TEST ON HAZELNUT BED BUG (*Gonocerus acuteangulatus AND Palomena prasina)*

AND ASIAN BED BUG(*Halyomorpha halys)*

WITH ESSENTIAL OILS OF ROSEMARY, SAGE, THYME AND MOUNTAIN *PINE* AND HYDROALCOHOLIC EXTRACT OF WORMWOOD IN REFERENCE TO ACETAMIPRID

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ABSTRACT

Bedbugs (*Cimicidae* subsp.) lead to considerable damage in agriculture and are a health problem.

The Asian bed bug that appeared a few years ago in the West does not respond well to treatments with common insecticides and suspended molecules have been readmitted to use due to their toxicity.

In this study the effectiveness of essential oils in the treatment of bedbugs applied directly to the metathorax of insects is demonstrated, with Acetamiprid as a positive control.

In agriculture, it is not possible to use essential oils as such, because they would cause damage to crops and would be dangerous for those who apply them, despite being used for the preparation of common foods, andultimately for the cost. Testsare carried out with some vehicles with low toxicity to humans with not encouraging results.

The composites contained in essential oils and synthetic active ingredients on human and midge acetylcholinesterase were also compared with computational tools (Flare Cresset).

INTRODUCTION

Bedbugs lead to considerable damage in agriculture and are a health problem.

The Asian bed bug that appeared a few years ago in the West does not respond well to treatments with common insecticides and suspended molecules have been readmitted to use due to their toxicity.

In this study , the effectiveness of essential oils in the treatment of bedbugs applied directly to the metathorax of insects is demonstrated.

Computational comparisons are also made of the compounds contained in essential oils, the active ingredients of wormwood extract and other inhibitory molecules of acetylcholinesterase commonly used as a target for insecticidesof

Piperine is used in combination with essential oils as an inhibitor of P450 (main enzymes that metabolize insecticides) but the results were not in line with expectations, urea was also used which generally facilitates the absorption of active ingredients, favoring the crossing of membranes but with not clear results.

Essential oils exert their insecticidal action in toto, although the individual compounds tested on acetylcholinesterase give a slight inhibition (ref 2). From the computational calculations listed below it would appear that several terpenoids could simultaneously occupy the bond pocket giving a synergistic action.

It would be interesting to test in vitro on acetylcholinesterase the individual compounds of essential oils combined with 2 or 3 to evaluate how synergism occurs.

A docking of the wormwood extract compounds is also performed highlighting the efficacy according to the scores as human acetylcholinesterase inhibitors in comparison to the drugs registered against Alzheimer's disease, and the results would seem encouraging.

Treatments used in the test:

Wormwood (*Artemisia absintium)* hydroalcoholic extract

2 liters mains water, 1.5 liters denatured alcohol (brand CRAI purchased at the supermarket in Italy)

500 grams of wormwood (*Artemisia absintium)* dried in pellets (grown on my farm) are mixed, heated to a boil for 30 min. and filtered

Obtained 1,4 liters extract

Absinthe grown in association with Nebbiolo from Barolo, Careglio farm, La Morra (CN) Italy

Essential oils of rosemary (*Salvia rosmarinus)*, sage(*Salvia officinalis)*, thyme, (Thymus vulgaris) mountain pine (*Pinus mugo)*

The essential oils are prepared using fresh aerial parts of the respective species (5 Kg), placed in a 35-liter distiller (35L Moonshine HERBS ESSENTIAL OIL KIT) purchased from galaxyond (China) and distilled in steam current.

Acetamiprid

An agropharmaceutical containing acetamiprid registered in Italy named EPIK is used, purchased from the provincial agricultural consortium of Gallo D'alba (CN) Italy

Indications on the label: 150 ml of product in 100 liters of water

= 1 ml in 0.666 liters of mains water

1 ml of product in 500 ml water is used here

Pesticide dilutions and wormwood extract

1/2 , 1/3, 1/5, 1/7 1/10, as is, mains water as negative control

So 7 groups X 2 (products) = 14 groups of insects

Test Execution Mode:

The insects are taken from the fixtures in the same day, inserted in a petri dish together with a slice of golden apple and then treated

39 capsules (Petri) total

1 bed bug *Cimex lectularius*

7 Asian bedbugs *Halyomorpha halys*

31 bedbugs of the *palomena prasina hazel*

Three drops are applied to the metathorax for each insect (wormwood extract and synthetic pesticide) and 1 drop for essential oils

