

La riscoperta dell'Officina: l'attività di ricerca sulle Piante Officinali dell'Istituto per i Sistemi Biologici del CNR

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Area della Ricerca Roma1
Montelibretti (RM)



in-Vitality 2022. 24 novembre 2022. MiCo_Milano

Fitoderivati: le fonti alternative.

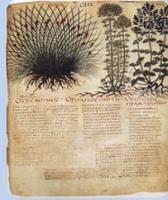
Filiere locali, economia circolare, nuove tecnologie: percorsi innovativi per la produzione di estratti vegetali.

Workshop promosso da Innovazione in Botanicals/CEC Editore.

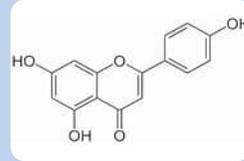
Le Piante Medicinali possono essere studiate da molti angoli visuali



Erbari



Etnobotanica



Biochimica

Medicina popolare

Botanica



Biodiversità



Piante Medicinali

Ambiente

Erboristeria

Culinaria

prodotti naturali



Fitoterapia

Aromi



Principi attivi

Aromaterapia

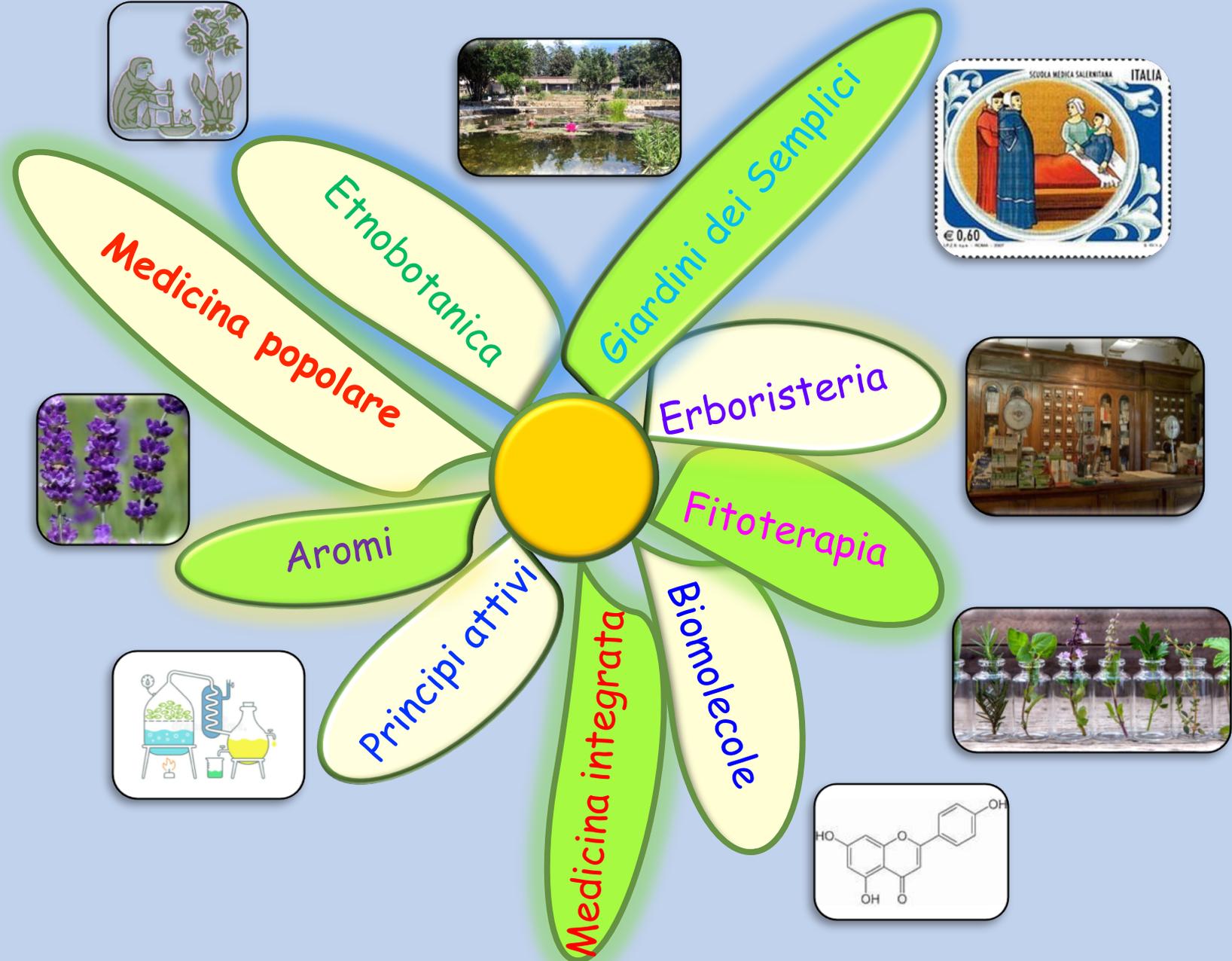
Risorse genetiche

Spezie

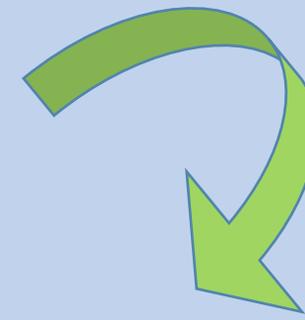
Piante tintorie



L'Istituto per i Sistemi Biologici ne studia gli usi terapeutici



Creazione di un "Orto dei Semplici"-Istituto Sistemi Biologici



Collezione di più di 100 specie di Piante Medicinali, raccolte nel periodo balsamico, quando sono più ricche di Principi Attivi.



Particolare cura viene prestata alle condizioni di crescita delle piante selvatiche della tradizione Mediterranea, e la gestione della domesticazione viene ridotta al minimo per simulare al meglio le condizioni degli habitat originari.

Orto selvatico per le specie
FITOALIMURGICHE



Il giardino fitoalimurgico per la valorizzazione delle piante spontanee

MARIA CLARA ZUIN* - GIAMPAOLO ZANIN** - GIUSEPPE ZANIN**

Phytoalimurgic Garden to Promote Wild Plants. *Wild plants have always been an important food source for people, not only in the distant past but also during more recent times of war. A wealth of knowledge linked to human traditions has been gained on these plants and which is worth preserving. The term phytoalimurgy derives from the Greek word phytūn = plant, plus the Latin words alimenta = food and urgentia = emergency - hence it means the study of ways to tackle a food crisis (famine, etc.) (Targioni-Tozzetti, 1767). To now create a Phytoalimurgic Garden (PG), as this emergency no longer exists in Europe, means to exploit and spread these plants, promoting popular customs, old flavours and uses. A PG is a place where edible wild plants are grown as if in their natural environment and is planned to produce food all year round. The PG is intended for agri-tourism and teaching purposes, to spread ethnobotanical knowledge and preserve biodiversity. Another advantage is that time and money can be saved by having the plants readily available for harvesting 'close to home' and which are unpolluted.*



MARIA CLARA ZUIN
Istituto Biologia Agroambientale
e Forestale, CNR (PD)

Il termine "alimurgia" è stato coniato da Giovanni Targioni-Tozzetti nel 1767 per indicare lo studio delle soluzioni da ricercare in caso di "urgenza" (necessità) alimentare (*alimenta urgentia* = alimurgia).

Questo termine è stato riproposto più recentemente da Oreste Mattioli nel 1918, con l'aggiunta del prefisso "fito", per rendere il termine più preciso e

definire meglio il campo di interesse; il termine non è riportato nei dizionari della lingua italiana.

In passato la cronica indigenza e soprattutto le frequenti carestie e le guerre costringevano l'uomo a ricercare soluzioni alimentari diverse dalle tradizionali e l'utilizzo delle piante spontanee era spesso l'unica soluzione disponibile.

Fortunatamente il nostro Paese non ha più urgenze simili, rimane però il fatto oggettivo che le piante spontanee sono ancora utilizzate per l'alimentazione umana. Le "piante alimurgiche" sono quindi "piante erbacee, suffrutici o alberi spontanei di cui uno o più organi o parti di essi vengono usati come alimento se raccolti ad un appropriato stadio del ciclo della pianta e

* Ist. Biologia Agroambientale e Forestale, CNR, Sez. Legnaro (PD), ** Dip. Agronomia ambientale e Produzioni vegetali, Università degli Studi di Padova.



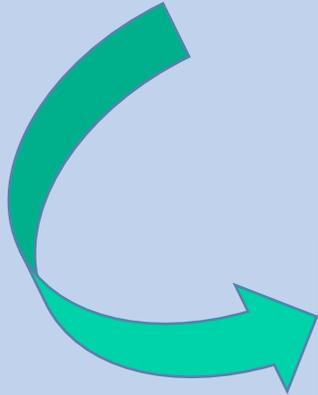
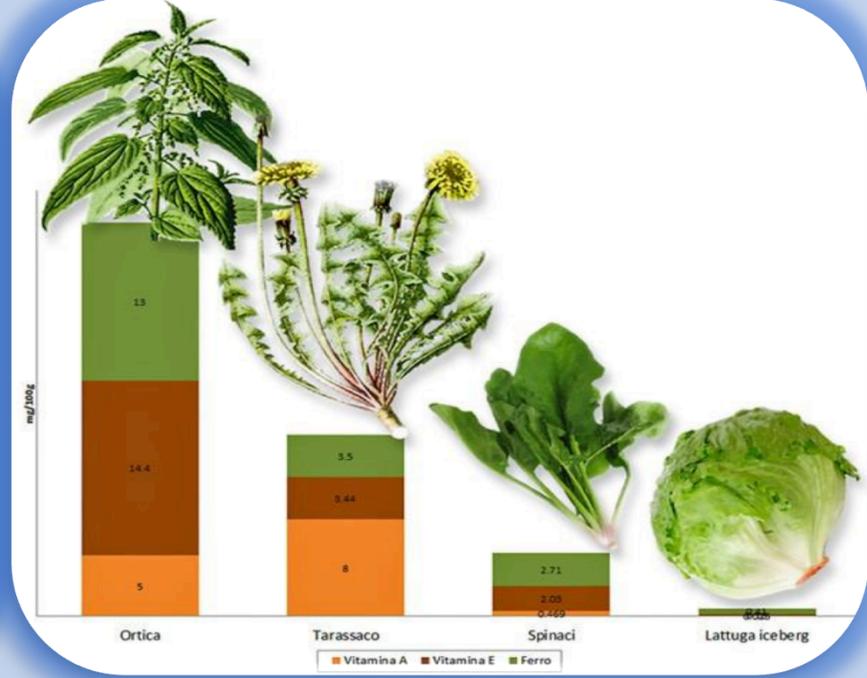
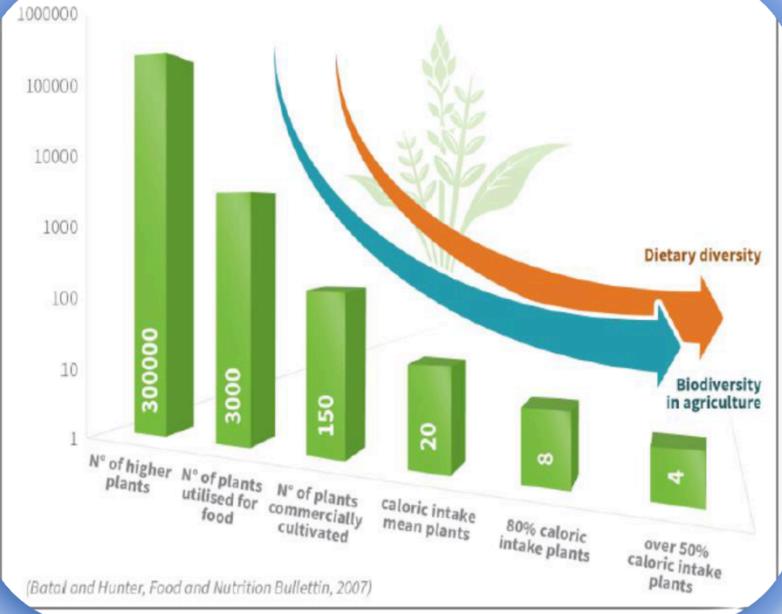
GIARDINO FITOALIMURGICO DI LEGNARO



eat
wild

SAFEGUARD OF WILD EDIBLE PLANTS
FROM TRADITIONS TO SUSTAINABLE AGRICULTURE
TOWARDS NEW POTENTIAL FOOD PRODUCTS

a proposal for H2020-SFS-07b-2015



Review

Born to Eat Wild: An Integrated Conservation Approach to Secure Wild Food Plants for Food Security and Nutrition

