UPLC-ESI-MS/MS Profiling, Antihaemolysin and Anti-Biofilm Activities of the underground parts of Common *Iris* Species

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Introduction

According to the world health organisation (WHO) priority programs, microbial resistance is a public health threat as the curative management of infections caused by resistant microorganisms is a difficult problem in medicine, mainly due to the depletion of conventional antibiotics.

Bacterial biofilms cause more than 60% of persistent and chronic microbial infections. Haemolysin protein is one of the most common virulence traits in *Staphylococcus aureus* bacteria.

The use of plant extracts and their phytochemicals as candidates for targeting the microbial resistance inhibition is increasingly focused in last decades. In Mongolian traditional medicine, Irises were long used for the treatment of bacterial infections. Irises have been used since the Ancient Egyptians.

Iris is the largest genus of family Iridaceae distributed mainly in temperate and tropical regions (Wilson, 2011). Although, *Iris* species are widely cultivated as ornamentals, since ancient times, yet several *Iris* species were presented as hot spot for research in 2020 reporting their medicinal activities, primary metabolites and secondary metabolites.

This study was designed to evaluate the *in vitro* antimicrobial potential of the polar (PFs) and non-polar (NPFs) fractions of the underground parts of the three *Iris* spp. (*I. confusa*, *I. pseudacorus* and *I. germanica*) against four prevalent pathogenic bacteria;. The biofilm inhibition and anti-haemolytic potentials of the aforementioned *Iris* species on methicillin resistant and sensitive (MRSA and MSSA) *S. aureus* bacterial strains were explored. Additionally, we targeted the qualitative and quantitative determination of these Irises metabolites using UPLC-ESI-MS/MS analysis in an attempt to correlate the detected metabolties with the observed activities.

Material & Methods

Plant material:

The flowering *I. confusa* Sealy was collected from Al-Mansouria, Giza, Egypt while, flowering *I. pseudacorus* L. and *I. germanica* L. were collected from the Experimental Station of Medicinal Plants of the Faculty of Pharmacy, Assiut University, Egypt. The underground parts were separated, air-dried in shade, and extracted according to Salem *et al*, 2016 (Figure 1)

UPLC-ESI-MS/MS analysis

This analysis was done according to Salem *et al*., 2016. The mass spectra of both the PFs and NPFs were acquired by full scan and all-ion-fragmentation MS in both ionization modes on an Exactive high resolution Orbitrap-type MS. The data matrices were obtained using Xcalibur software.

Antibacterial and virulence inhibitory activity

Staphylococcus aureus MTCCoo 87, *Bacillus sphaericus* MTCC 511, *Escherichia coli* MTCC 443 and *Enterobacter aerogenes* MTCC 111 were selected for the antibacterial screening. *S. aureus* ATCC 29213 highly haemolysin producing strain and two high biofilm producing MSSA and MRSA isolated from wound and abscess skin infections, respectively and identified by Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF Vitek MS) and confirmed by 16s rRNA genetic identification were used.

Statistical Analysis

Multi-variate data analysis was performed using Metaboanalyst 3.0. Concerning the microbiological assays, Student's t-test was used and the differences were considered significant if the *p* values were <0.05.