Table with indication of group number, type of treatment and dilution

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
In Tal Which one	**And as it is**	A 1/2	**E 1/2**	A 1/3	**E 1/3**	**A 1/5**	**And 1/5**	**A 1/7**	**And 1/7**	**A 1/10**	**And 1/10**	**A THYME**	**C**
*	*	*	*	*		*				*			

*= ASIAN BED BUG

So fourteen groups with an Asian bed bug in seven

C= control= mains water

A= wormwood, A1/2= diluted wormwood extract 50%

A(1/3....1/10)

E= Epik as above

TO THE BED BUG IS USED WORMWOOD EXTRACT AS IT IS

FOR EACH GROUP 3 SPECIMENS

Results

Epik (pesticide):

Champion N° 12 alive

Champion N° 10 dead 1 insect out of 3

Sample N° 6 deaths 2 insects out of 3

Champion No. 2 and 4 all dead

Absinthe alcohol extract:

sample 1, 3, 5, 7, 9, 11 all alive

Bed bug(1 specimen) died

Sample 13 with **thyme** alcohol extract at life

Essential oils

1 drop on the metathorax

Sample num 15 rosemary essential oil, 2.3 3 drops 2.2 2 drops 2.1 1 gtc

Champion num 16 sage all 1 drop

Sample number 17 thyme 1 drop

Sample num 18 mountain pine 1 drop

Undiluted essential oils test result

The treated bedbugs died almost immediately with the essential oil of rosemary and sage, 2 videos uploaded on YOUTUBE (visible only to those who have the link)

https://youtu.be/u3w5M-hhf1A

https://youtu.be/_i_TJmQlokk

With **thyme essential oil** insects died in about 1/2 hour

With **the essential oil of mountain pine** (test on 2 insects) one died almost instantly the other in a few minutes

Dilution of rosemary essential oil with tween (polysorbate) 20 (farmalabor)

Polysorbate is used to solubilize essential oil in water (purchased at the pharmacy)

2 ml of rosemary essential oil is added to 1 ml of tween 20

Water is added to the essential oil + tween solution (Sigma Aldrich analytical grade purity)

Tween +R oil	1/2	1/3	1/5	1/7	1/10	1/15	1/20	1/30	1/40	1/70	1/100
M	M	V	M	V	V	V	M	M	M	V	V
		**		**	**	**					

V= live (insect)

M= dead

** = not very viable, 1 drop on the hemithorax, only 1 bed bug for each dilution

Dilution with extra virgin olive oil rosemary essential oil

1/2	1/5	1/10	1/20	1/50	1/100
V **	V **	V **	V	V	V

Only 1 bed bug per dilution

After the application of the drop of solution there is a reaction comparable to convulsions for vertebrates, and then a slow recovery in 15-20 hours

Sage essential oils diluted with acetone

2 ml of essential oil is diluted with 2 ml of acetone, followed by subsequent dilutions with acetone

1/2	acetone	1/5	1/7	1/10	1/15	1/20	1/30	1/40	1/70	1/100
M	V	V**	M	V**	V **	V	V	V	M	V

Only 1 bed bug per dilution

After the application of the drop of solution there is a reaction comparable to convulsions for vertebrates, and then a slow recovery in 15-20 hours

Sage essential oils diluted with alcohol

2 ml of essential oil is diluted with 2 ml of alcohol

1/2	alcohol	1/5	1/7	1/10	1/15	1/20	1/30	1/40	1/70	1/100
M	V	V**	V**	V	V	V	V	V	M	V

Only 1 bed bug per dilution

After the application of the drop of solution there is a reaction comparable to convulsions for vertebrates, and then a slow recovery in 15-20 hours and death after a few days for less viable insects (**)

CONCLUSIONS

A treatment test of the green bed bug and hazel (*Palomena presina, Gonocerus acuteangulatus) and* Asian bed bug (*Halyomorpha halys)* is carried out with essential oils of rosemary, sage, thyme, mountain pine, and hydroalcoholic extract of wormwood, using as positive control (Epik), synthetic insecticide (acetamiprid active ingredient 50 g / L) and negative water control.