Teresa Borelli ^{1,*}, Danny Hunter ¹, Bronwen Powell ², Tiziana Ulian ³, Efsio Mattana ³, Céline Termote ¹, Lukas Pawera ^{4,5}, Daniela Beltrame ⁶, Daniela Penafiel ^{7,8}, Ayfer Tan ⁹, Mary Taylor ¹⁰ and Johannes Engels ¹

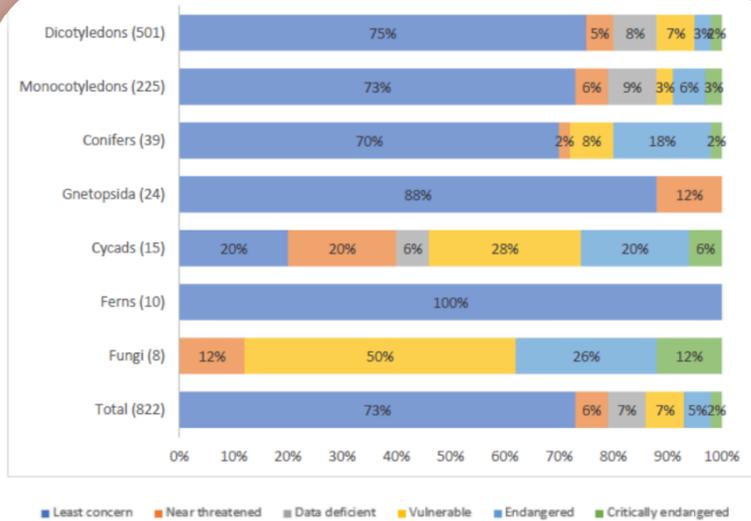


Figure 1. Number of WFPs and fungi on the IUCN Red List of Threatened Species classified by class and risk category Source: IUCN Red List 2017. Adapted from FAO [45].



Cite this: DOI: 10.1039/d2fo02593k

Science and claims of the arena of food bioactives: comparison of drugs, nutrients, supplements, and nutraceuticals

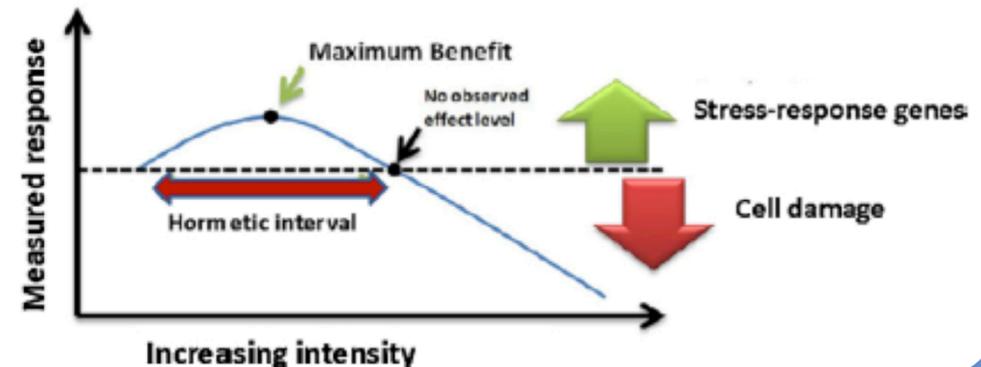
Francesco Visioli ^{a,b}

Nutritional hormesis

What has been discussed above fits very well with the concept of nutritional hormesis. The ingestion of molecules that act as electrophiles triggers the nucleophile response, in turn contributing to homeostasis via the redox mechanism and, hence, anti-inflammatory feedback.

The notion of hormesis refers to the **non-linear relationship between the dose and the effect: a substance that is toxic at high doses might be healthy at lower ones.** From a nutritional viewpoint, the “hormetic zone” is the optimal dose range between ineffective and toxic.

Hormetic response to stress-stimuli (CR, Exercise, ROS, radiation, xenometins)





L'ISB partecipa al Progetto Biomemory CNR e cura una collezione di 102 specie medicinali

- Medicinal Herb Garden
- CNR-ISB-MGH**
- Medicinal and Wild Edible Plants
- Field collection, Seed collection



Campanula rapunculus



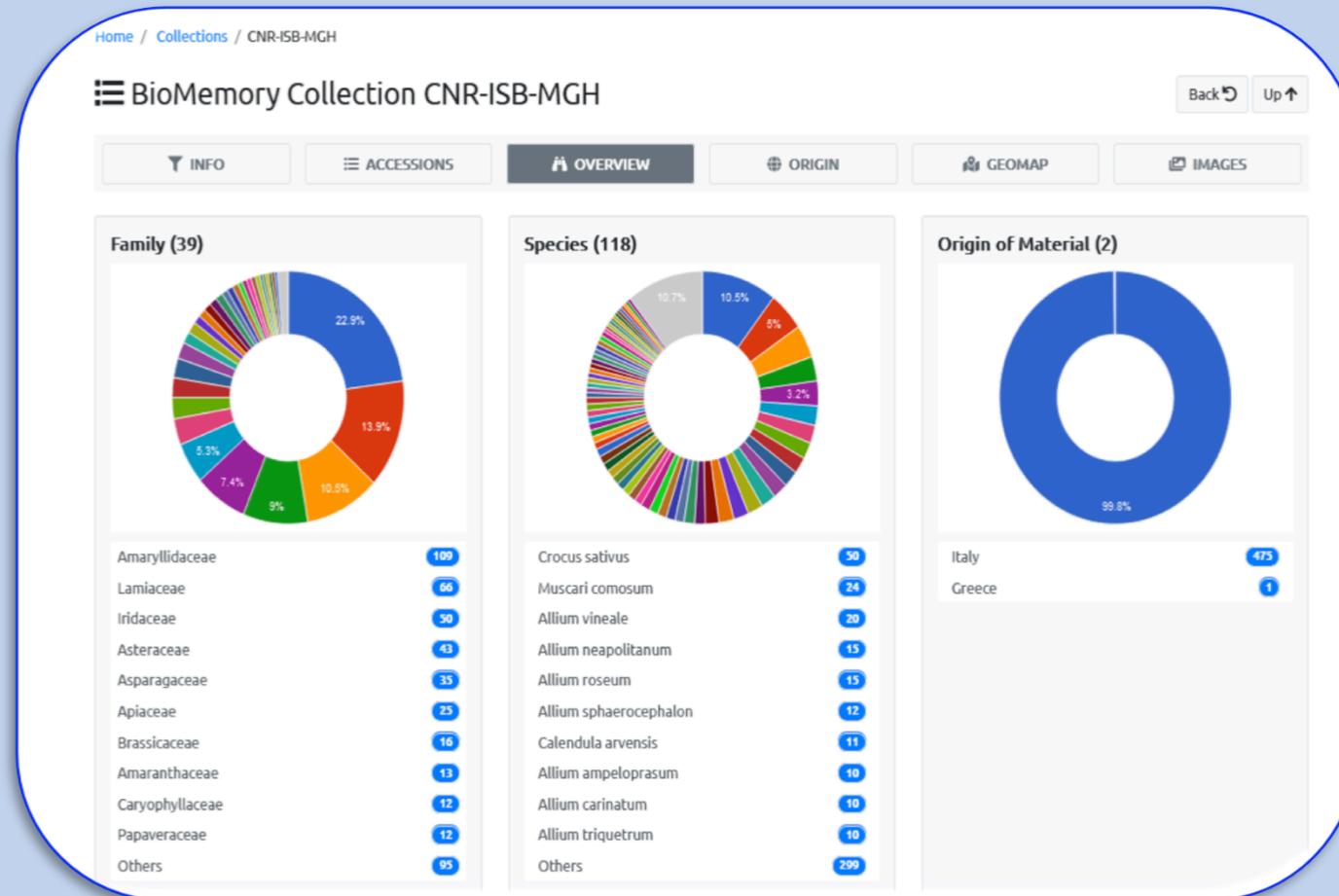
Allium ursinum



Allium ampeloprasum



Allium nevs kianum



Aromatiche
Alimurgiche
Tintorie

Piante:

Spontanee
Raccolte
Seminate

in situ
ex situ

Attività:

- Conservazione del germoplasma
- Raccolta nel periodo balsamico
- Preparazione estratti



Plantago coronopus



Hypericum perforatum



Allium flavum



Centaurium erythrae



Geranium sanguineum



Allium vineale



Daucus carota



Silene dioica



Glechoma hederacea



Allium roseum



Allium carinatum



Allium sphaerocephalon

Preparazione di Estratti alcolici e Olii Essenziali



Piante Medicinali Aromatiche_ *L. angustifolia*
Parti della pianta con proprietà fitoterapiche (fiori),
raccolte nel periodo balsamico (fine dell'estate)



Essiccazione e
frantumazione



Sminuzzamento in N2 liquido
e determinazione del peso



Macerazione in Solvente idro-alcolico
per preparare Estratto



Distillazione in corrente di vapore
per estrarre l'Olio Essenziale (OE)

