AIT Research

AN INVESTIGATION INTO THE SAFETY AND THERAPEUTIC EFFICACY OF HERBAL PRODUCTS FOR ANXIETY AND DEPRESSION

Tomasz Szank, Gary Stack, Therese R. Montgomery
Bioscience Research Institute, Athlone Institute of Technology

Background

12 % of the Irish population is believed to be suffering from depression[1-3], whilst 800,000 deaths are reported annually worldwide[4]. Depression will, therefore, be the largest contributor to disease burden by 2030 and there is an urgent need to develop improved treatments. Current pharmacotherapies are based upon the monoamine theory of depression. Conventional antidepressants can induce unpleasant side-effects, resulting in low patient compliance and a high failure response rate[1,5].

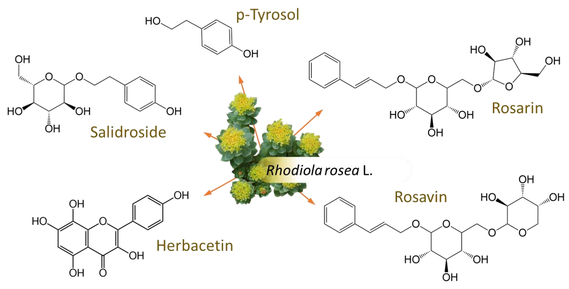


Figure 1. *Rhodiola rosea L. and its active compounds Tyrosol, Salidroside, Rosavin, Rosarin and Herbacetin[6,7]*

Herbal extracts of the medicinal plant *Rhodiola rosea* have been used traditionally to treat anxiety, stress and depression with few reported side effects, yet little is known as to which plant constituent is responsible for this action[8-11]. The primary goal of this research is to determine the safety and potential anti-depressant efficacy of selected *Rhodiola rosea* bioactive compounds (Fig.1).

Research Question

Can specific bioactive compounds within the medicinal plant *Rhodiola rosea* modulate monoamine neurotransmission, cellular inflammation and antioxidative status, allowing for the alleviation of depressive symptoms without side effects?

Methodology

Cell Models

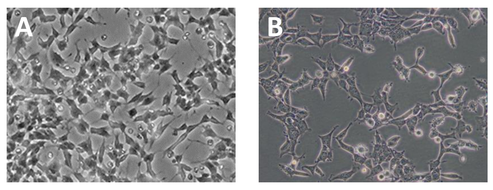


Figure 2. *Undifferentiated SH-SY5Y (A) and T-REx SERT HEK293 (B) cell lines at low density.*

SH-SY5Y

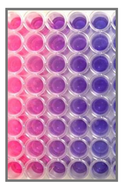
- Human neuroblastoma cell line with catecholaminergic phenotype; *in vitro* neuronal cell model.
- Express noradrenaline transporter (NET).

T-REx SERT HEK293

- Transfected human embryonic kidney (HEK293) cell line expressing serotonin transporter (SERT) under inducible tetracycline (TET) promoter[12].

Phase 1. Safety – *neurotoxic and neuroprotective effects*

- Viability via resazurin reduction assay
- Cellular antioxidant activity in SH-SY5Y cells via 2',7'- dichlorodihydrofluorescin diacetate (DCFH-DA) assay.



Phase 2. Neuromodulation – *effects on serotonin and noradrenaline reuptake*

- Characterisation of SERT and NET dependent uptakes.
- SERT and NET dependent uptake of radiolabelled substrate, [^{3}H]MPP+ *in vitro.*

Phase 3. Market Evaluation – *commercial extracts content.*

- High Performance Liquid Chromatography (HPLC) Dual Absorbance Detector (DAD) method development.
- Qualitative and quantitative evaluation of commercial *Rhodiola rosea* extracts.

Results

Phase 1. Rhodiola rosea: a safe, potent antioxidant.