Applying a drop of essential oil to the metathorax of insects caused their death in some cases almost instantly video https://youtu.be/u3w5M-hhf1A

https://youtu.be/_i_TJmQlokk

The alcoholic extract of wormwood was lethal for the bed bug *Cimex lectularius* while for the other species of bedbugs caused slight numbness for the highest concentrations.

The synthetic insecticide was totally lethal for the concentration indicated on the label and lethal at 50% for the dose indicated on the label diluted to 1/5

Diluting the essential oils with extra virgin olive oil has a considerable reduction in toxicity equally for acetone and ethyl alcohol, for dilution to 50% there is almost immediate death, for the other dilutions to a first "convulsive" phase there is a slow recovery.

The essential oils tested here are all effective and although they are natural, the cost hinders their use, which in the retail purchase is around the euro per gram and this discourages their use.

If the essential oil, however, for example for the treatment of the stone bed bug is produced on the farm, the aromatic waters are recovered, that is, the aqueous residues of the hydrodistillation that contain 1% of essential oils as a basis for the treatments, perhaps it is possible to obtain a good result without residues with acceptable costs.

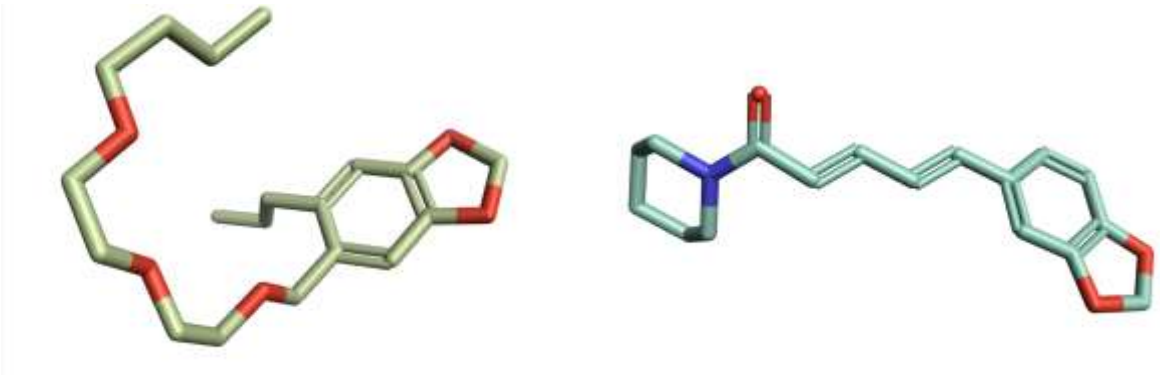
An unknown remains the organoleptic aspect, the oils could alter the taste or aroma of food

PIPERNA

In some formulations of natural insecticides, piperonyl butoxide is added as a co-formulant, as an inhibitor of cytochromes responsible for insecticide metabolism, increasing their effectiveness

The idea of adding piperine to essential oils (whose 2D structure formula is similar) to increase their effectiveness, given the cost of the essential oil.

Piperine (a yellow solid) dissolves well in alcohol, while essential oils do not form clear solutions in alcohol.