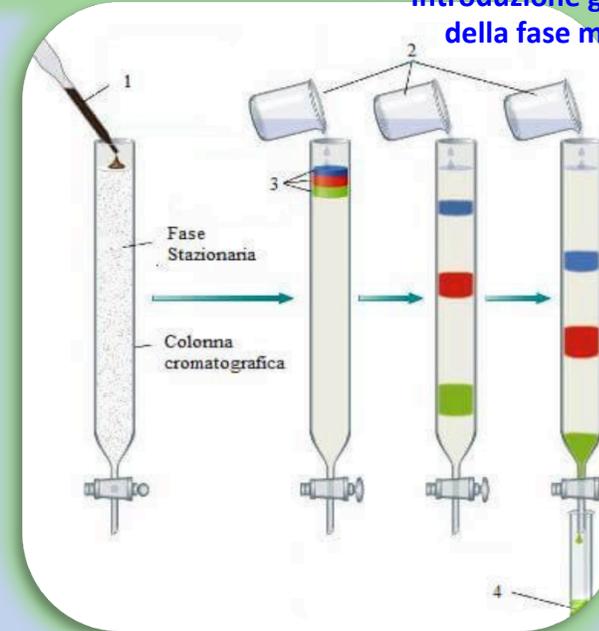
Identifichiamo le sostanze contenute in un estratto



La **CROMATOGRAFIA** è una tecnica che permette la separazione delle sostanze contenute in una miscela in seguito alla loro distribuzione tra due fasi, una solida (fase stazionaria) e una liquida in movimento lungo una direzione definita (fase mobile)

Introduzione della miscela

Introduzione graduale della fase mobile

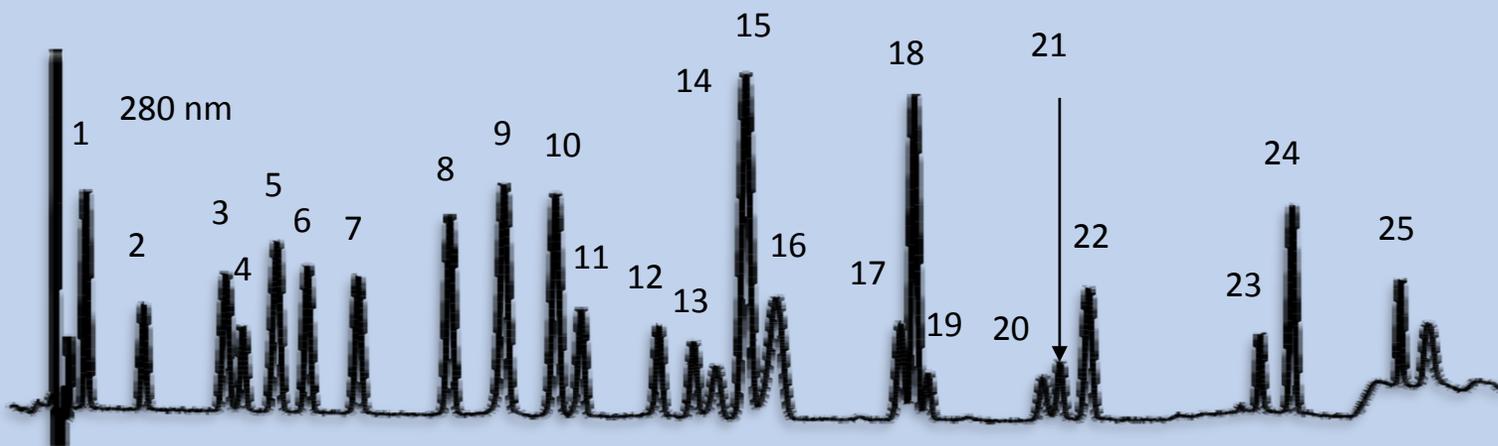


Recupero singole sostanze in frazioni diverse



Gli stessi principi che separano le sostanze nella colonna di vetro vengono applicati in apparecchiature più sofisticate, in grado di lavorare anche su quantità molto piccole (**UHPLC**)

Nel cromatogramma ogni segnale corrisponde ad una sostanza



1. Acido gallico, 2. Acido 3,4-diidrossibenzoico, 3. **Acido caftarico**, 4. Acido 4-idrossibenzoico,
5. Catechina, 6. Acido 3-idrossibenzoico, 7. Acido caffeico, 8. Epicatechina,
9. **Delfinidina-3 glucoside**, 10. **Acido cumarico**, 11. **Cianidina-3-glucoside**,
12. **Pelargonidina-3-glucoside**, 13. **Acido sinapico**, 14. **Peonidina-3-glucoside**, 15. **trans-Piceide**,
16. **Malvidina-3-glucoside**, 17. Naringenina-7-glucoside, 18. **Rutina**, 19. **Quercitina-3-glucoside**,
20. **Miricetina**, 21. **Kamferolo-3-glucoside**, 22. **trans-Resveratrolo**, 23. **Quercitina**,
24. Naringenina, 25. **kamferolo**



Strumentazione

- Spettrometro Bruker AVANCE III HD 600
- Frequenza: 600 MHz ($B_0=14.1$ T)
- Campioni liquidi, semi-solidi (HR-MAS)

Applicazioni

- La Risonanza magnetica è un metodo analitico avanzato che è in grado di identificare la struttura e la composizione molecolare di metaboliti nelle matrici biologiche (vegetali).
- Utile sia per composti isolati, che per lo studio di miscele complesse, senza separazione fisica dei componenti
- Può essere applicata per lo studio di estratti vegetali di diversa polarità (metaboliti idrosolubili, idrofobi (liposolubili), olii essenziali, ecc.)

Esempi di classi di composti studiati con NMR

Estrazioni non specifiche

Metaboliti idrosolubili

Amino acidi liberi,
Acidi organici,
Zuccheri,
Polifenoli, Vitamina C

Metaboliti liposolubili:

Un profilo di acidi grassi,
Steroli,
Fosfolipidi,
Tri- e digliceridi,
Terpeni,
Carotenoidi

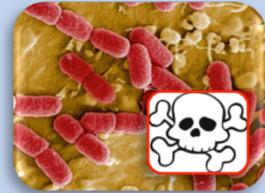
Estrazioni/metaboliti target

Glucosinolati,
Antocianine, Flavonoidi,
Sesquiterpeni lattoni,
Terpeni

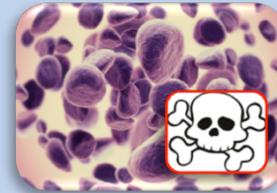
Analisi delle proprietà antimicrobiche e immunoadiuvanti delle Piante Aromatiche Medicinali (PAM)

L'Istituto per i Sistemi Biologici dispone di una BIO Banca di 200 estratti di PAM

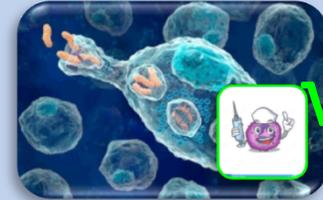
L'ISB studia l'attività



antibatterica



antitumorale



Immunomodulante
(macrofago attivato)



antimicrobica

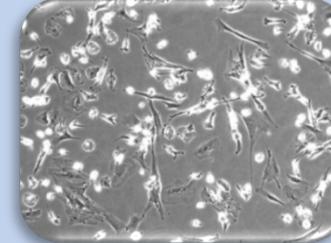
degli estratti vegetali su :



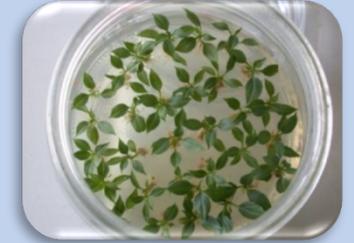
Batteri su
piastra



Cellule tumorali



Macrofagi umani



Cellule vegetali

Alcune delle piante studiate



Lavandula angustifolia



Cinnamomum zeylanicum

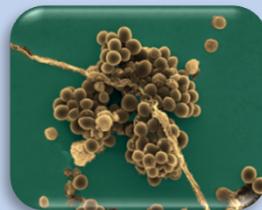


Rosmarinus officinalis

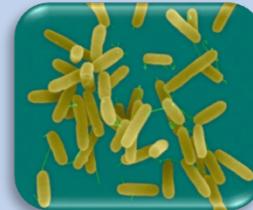


Cannabis sativa

Batteri bersaglio



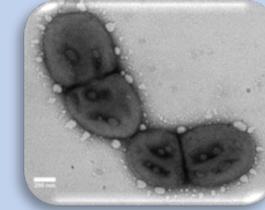
Staphylococcus aureus



Pseudomonas aeruginosa



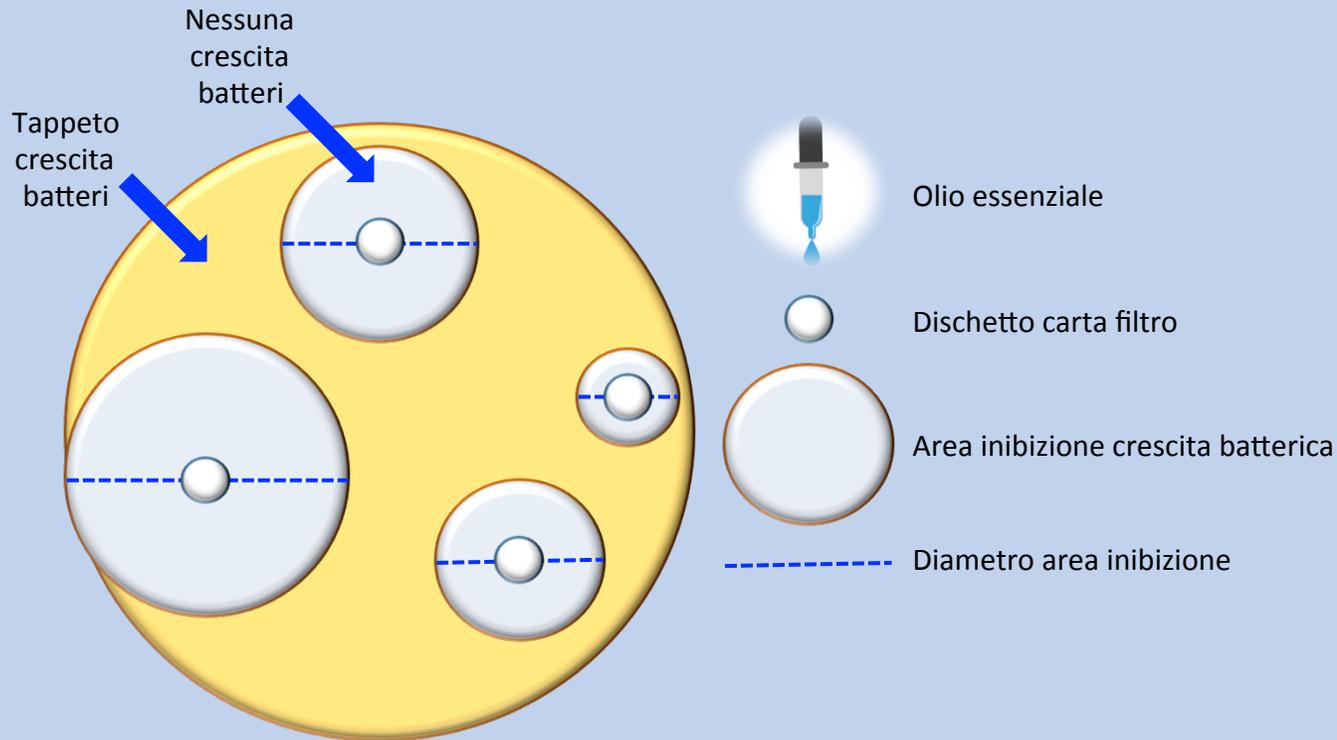
Staphylococcus epidermidis



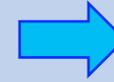
Porphyromonas gingivalis

Oli Essenziali vs Antibiotici

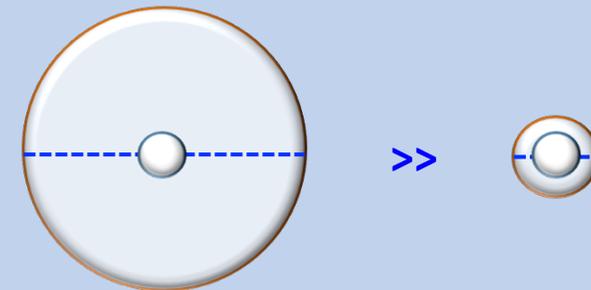
Il vantaggio dell'utilizzo di Oli Essenziali per debellare le infezioni batteriche sta proprio nella loro complessa composizione, che **non permette ai batteri patogeni di sviluppare resistenze**, come nel caso degli **Antibiotici** convenzionali, costituiti prevalentemente da una singola molecola.