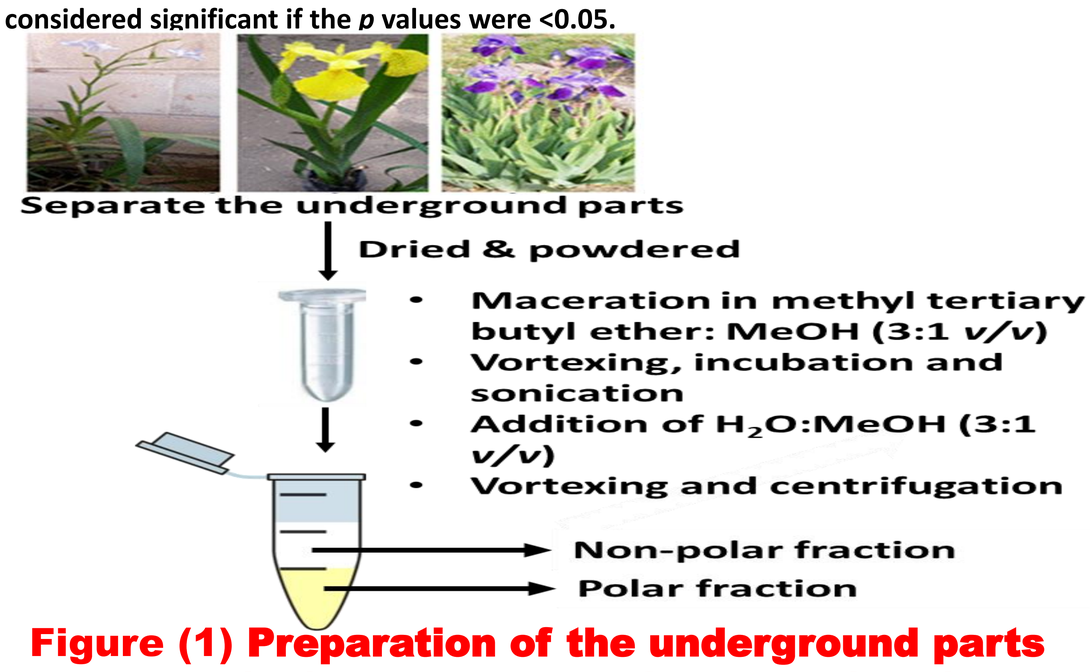

Figure (1) Preparation of the underground parts polar (PF) and non-polar fractions (NPF)

Results

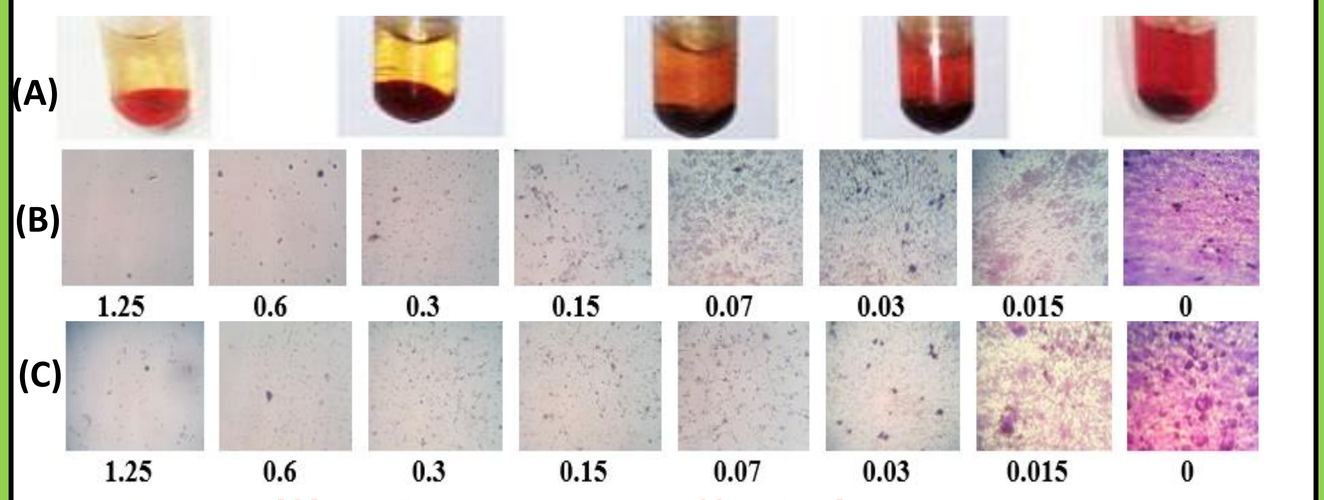

Figure (2) *I. Pseudacorus* effect A) on haemolysin production B) MRSA C) MSSA biofilm inhibition percentage