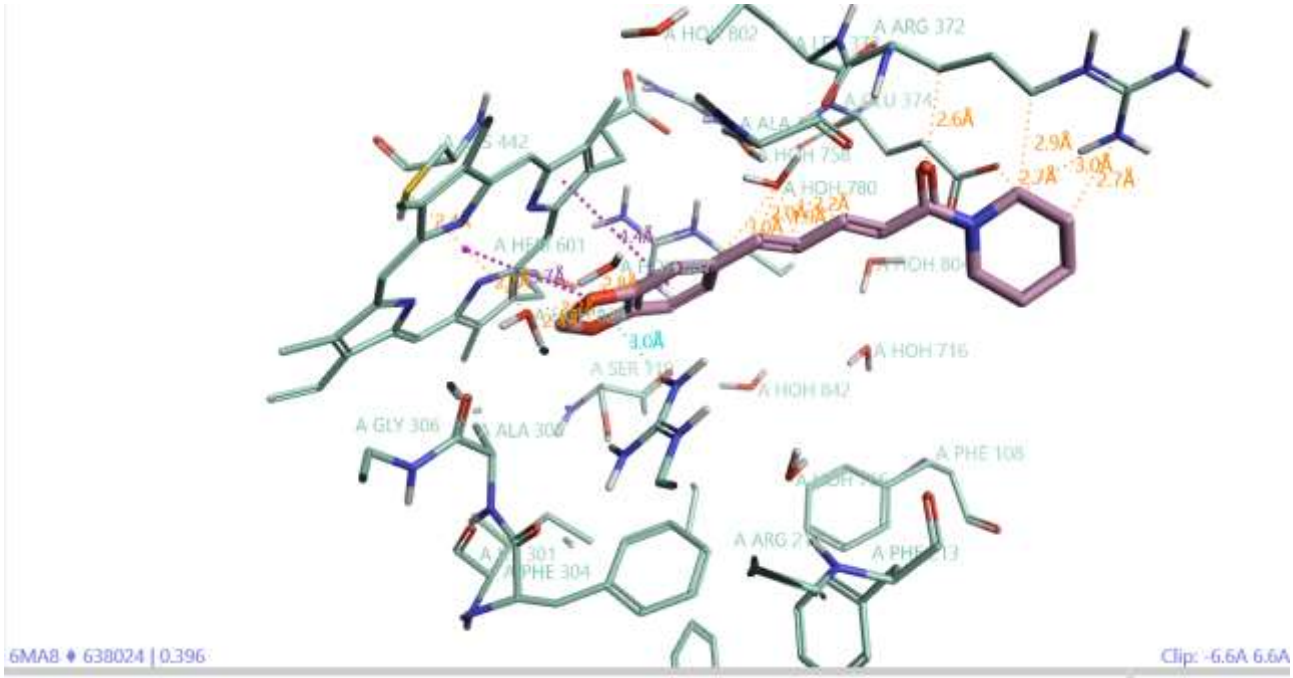


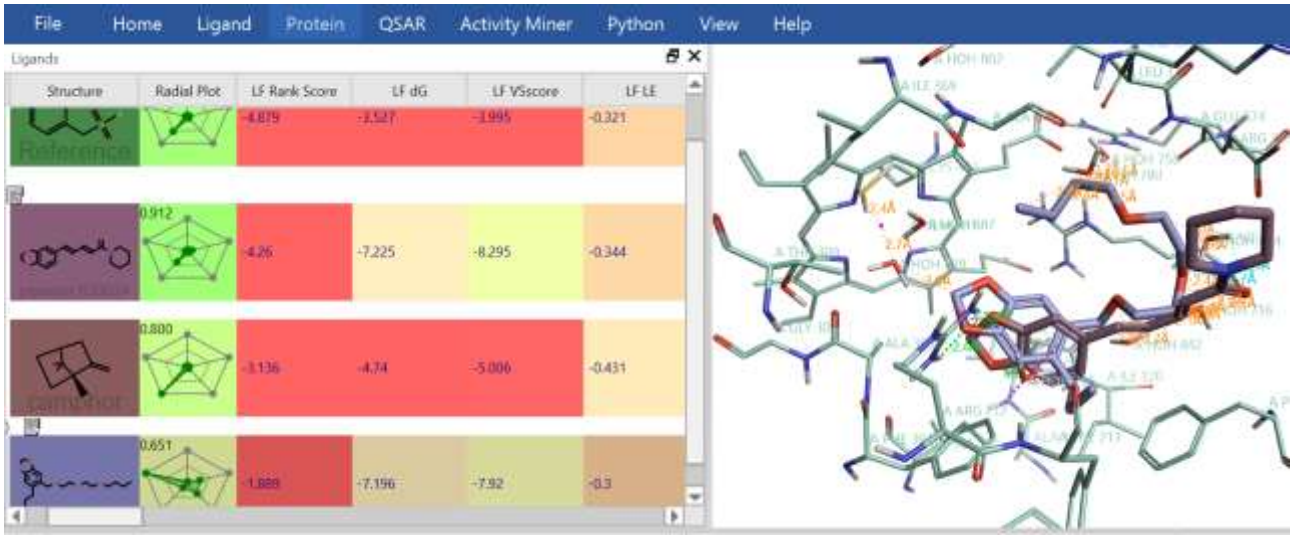
Piperonyl Butoxide Piperine

(green carbon atoms, osigenous red, nitrogen blue)