Sensibili vs Resistenti

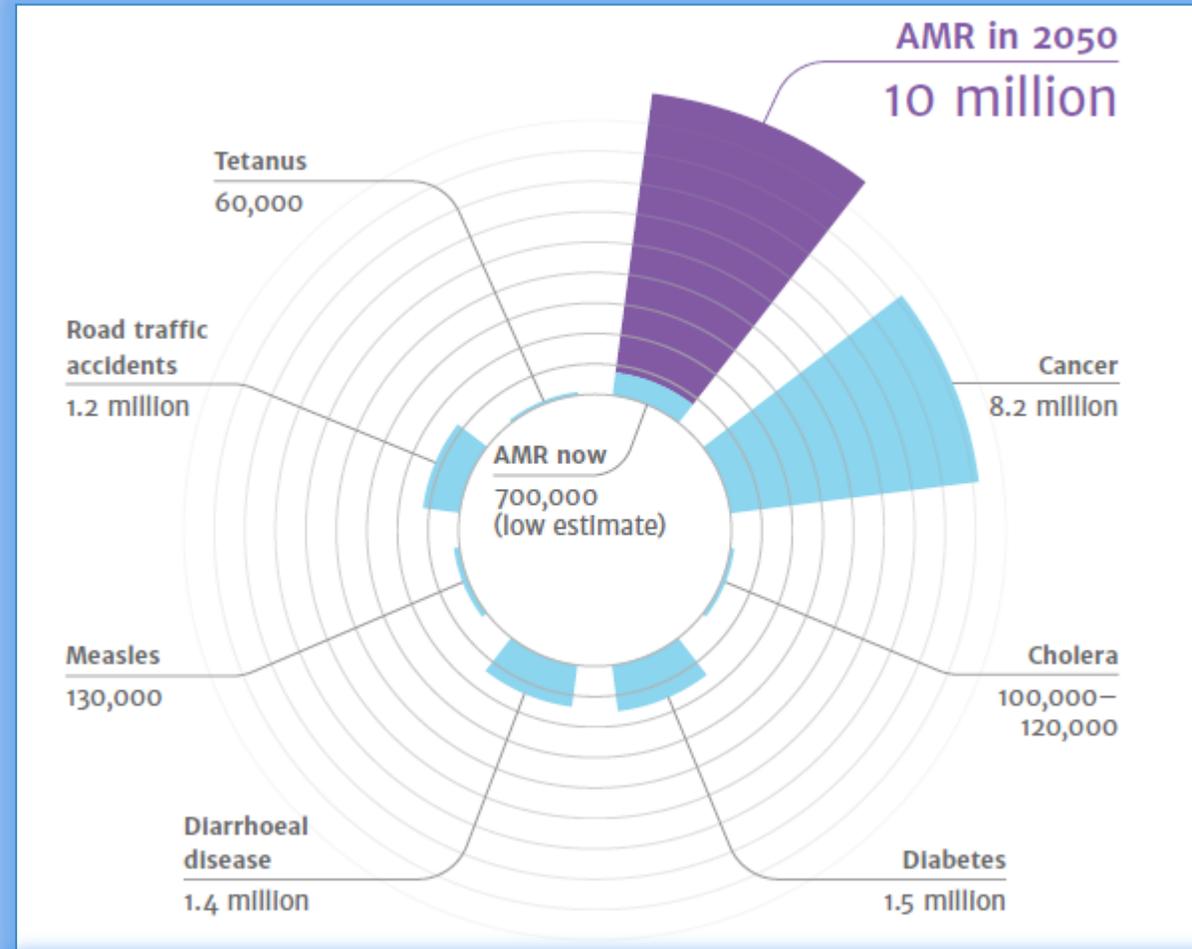
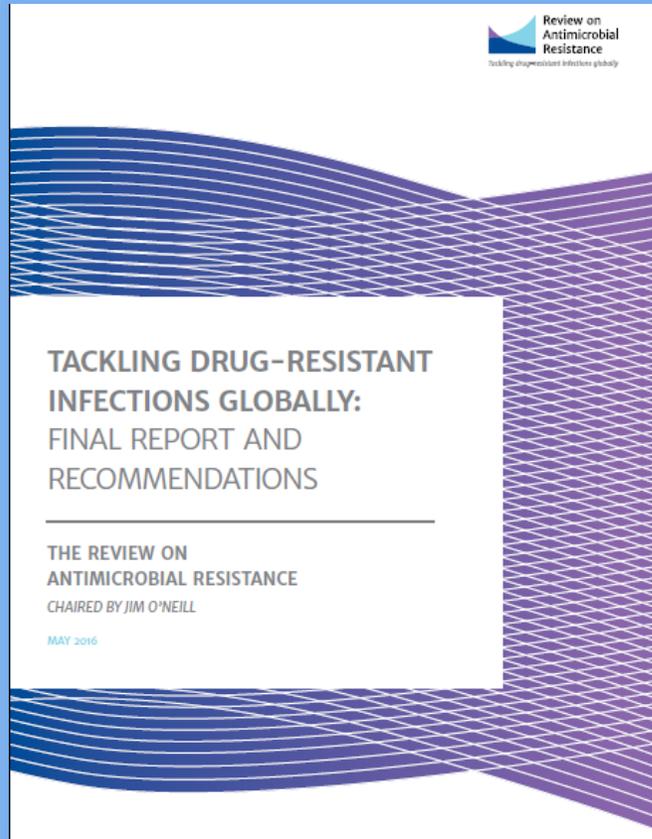


Diametro area di inibizione della crescita
Batteri Sensibili >> Batteri Resistenti



Maggiore è il potere antibatterico di un OE e maggiore sarà il diametro di inibizione della crescita su una piastra con terreno solido

DEATHS ATTRIBUTABLE TO AMR EVERY YEAR

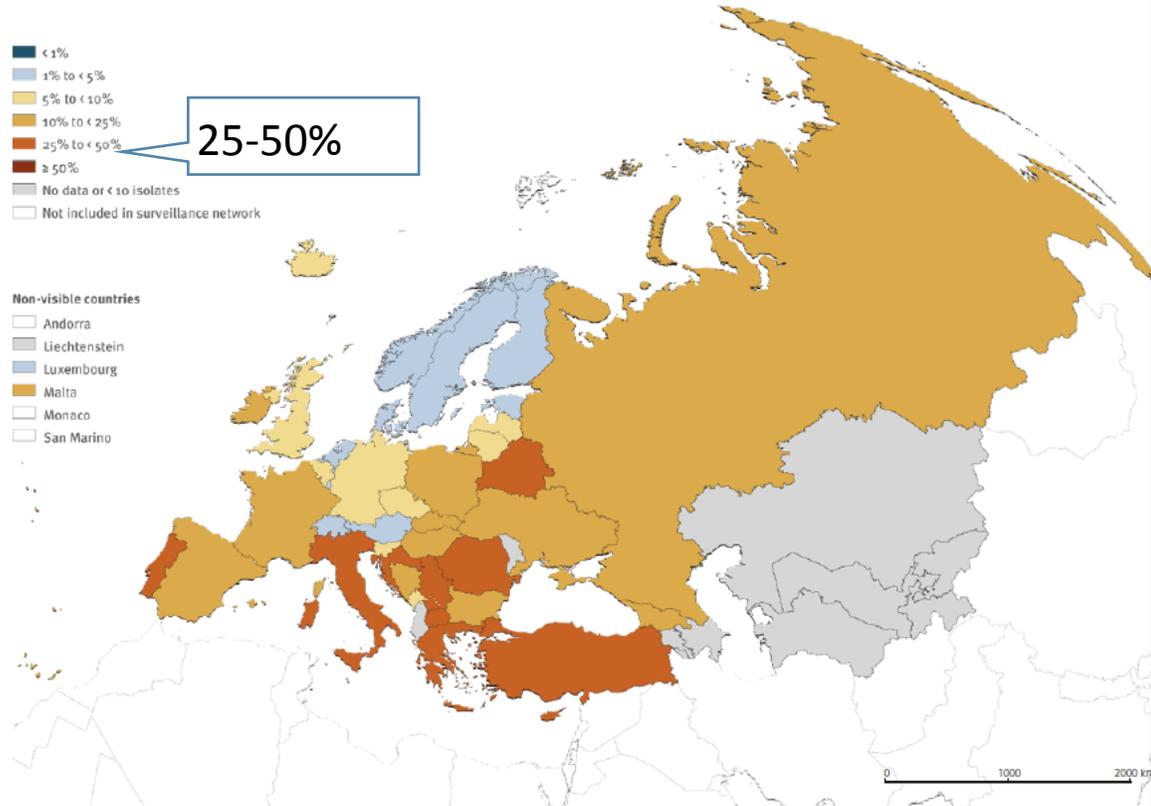


Sources:

Diabetes: www.who.int/mediacentre/factsheets/fs312/en/ Cancer: www.who.int/mediacentre/factsheets/fs297/en/
Cholera: www.who.int/mediacentre/factsheets/fs107/en/ Diarrhoeal disease: www.sciencedirect.com/science/article/pii/S0140673612617280
Measles: www.sciencedirect.com/science/article/pii/S0140673612617280 Road traffic accidents: www.who.int/mediacentre/factsheets/fs358/en/
Tetanus: www.sciencedirect.com/science/article/pii/S0140673612617280