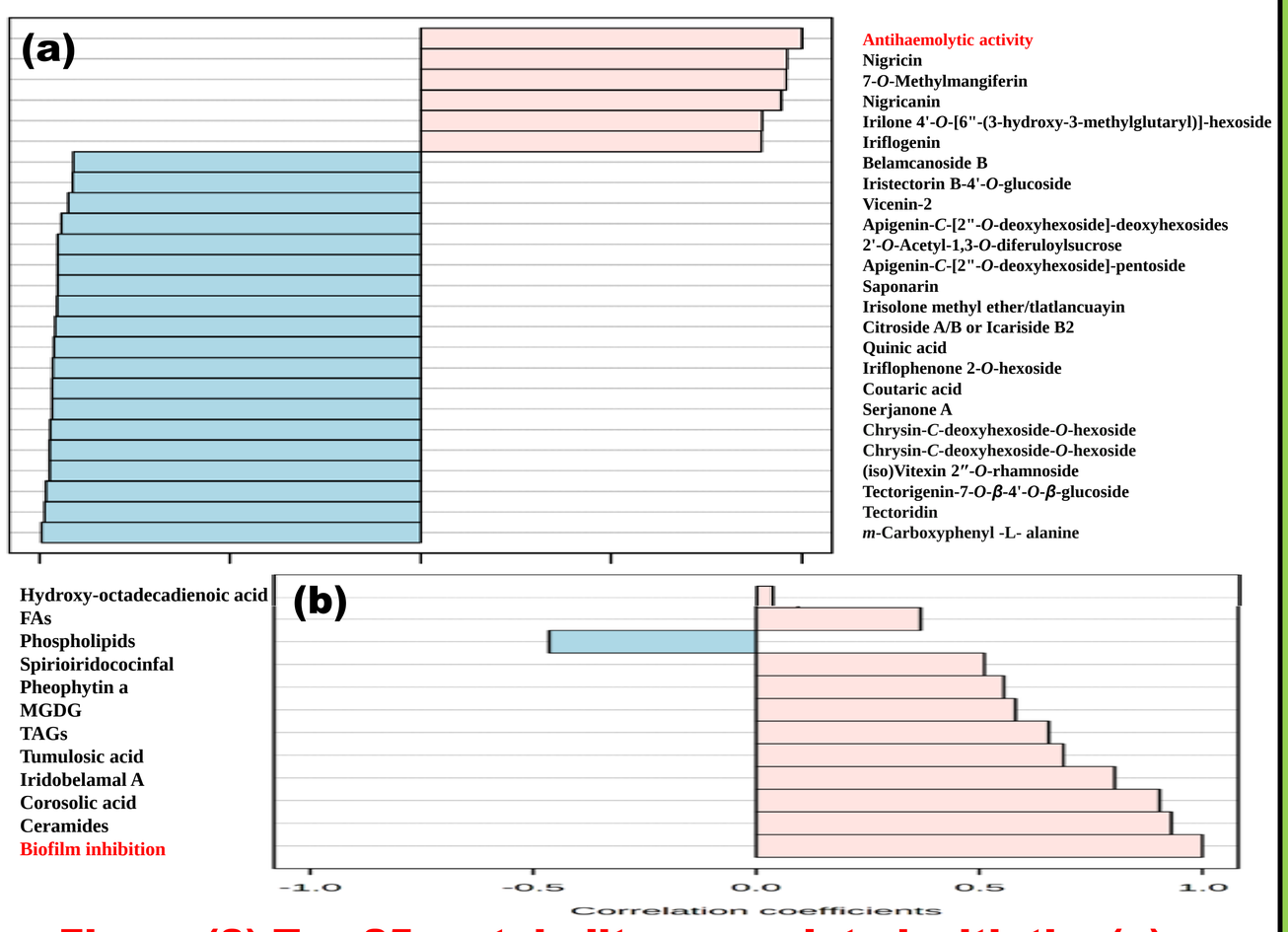

Figure (3) Top 25 metabolites correlated with the (a) antihaemolytic and (b) antibiofilm activities

Conclusion

***I. pseudacorus* showed the most potent biofilm inhibition and antihaemolytic potentials against MRSA and MSSA. Isoflavonoids and xanthones** were correlated with the anti-haemolytic activity. While, **triterpene acids, iridals, triacylglycerols and ceramides** were correlated with the biofilm inhibition potential.**It is highly advised to** isolate PFs and NPFs major compounds and to explore their possible antimicrobial potencies both via *invitro* and *invivo* models.

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References

*Salem, M.A., *et. al.*, 2016. Protocol: a fast, comprehensive and reproducible one-step extraction method for the rapid preparation of polar and semi-polar metabolites, lipids, proteins, starch and cell wall polymers from a single sample. Plant Methods 12(1), 1-15.

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