Computational cytochrome P450 comparison of PMF inhibitor with piperine and piperonyl butoxide and camphor performed with Flare Cresset

Piperine to cyp3a4





Piperine docking scores compared to PMF (phenylmethylsulfonyl fluorurro) inhibitor p450 and camphor and piperonyl butoxide in PDB (Protein data bank) ID 6MA8

Piperine scores better than the PMF inhibitor and piperonyl butoxide, and camphor in essential oils has an inhibitory activity

Test on bed bug with essential oil of rosemary, piperine and urea

P piperine solution (0.18 g) in 3.5 ml ethanol (98% Sigma aldrich analytical grade)

A technical grade urea solution with 50% mains water

R rosemary essential oil obtained from the steam distillation of *Salvia rosmarinus* grown on the farm (agr. Careglio)

Mains water

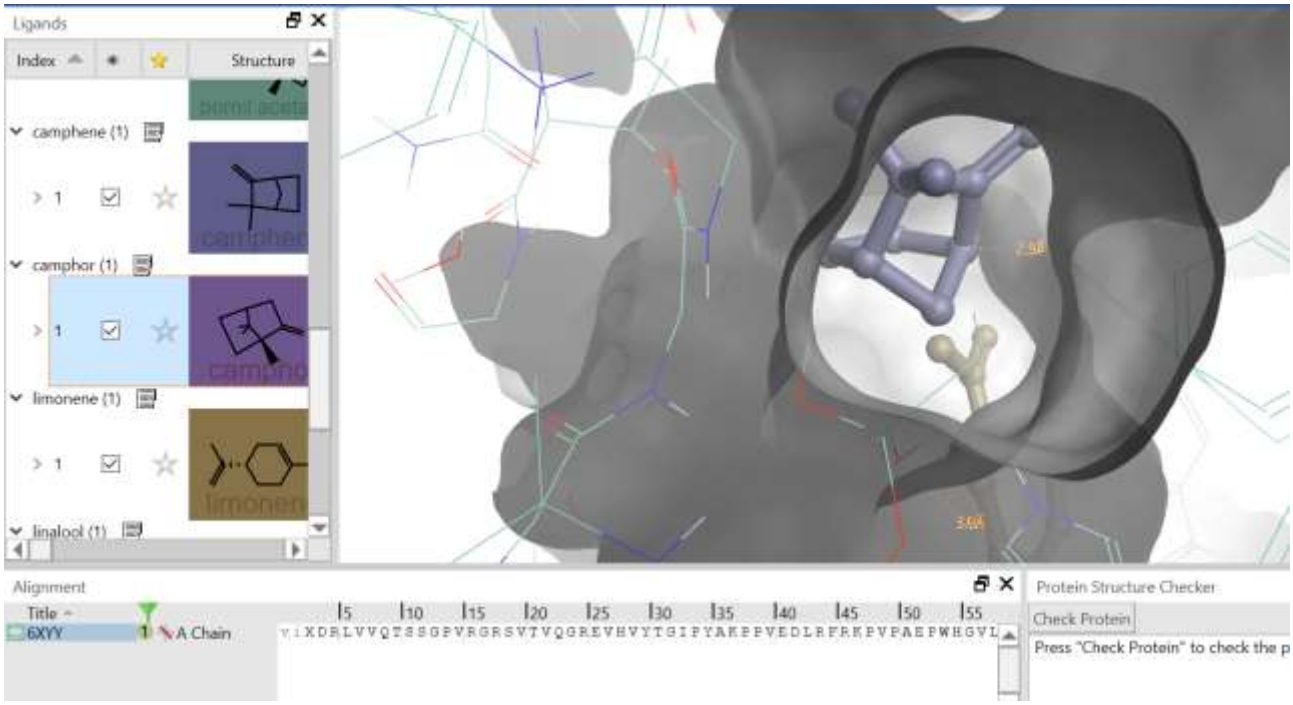
P	R	U	R+P 50%	R+U 50%	R+P+U 1/3,1/3,1/3	R+P+A 1/3,1/3,1/3	R+P+U 1/3,1/3,1/3 + 50% A
	#		#	##	##	##	

death of the insect in 1 h

death of the insect within 2

Docking sage essential oil compounds inside the acetylcholinesterase binding pocket

Si note from the representation that more than one compound (even 3) can occupy the pocket at the same time being able to give a synergistic action on the receptor.