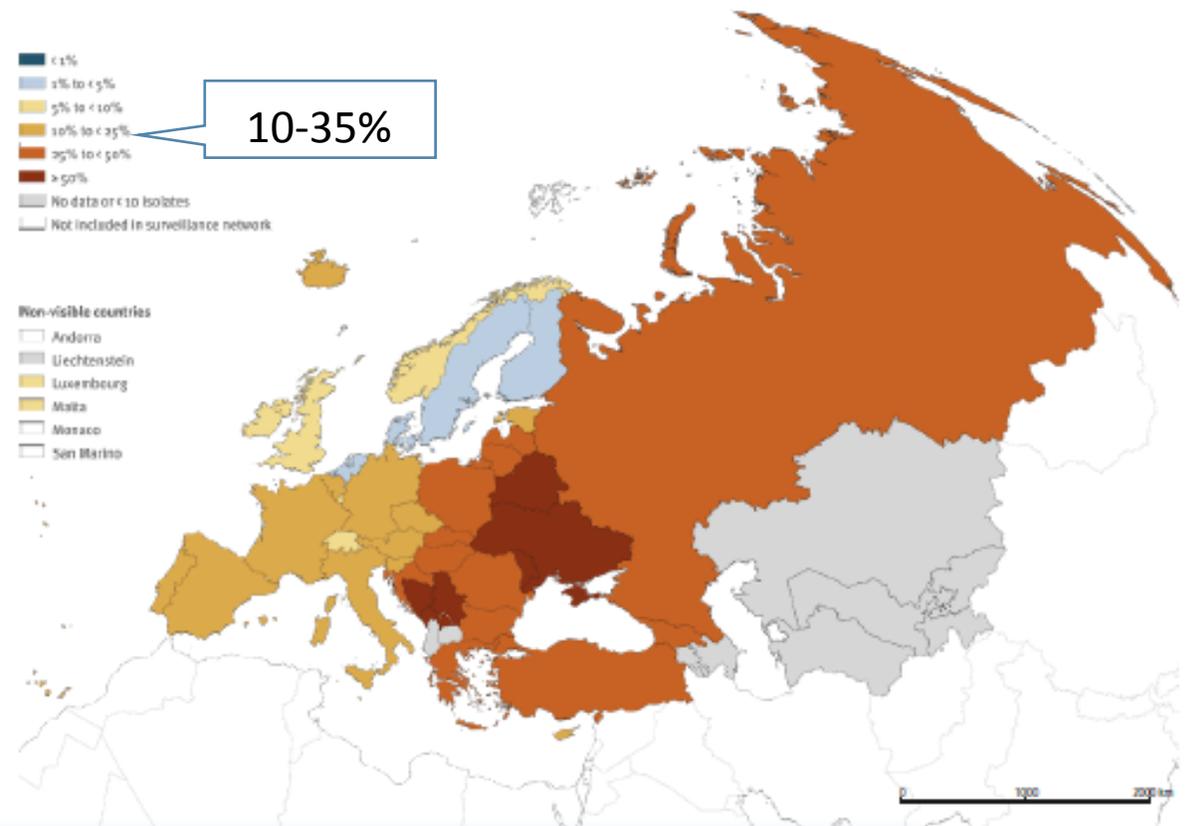
*S.aureus*_Gram positive

Fig. 8 *S. aureus*: percentage of invasive isolates resistant to methicillin (MRSA),^a by country/area, WHO European Region, 2020



*P.aeruginosa*_Gram negative

Fig. 6 *P. aeruginosa*: percentage of invasive isolates with resistance to carbapenems (imipenem/meropenem), by country/area, WHO European Region, 2020



SURVEILLANCE REPORT

Antimicrobial resistance surveillance in Europe 2022 – 2020 data

European Centre for Disease Prevention and Control_An agency of the European Union

Un caso studio.

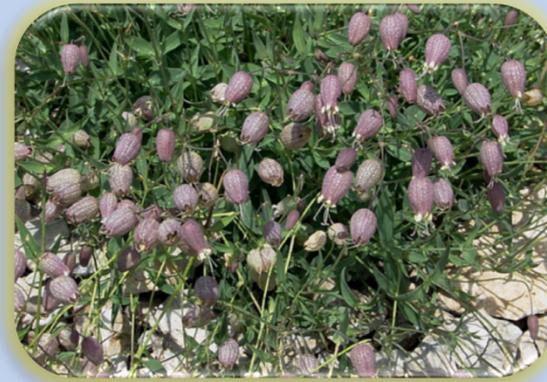
Analisi delle proprietà inibenti sulla crescita e sulla formazione del biofilm batterico di *S.aureus* ATCC 25923 degli estratti di sei specie di piante fitoalimurgiche e composizione metabolica degli estratti



G. h.



C. a.



S. v.



S. l.



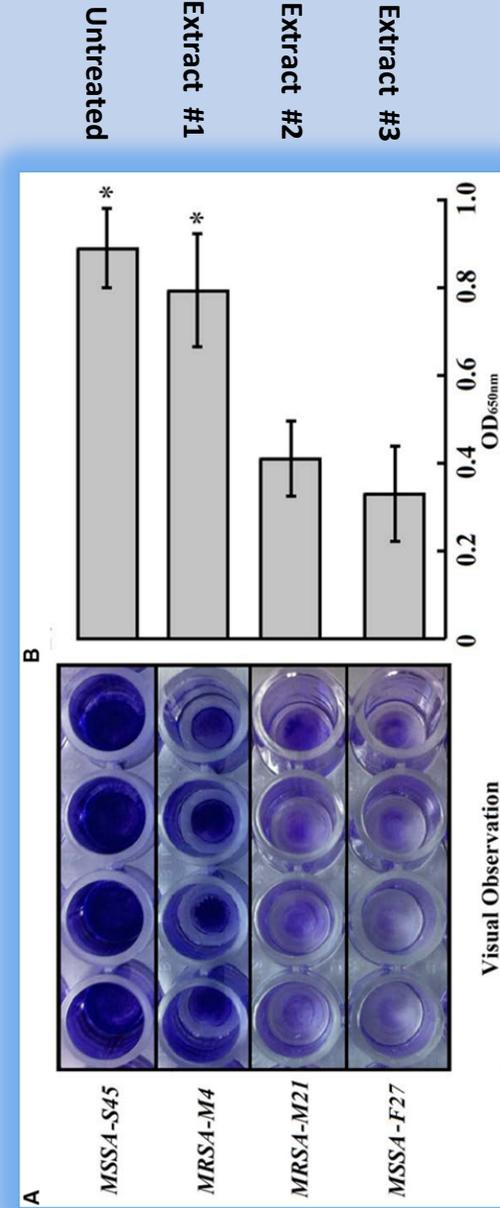
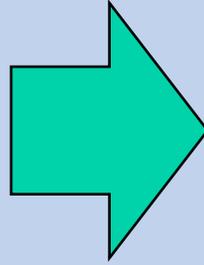
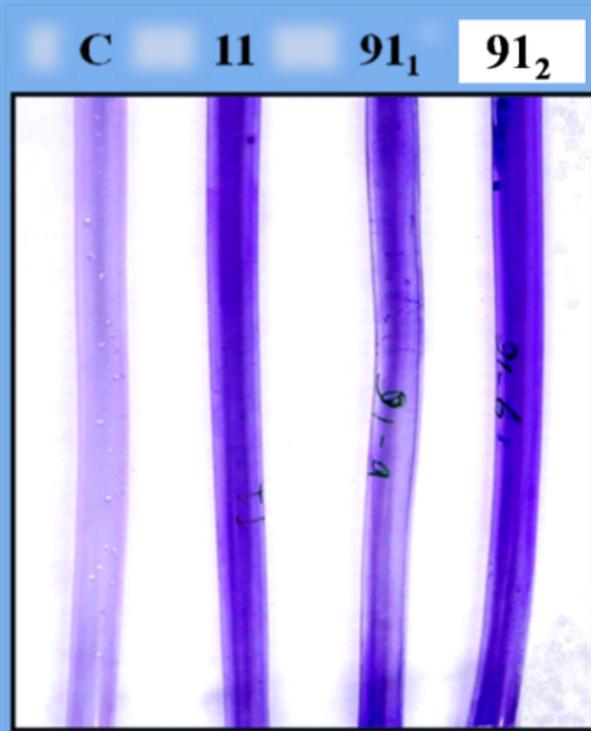
S. o.



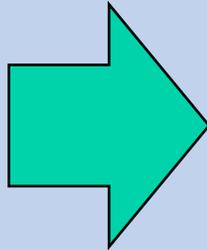
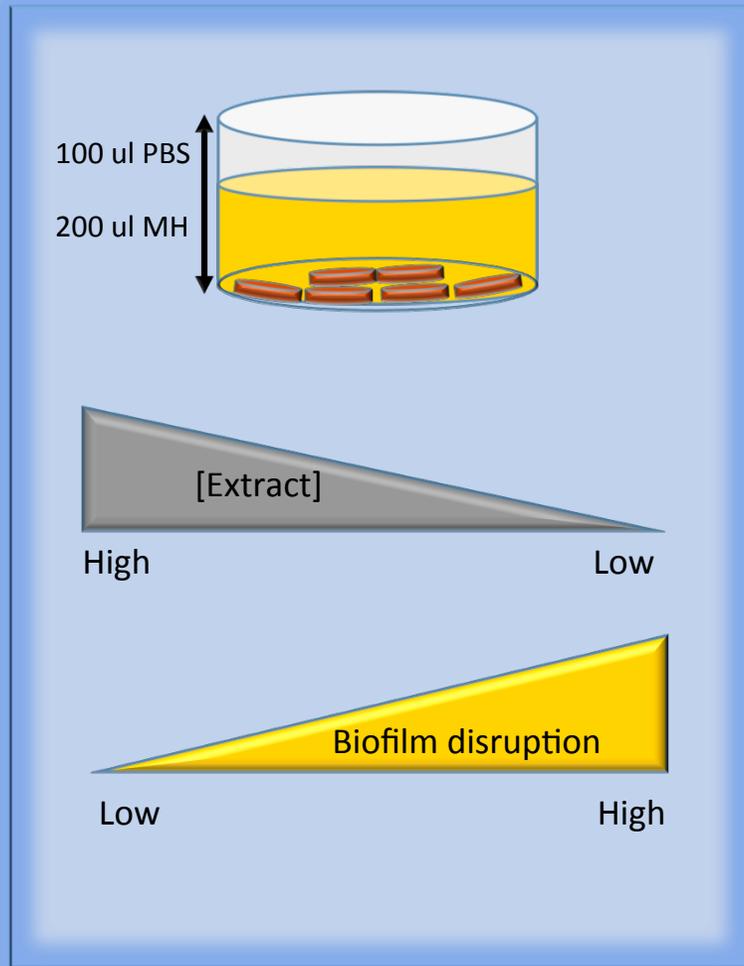
D. e.

