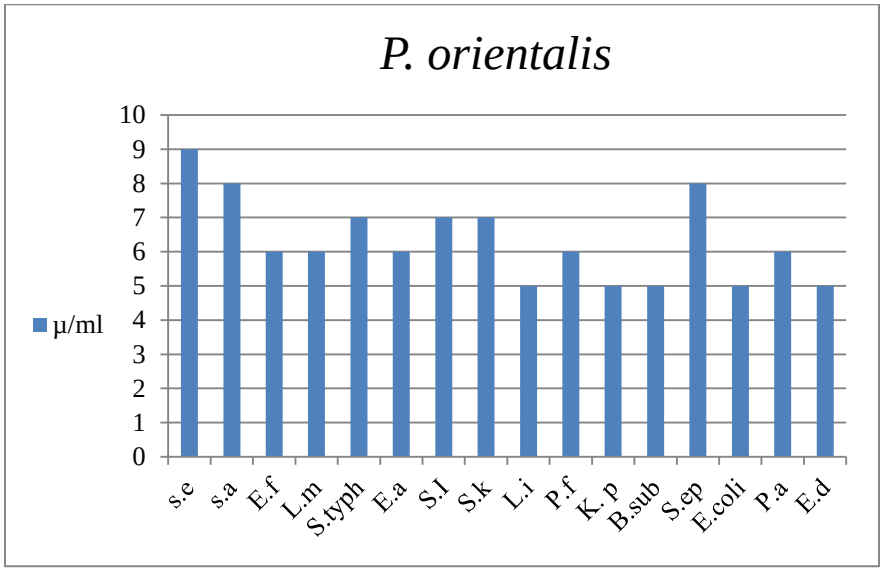


Photo 16: MIC of *P. orientalis*



Graphic 5: Antimicrobial Activity of *P.orientalis*

A. equi-trojani have affected against Gram-positive bacteria*: S. aureus, S. epidermidis*, *E.durans*, *B. subtilis*, L.*monocytogenes* and *L.innoeua where were 10* µ/ml, 8µ/ml, 4 µ/ml, 8 µ/ml, 2 µ/ml, 8 µ/ml and 2µ/ml respectively. But have no affect against E. facium . And Gram-native bacteria *Salmonella typhimurium*, *Salmonella kentucky*, *Salmonella infantis*, *S. enterlidis*, *E.coli*, *E. aerogenes*, *P.aeruginosa*, *P. fluorescens* and *K. pneumonia* where were 1 µ/ml, 4µ/ml, 4µ/ml, 4µ/ml, 5µ/ml, 3µ/ml, 4µ/ml, 2µ/ml and 3µ/ml. On the other hand have no affect against C. albicans. As shown in (Graphic 5).