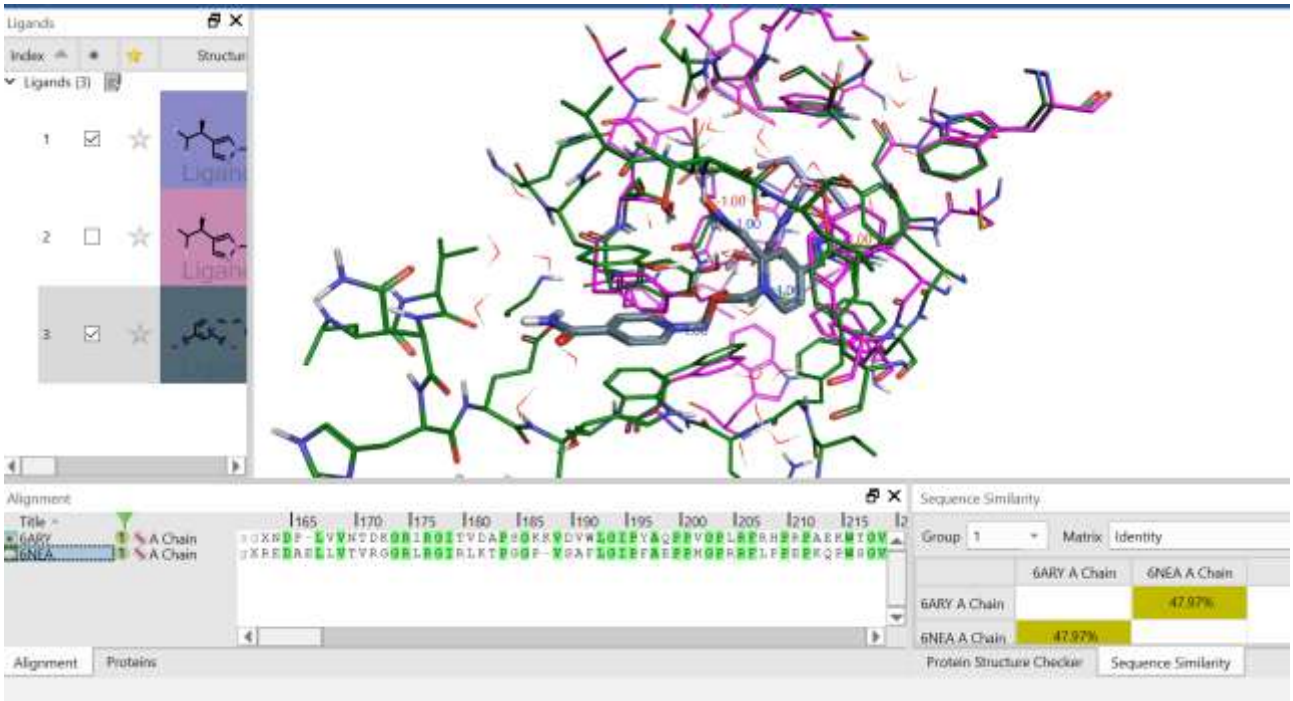
In the figure camphene and limonene inside the pocket of acetylcholinesterase (PDB 6XYY)

The insecticidal activity of the essential oil is mainly due to the main terpenoid constituents inhibitors of aceticolinesterase, there is an important synergistic effect between the constituents according to Perry et al, (ref 2) poiché no single constituent is particularly powerful to justify the inhibitory action of essential oils (ref 2).