Crystal Violet staining of bacterial mature biofilm

Nosocomial catheters-associated infections

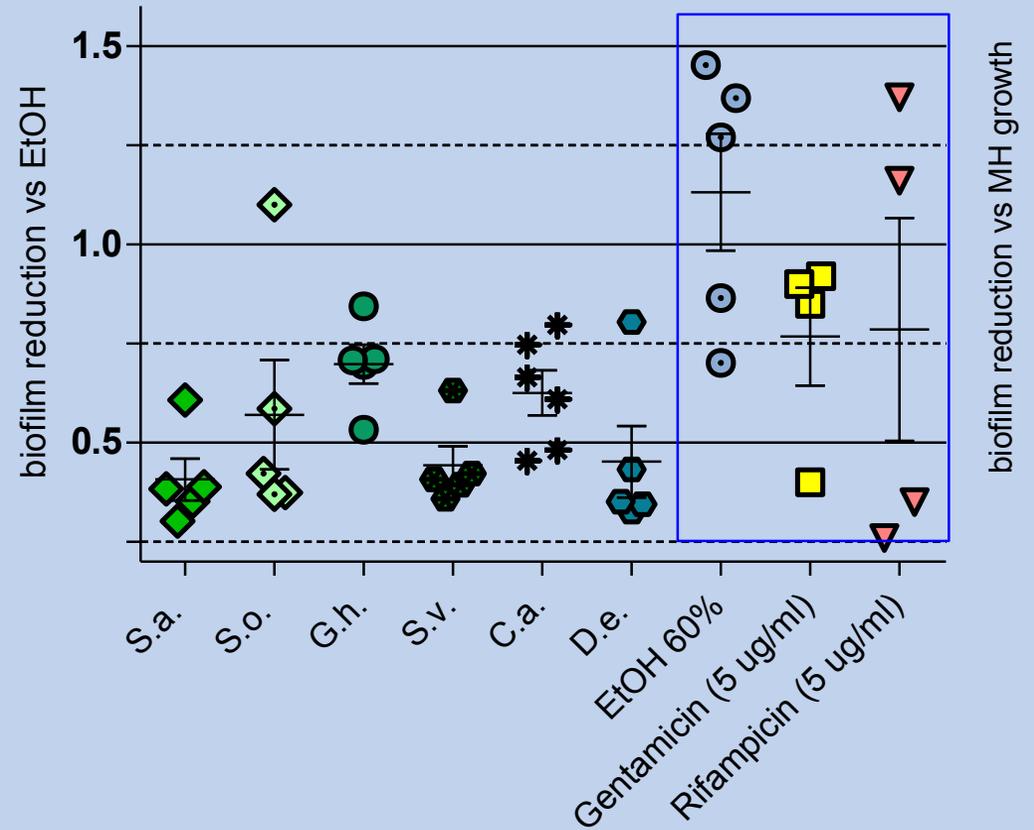


Piastra 96 hr
(ultime 24 hr + estratti in MH)

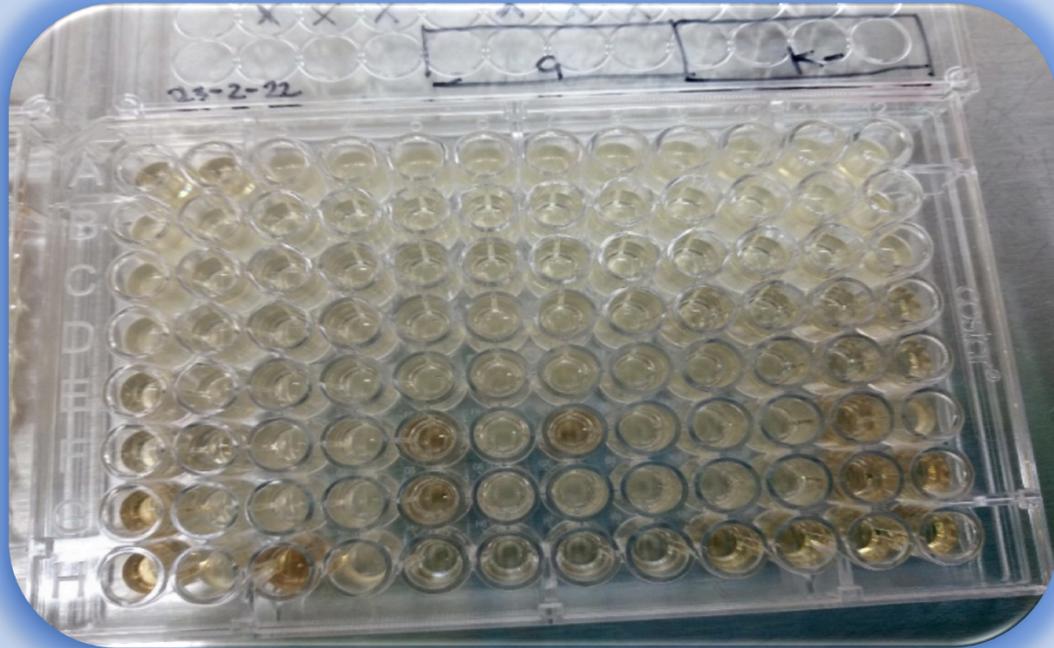


Anti-biofilm activity

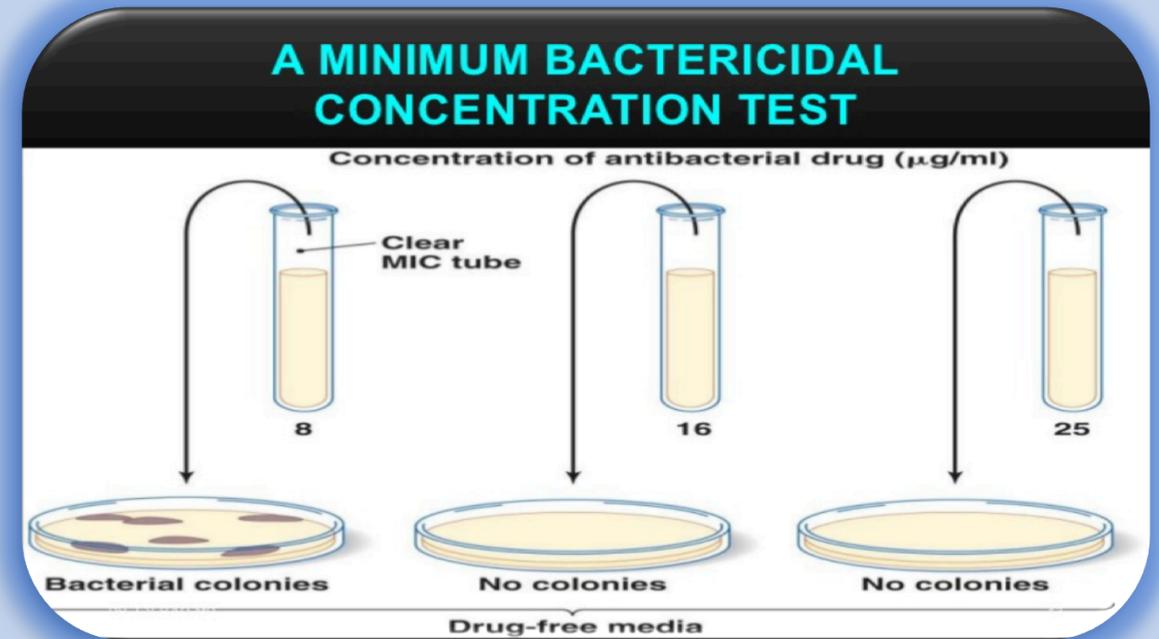
S.aureus Biofilm
96 hr in MH



Minimal Inhibiting Concentration (MIC) and Minimal Bactericidal Concentration (MBC)



t1 MIC



t2 MBC

Plant Species	Extract	Planktonic bacteria				Mature biofilms
		<i>S.aureus</i>		MRSA		<i>S.aureus</i>
		MIC	MBC	MIC	MBC	MBIC
		[mg/ml]		[mg/ml]		96 h
<i>S. a.</i>	C51	>4,5<5	5	>4,5<5	5	nd
	C72	>4,5<5	5	>4,5<5	5	0,25
	C215	>20	>20	>20	>20	nd
	B216	>20	>20	>20	>20	nd
<i>S. o.</i>	C64	>8<9	9	9	10	0,25
	C56	>20	>20	>20	>20	0,25
<i>G. h.</i>	C43	<10	10	<10	10	0,25
<i>C. a.</i>	C210	5	10	20	>20	nd
	C206	2	20	20	>20	0,5
	C133	>20	>20	>20	>20	0,25
	C46	>20	>20	>20	>20	
<i>S. v.</i>	C73	15	20	15	20	0,25
	C49	20	>20	20	>20	0,25
<i>D. e.</i>	C171	>20	>20	>20	>20	0,25

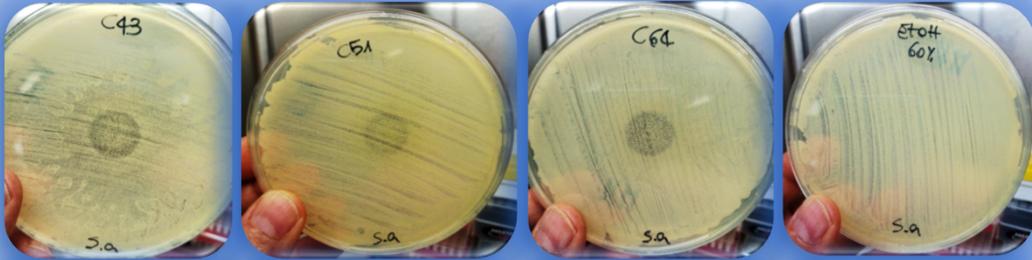


MIC
MBC

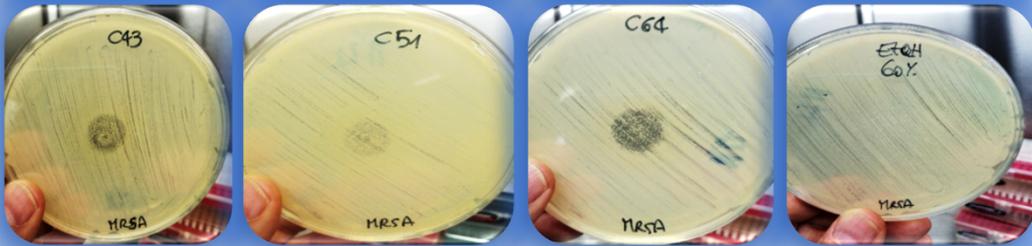
Ordine
decescente
proprietà
antimicrobiche

Antiadesività 1/125 MIC

S. aureus
ATCC 25923



MRSA
ATCC 33591

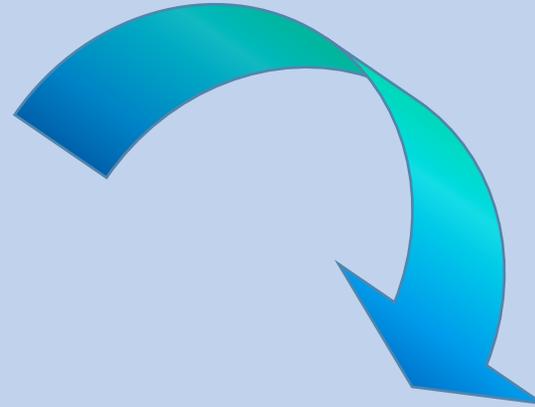


G. h.

S. a.

S. o.

Solvente



Acquisizione con Paint 3D, stessa area della piastra,
(1053 x 840 px)