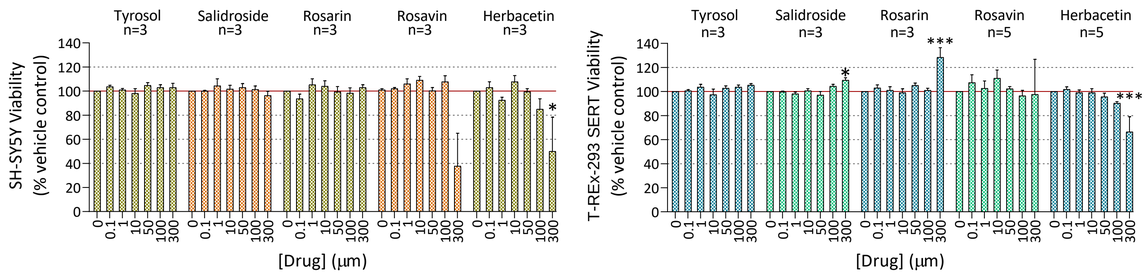


Figure 3. Drug effect on resazurin reduction in SH-SY5Y (LEFT) and T-Rex-293 SERT cells after 24-hour drug treatment. *Data = means of n individual experiments performed in quadruplicate ±SEM. One way ANOVA with Dunnett's post hoc (drug vs untreated control: *P<0.05, **P<0.01, ***P<0.001).*

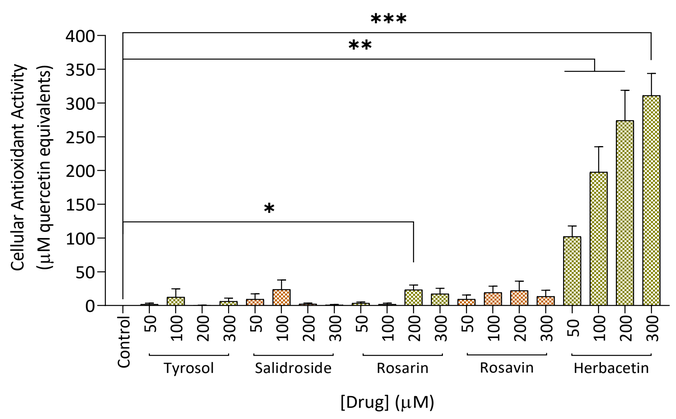
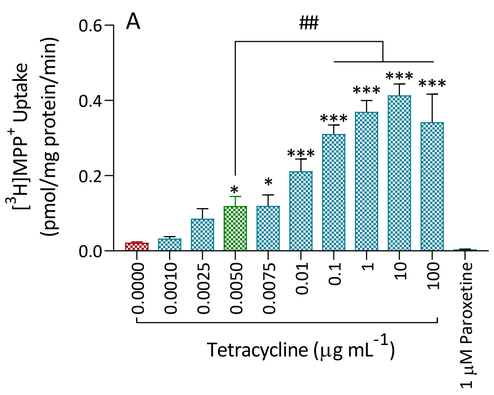


Figure 4. Cellular antioxidant assay in neuronal SH-SY5Y cells. *Mean of n=4 ±SEM in triplicate. One-way ANOVA+ Dunnett's post hoc (treatment vs untreated control) (*P<0.05, **P<0.01, ***P<0.001). The cellular antioxidant activity represented as μM quercetin equivalents.*

Phase 2.A. TET induced [^{3}H]MPP+ uptake (A) and SERT expression (B) in T-REx-293 SERT is dose-dependent.

Figure 7.A. TET induced SERT dependent uptake of radiolabelled substrate [^{3}H]MPP+ in T-REx-293 SERT cell line. *Data = mean of at least two independent experiments performed in quadruplicate ±SEM. One-way ANOVA with Tukey post hoc (vs untreated control: *P<0.05, **P<0.01, ***P<0.001; between treatments:#P<0.05, ##P<0.01). Optimal [TET] was established to be 5 ng/mL (highlighted in green).*