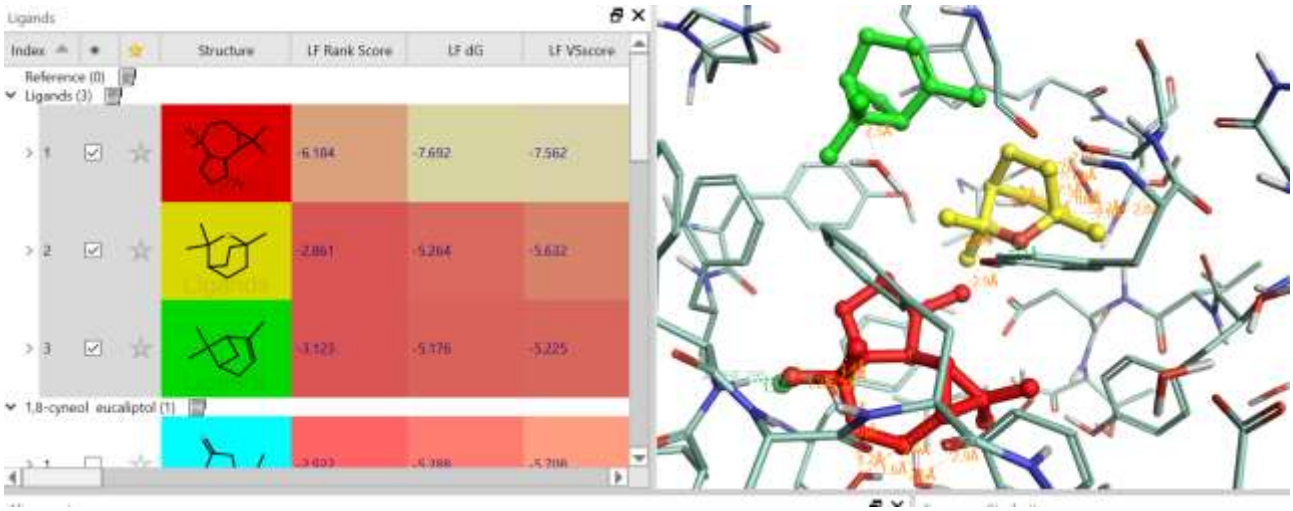
The terpenoids of essential oils being molecules with a low molecular weight, can bind simultaneously in the binding pocket

It would be interesting to test in vitro on acetylcholinesterase the individual compounds combined with 2 or 3 to evaluate how synergism occurs.

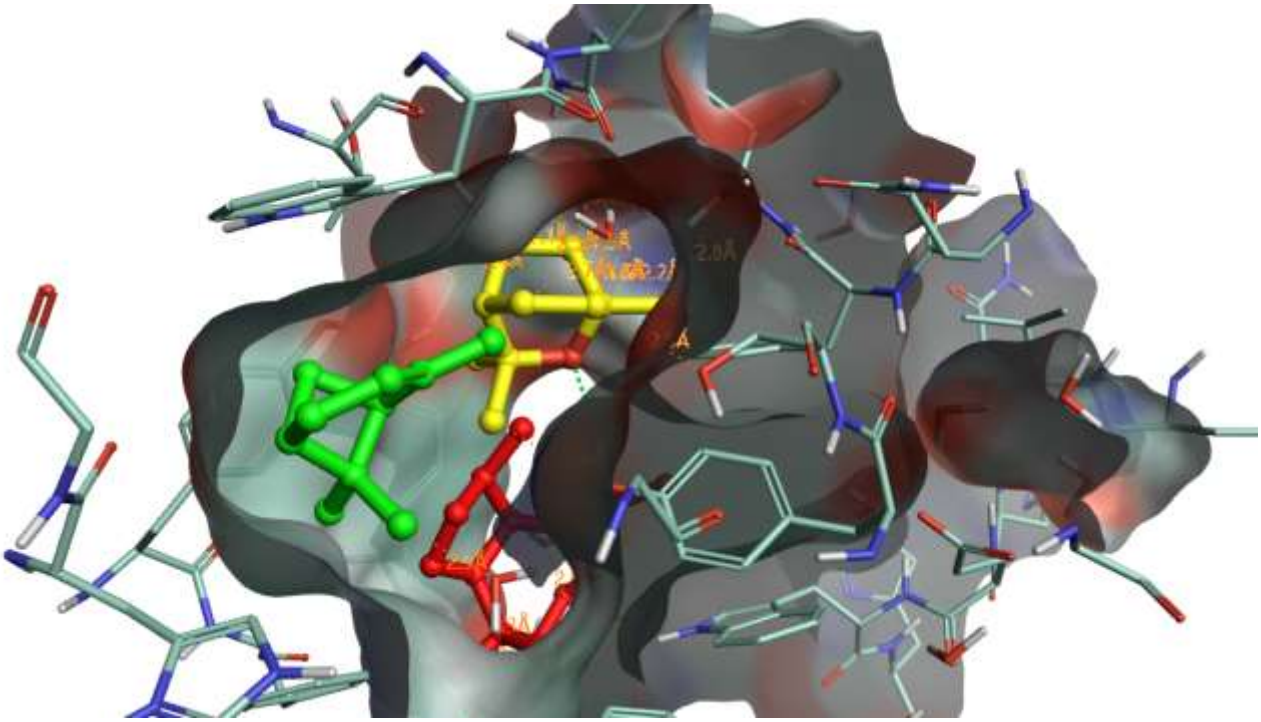
Computational comparison between the binding pocket of human acetylcholinesterase and fruit fly