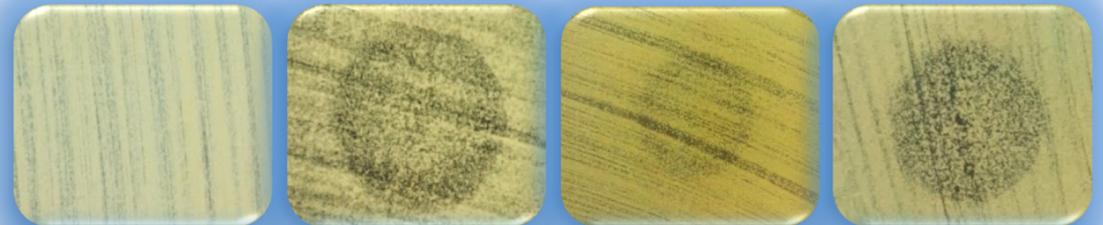
Et-OH 60%

G. h.

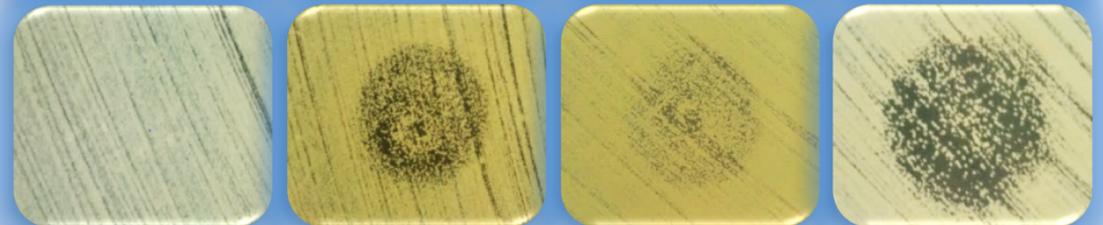
S. a.

S. o.

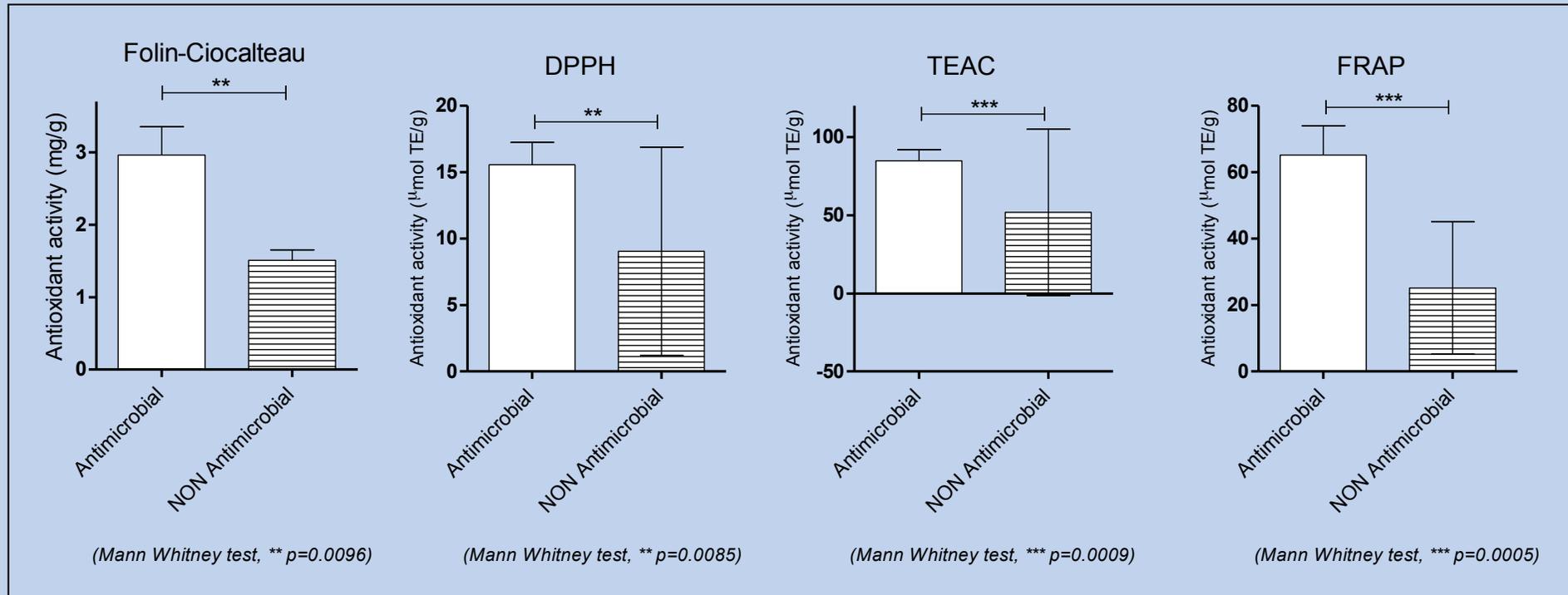
S. aureus
ATCC 25923



MRSA
ATCC 33591



Proprietà antiossidanti



p value

Statistical
significance

Se tolgo C49 e C46: Folin, $p>0.05$, DPPH, **** $p<0.0001$, TEAC, **** $p<0.0001$, FRAP, **** $p<0.0001$

Bioprospecting

Ordine decrescente proprietà antimicrobiche



S. a.>



S. o.-*G. h.*>



C. a.>



S. v.>



S. v. (C49)



Estratti no MIC-MBC

C. a. (C46)



C. a. (C133)



S. o. (C56)



D. e. (C171)



Linea di ricerca sulle Piante Medicinali

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Commentary

A manifesto for the valorization of wild edible plants

Loretta Bacchetta^{a,1}, Francesco Visioli^{b,k,*}, Giulia Cappelli^c, Emily Caruso^d, Gary Martin^d, Eva Nemeth^e, Gianni Bacchetta^f, Gianni Bedini^g, Alexander Wezel^h, Tedje van Asseldonkⁱ, Leo van Raamsdonk^j, Francesca Mariani^c, on behalf of the Eatwild Consortium^{a,2}

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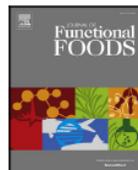


Journal of Functional Foods 45 (2018) 499–511

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A *Corylus avellana* L. extract enhances human macrophage bactericidal response against *Staphylococcus aureus* by increasing the expression of anti-inflammatory and iron metabolism genes

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Review

A Systematic Review on the Antimicrobial Properties of Mediterranean Wild Edible Plants: We Still Know Too Little about Them, but What We Do Know Makes Persistent Investigation Worthwhile

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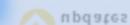
^{*} Correspondence: francesca.mariani@cnr.it; Tel.: +39-06-90672518

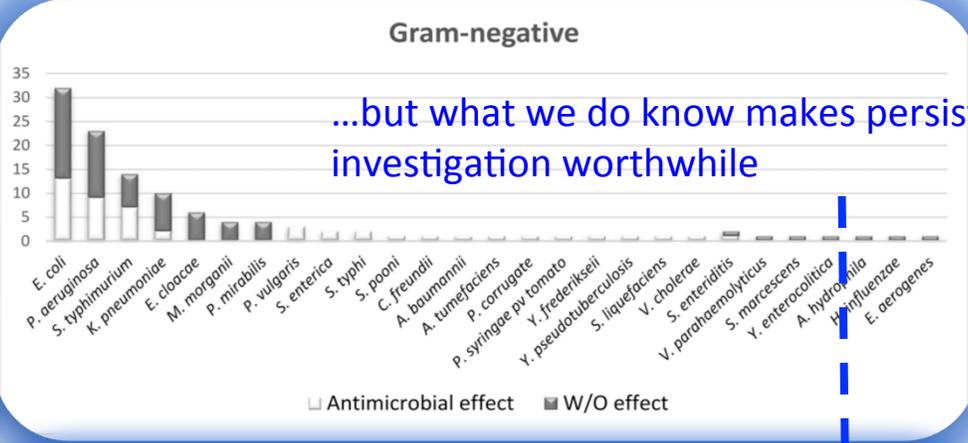
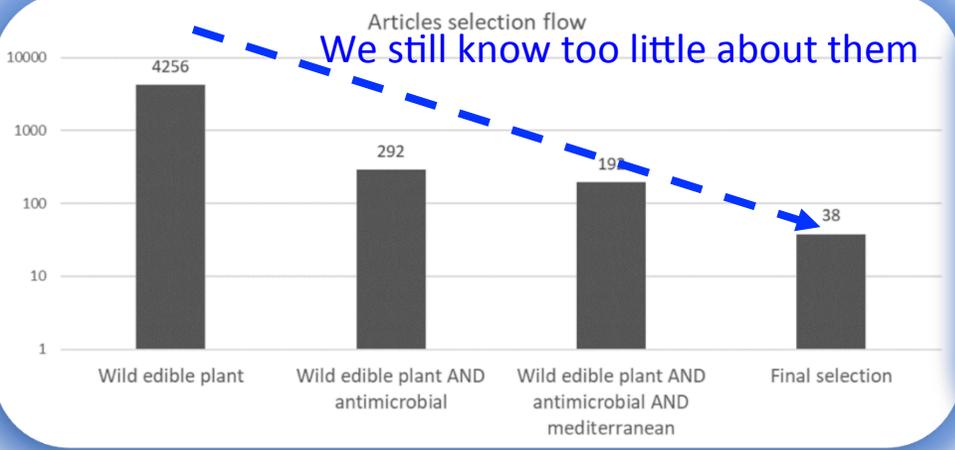
Abstract: (1) Introduction: Bacterial resistance to antibiotics is estimated to be the cause of a major number of deaths by 2050 if we do not find strategies to slow down the rise of drug resistance. Reviews on Mediterranean wild edible plants (MWEPs) with antimicrobial properties are scarce in the main databases (PubMed, Scopus, and WoS). Hence, we proceeded to conduct a new review of the studies on MWEPs. (2) Methods: We used 'wild edible plant' and 'antimicrobial' as keywords. Within this group, exclusion criteria were reviews, studies concerning non-Mediterranean plants or non-edible plants, studies on topics other than plants or containing no description of antimicrobial properties, or off-topic studies. (3) Results: Finally, out of the one hundred and ninety-two studies we had started with, we reviewed thirty-eight (19.8%) studies concerning the antimicrobial properties of seventy-four MWEPs species belonging to twenty-five Families. Fifty-seven (77%) species out of seventy-four proved to be antimicrobial, with a stringent threshold selection. (4) Conclusions: Studies are still very heterogeneous. We still know too little about MWEPs' properties; however, what we already know strongly recommends carrying on investigation.