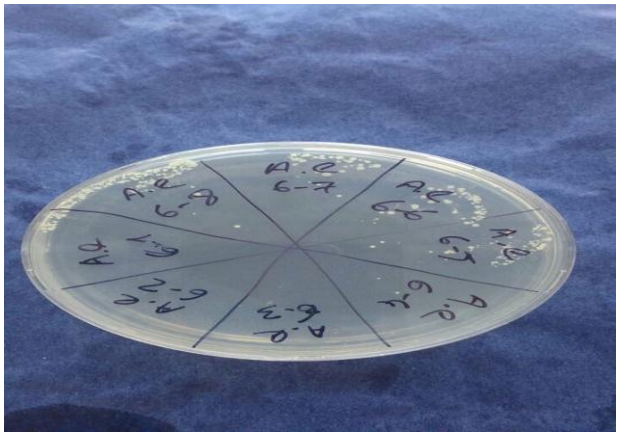


Photo 19: MBC of A. equi-trojani

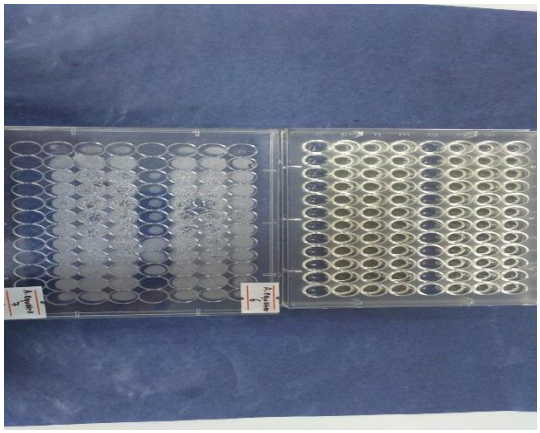
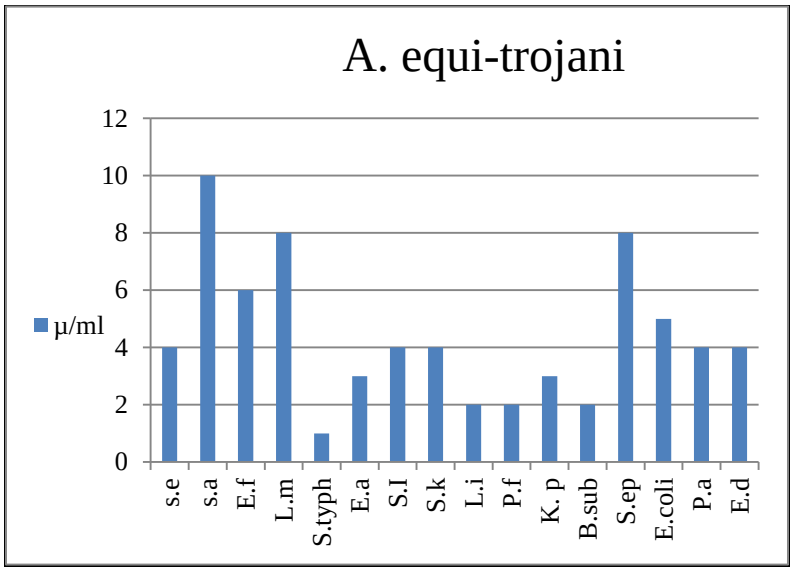


Photo 18: MIC of *A. equi-trojani*



Graphic 6: Antimicrobial Activity of *P.orientalis*

V. CONCLUSION & DISCUSSION

Czerwińska, E., & Szparaga, A. (2015) tested the antimicrobial activity of the leaves, flowers and young sprouts of *Pinus sylvestris* flowers and leaves *essential* oil against Gram-positiveand 12 different microorganisms of fungi. They have found a disc-diffusion method inhibition zone of *Staphylococcus aureus* (12.08 mm), Lavandula vera (10.30 mm), Listeria monocytogenes(12.08 mm) and *Escherichia coli* (ø 8.22 mm) [14]. In our study we determined of MIC value for Staphylococcus aureus 9µg/mL, *Listeria monocytogenes* 6 µg/mL and 1 µg/mL of *E. coli, but no affect againsy fungi.* This difference is possibly related to they used 5 g of driedplant poured over 100 ml of cold water and left for 24 h at 20°C, then filtered.(19)

Hakkı Alma et al. (1999) tested the antimicrobial activity of many parts of various trees grown in the Kahramanmaraş region of Turkey of *Pinus brutia, Cedrus libani, Pinus nigra* and other Chloroform, CH3)2CO and methanol concentrates of leaves in opposite to 15 microorganisms. The outcomes demonstrated that antifungal impacts were not watched for the entire concentrates, E. coli was not repressed by any of the plant separates aside from by the chloroform and CH_3) CO_2 concentrates of the leaves of A. cilicia, which separately demonstrated hindrance zones of 16–18 mm [30]. In our study we determined of MIC value for *Pinus brutia, Cedrus libani and Pinus nigra* (1-9 µg/mL), (1-10 µg/mL) and (1-8 µg/mL), respectively. This difference is possibly related to they used other Chloroform, acetone and methanol extracts of leaves while in our study Steam distillation by used Clevenger apparatus oil.(18)

Eryilmaz, M., Tosun, A., & Tümen, İ. (2016) tried the movement of antimicrobial ethereal concentrates of some Pinaceae and Cupressaceae species gathered from Turkey. The concentrates from *Pinus nigra* Arn., *P. brutia* Ten., *Cedrus*

libani A, Abies equi-trojani, Picea orientalis and other against 7 microorganisms. They have found a disc-diffusion method inhibition zone of *Pinus nigra* Arn agains *Staphylococcus aureus, Staphylococcus aureus* (MRSA) were 8mm. *P. brutia* Ten against Pseudomonas aeruginosa 8mm. *Cedrus libani* has no affect. *Picea orientalis* aganst *Staphylococcus aureus, Staphylococcus aureus* (MRSA), *Escherichia coli*, Klebsiella pneumonia and Pseudomonas aeruginosa werw 7mm, 7mm, 7mm 8mm and 7mm, respectively, but have no affect against Candida albicans with all extract [16]. In our study we determined of MIC value for *Pinus brutia, Cedrus libani, Pinus nigra Abies equi-trojani and Picea orientalis* were (1-9 µg/mL), (1-10 µg/mL), (1-8 µg/mL), (1-10 µg/mL) and (1-8 µg/mL) respectively. Except Candida albicans have no affect.(17)

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