Alignment and overlap of the A chain of human acetylcholinesterase (PDB ID 6NEA in green) and fruit fly (PDB ID 6ARY in fuchsia) similar to 47.9% of the binding site.



Sage essential oil compounds simultaneously inside the acetylcholinesterase binding pocket in viridiflor red, eucalyptol yellow and alpha pinene green



Like the previous figure with the surface of the binding pocket highlighted.

Docking human acetylcholonesterase compounds wormwood extract using as a comparison the main drugs used in alzheimer's


Ligands
Index
Structure
LF Rank Score
LF dG
LF VScore
LF LE
Radial Plot
1
Reference
-5.949
-7.393
-8.856
-0.308
Ligands (0)
absintina (1)
1
-7.492
-9.044
-9.419
-0.251
0.259
anabasine (1)
1
-4.337
-5.866
-6.613
-0.489
0.063
anabsinthin (1)
1
-8.997
-10.118
-11.247
-0.281
0.374
artabsin (1)
1
-7.591
-7.634
-7.982
-0.424
0.977
artemisinin (1)


Ligands
Index
Structure
LF Rank Score
LF dG
LF VScore
LF LE
Radial Plot
artemisinin (1)
1
-4.750
-7.721
-7.814
-0.386
1.000
Caffeic acid (1)
1
-5.43
-6.021
-6.385
-0.463
0.997
quercetina (1)
1
-10.094
-8.713
-10.288
-0.396
0.773
rutina (1)
1
-9.158
-8.471
-14.615
-0.197
0.200
thiophene (0)
tujone (1)
1
-2.741
-5.11
-5.21
-0.465
0.800

(ref 3) The knowledge of these two parameters allowed to evaluate the free energy of the bond of the detergent to the P glycoprotein in the lipid membrane, Δ G tl 0 , which reflects the direct detergent-transporter affinity. It increased as the number of ethoxylic groups increased, suggesting that these hydrogen bond acceptor groups are the key elements for the cleansing-transporter interaction in the lipid membrane. The free binding energy to P-glycoprotein by ethoxyl group (EO) was determined as about Δ G EO 0 = − 1.6 kJ/mol. The present results also document that, depending on the concentration applied, detergents are intrinsic substrates or inhibitors of P-glycoprotein.

This explains why piperonyl butoxide is effective as a co-formulant in insecticides, because it blocks the cellular outflow of insecticides by inhibiting p-glycoprotein but also piperine see ref 4.

CONCLUSIONS

The insecticidal action of essential oils of rosemary, sage, thyme, mountain pine, and hydroalcoholic extract of wormwood on bedbugs has been tested and the results are surprising for the oils as seen in the videos, applied as such,

https://youtu.be/u3w5M-hhf1A

https://youtu.be/_i_TJmQlokk

however, if they are diluted, the effectiveness collapses. Essential oils have a significantly lower toxicity than synthetic compounds, but hinder their use in agriculture the cost.

Piperine is also tested as a synergist with slight results.

Some compounds in wormwood extract (quercetin, rutin, anabsyntina) could be used against Alzheimer's disease as acetylcholinesterase inhibitors according to the scores of the docking performed on acetylcholinesterase umama (PDB 6NEA).

The study project is self-financed

Computational calculations and representations of proteins and molecules are performed with Flare Cresset (academic license)

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Juliana Dameli Nascimento

2 In-vitro inhibition of human erythrocyte acetylcholinesterase by salvia lavandulaefolia essential oil and constituent terpenes

N S Perry

3 Detergents as intrinsic P-glycoprotein substrates and inhibitors

XiaochunLi-BlatterPierluigiNerviAnnaSeelig

4 Piperine, one of the main constituents of black pepper, inhibits human P-glycoprotein and CYP3A4

Rajinder K. Bhardwaj , Hartmut Glaese