Keywords: wild edible plants; antimicrobial effect; Mediterranean plant; Gram+ bacteria; Gram– bacteria; extraction protocols; bioactive compounds; essential oils



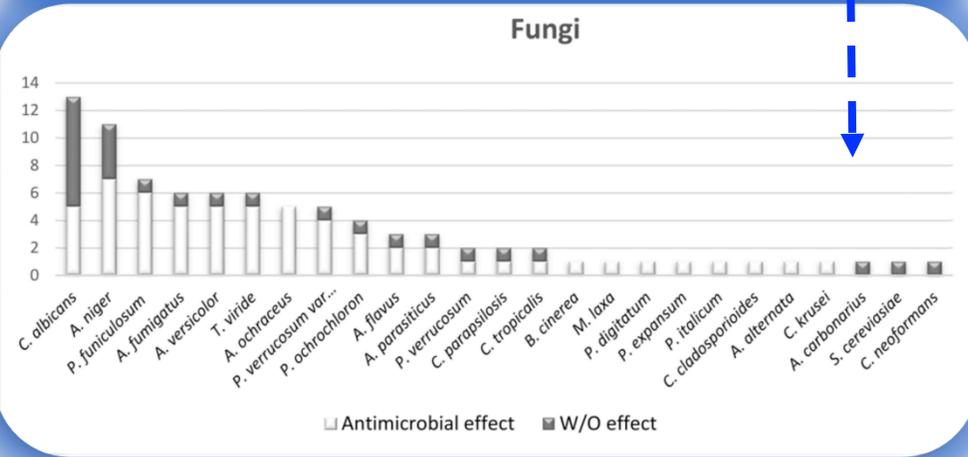
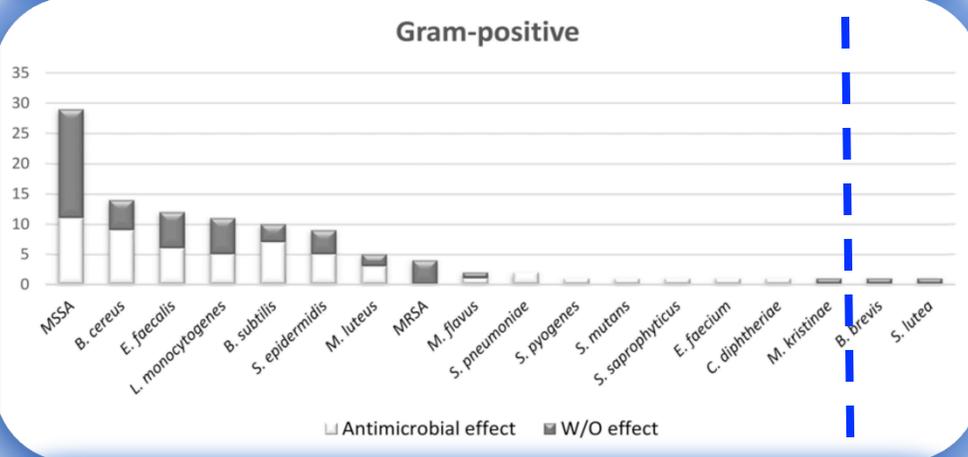
Citation: Cappelli, G.; Mariani, F. A Systematic Review on the Antimicrobial Properties of Mediterranean Wild Edible Plants: We Still Know Too Little about Them, but What We Do Know Makes Persistent Investigation Worthwhile. *Foods* 2021, 10, 2217. <https://doi.org/10.3390/foods10092217>





Families with the most studied species	Species	N° publications	Ref.
Amaranthaceae	<i>C. album</i>	2	[11,44]
Amaranthaceae	<i>C. murale</i>	2	[29,41]
Amaryllidaceae	<i>A. roseum</i>	4	[21,27,32,33]
Apiaceae	<i>F. vulgare</i>	3	[24,29,31]
Asphodelaceae	<i>E. spectabilis</i>	2	[18,22]
Asteraceae	<i>C. raphanina</i>	4	[16,17,20,37]
Asteraceae	<i>C. coronarium</i>	2	[24,35]
Asteraceae	<i>C. pumilum</i>	2	[13,24]
Asteraceae	<i>S. asper</i>	2	[7,19]
Asteraceae	<i>S. oleraceus</i>	4	[7,8,13,19]
Asteraceae	<i>T. officinale</i>	2	[8,19]
Brassicaceae	<i>N. officinale</i>	2	[41,44]
Caryophyllaceae	<i>S. vulgaris</i>	2	[19,39]
Lamiaceae	<i>Z. clinopodioides</i>	2	[25,40]
Papaveraceae	<i>P. rhoeas</i>	2	[29,44]
Polygonaceae	<i>P. aviculare</i>	2	[30,44]

Table S2. List of the 16 most studied species (described in more than one publication), belonging to 10 out of the total 25 botanical families. Four species (in bold) are analysed in more than two studies each, accounting for 39.5% of the whole thirty-eight studies reviewed.





Lavandula angustifolia Mill. Essential Oil Exerts Antibacterial and Anti-Inflammatory Effect in Macrophage Mediated Immune Response to Staphylococcus aureus

D. Giovannini, A. Gismondi, A. Basso, L. Canuti, R. Braglia, A. Canini, F. Mariani & G. Cappelli



submitted



Cinnamomum zeylanicum Blume essential oil inhibits metastatic melanoma cell proliferation by triggering an incomplete tumour cell stress response

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Article

A Rapid Nano-Liquid Chromatographic Method for the Analysis of Cannabinoids in Cannabis sativa L. Extracts

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Abstract: *Cannabis sativa L.* is an herbaceous plant belonging to the family of Cannabaceae. It is classified into three different chemotypes based on the different cannabinoids profile. In particular, fiber-type cannabis (hemp) is rich in cannabidiol (CBD) content. In the present work, a rapid nano liquid chromatographic method (nano-LC) was proposed for the determination of the main cannabinoids in *Cannabis sativa L.* (hemp) inflorescences belonging to different varieties. The nano-LC experiments were carried out in a 100 μm internal diameter capillary column packed with a C18 stationary phase for 15 cm with a mobile phase composed of ACN/H₂O/formic acid, 80/19/1% (v/v/v). The reverse-phase nano-LC method allowed the complete separation of four standard cannabinoids in less than 12 min under isocratic elution mode. The nano-LC method coupled to ultraviolet (UV) detection was validated and applied to the quantification of the target analytes in cannabis extracts. The nano-LC system was also coupled to an electrospray ionization–mass spectrometry (ESI-MS) detector to confirm the identity of the cannabinoids present in hemp samples. For the extraction of the cannabinoids, three different approaches, including dynamic maceration (DM), ultrasound-assisted extraction (UAE), and an extraction procedure adapted from the *French Pharmacopeia's* protocol on medicinal plants, were carried out, and the results achieved were compared.

Keywords: cannabinoids; *Cannabis sativa L.*; hemp inflorescences extracts; nano-LC-UV; nano-LC-MS

Citation: Žampachová, L.; Aturki, Z.; Mariani, F.; Bednář, P. A Rapid Nano-Liquid Chromatographic Method for the Analysis of Cannabinoids in *Cannabis sativa L.* Extracts. *Molecules* **2021**, *26*, 1825. <https://doi.org/10.3390/molecules26071825>

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Progetti in corso

Olea europaea nano-formulated leaves extracts
antimicrobial properties



Resveratrol anti-biofilm activity (“Liposomes as drug delivery systems of antimicrobial biomolecules obtained from F&V co-products”.)

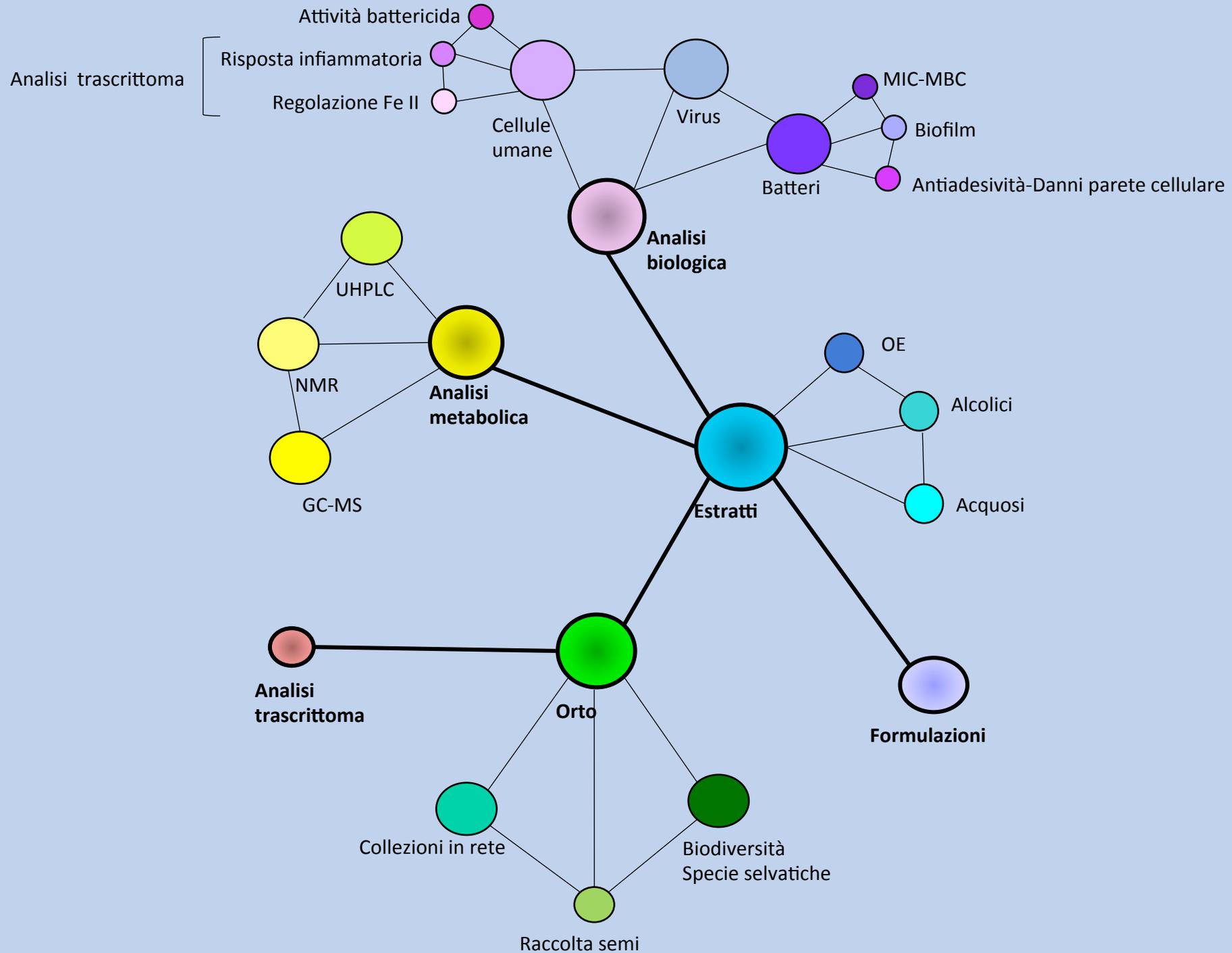


Six **wild edible plants** antimicrobial and antioxidant properties



Cannabis sativa (EOs and Idro-alcoholic extracts)
antimicrobial properties







F. Mariani



Z. Aturki



G. Cappelli



E. Donati



G. D'Orazio



I. Nicoletti



A. Sobolev



G. Testone

Grazie per l'attenzione.



G. Mancini



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