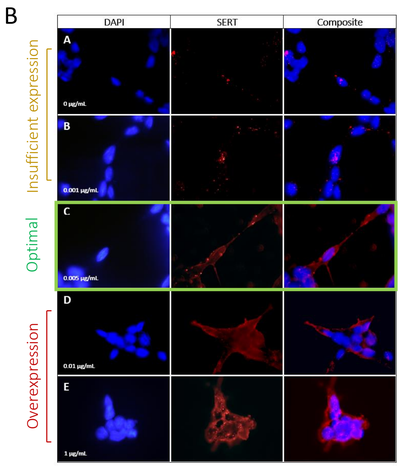


Figure 7.B. Immunocytochemistry and fluorescent microscopy analysis of TET induced SERT expression in T-REx-293 SERT cell line

- 0 – 1 μg/mL TET for 24 hours
- Fix: Acetone:MeOH (1:1), Block 1% BSA in PBST
- 1°Ab – SERT H-45 mouse polyclonal
- 2°Ab – chicken-anti-mouse IgG (H+L), CF™568 (RED)
- Nuclei counterstained with DAPI (BLUE)
- Leica DM2500 fluorescent microscope.
- Image composites created using Image-Pro Plus 6.0 for Windows

Phase 2.B. Rhodiola rosea: a potential NET and SERT inhibitor.

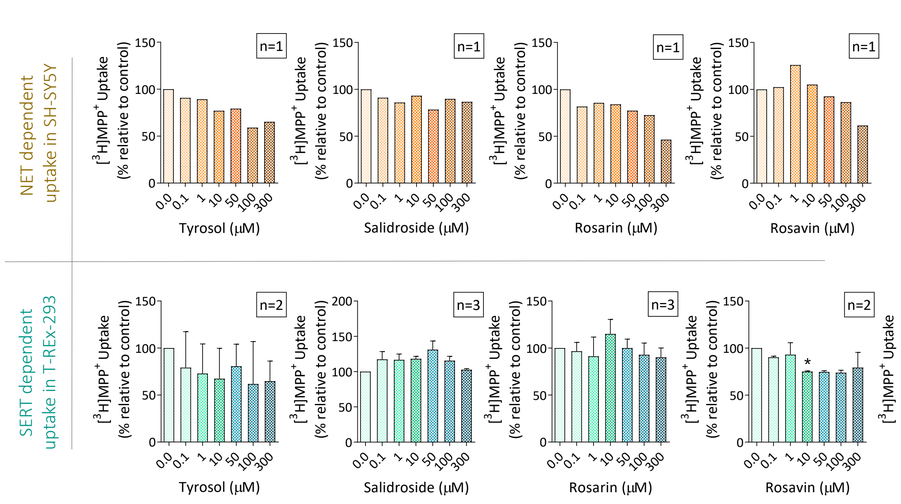


Figure 8. Drug effects on NET and SERT dependent [^{3}H]MPP+ uptake. *Data = mean of n independent experiments ±SEM in quadruplicate. One-way ANOVA with Dunnett's post hoc (*P<0.05).*

Phase 3. Market analysis of commercial *Rhodiola* extract.

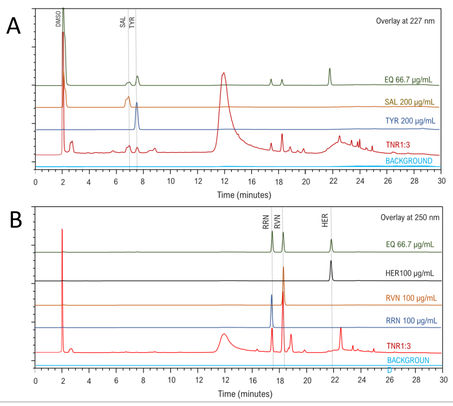


Figure 9. Chromatograms overlay of commercial Rhodiola extract. *Tyrosol and salidroside detected at 227 nm (A), rosavin, rosarin and herbacetin detected at 250 nm (B). Analysis performed on Waters 2695 HPLC DAD system.*

Table 1. Commercial sample analysis and label claim.

Item:	TNR	0.31 g/capsule	
Compound	mg/capsule	% content	% label claim
Tyrosol	1.782	0.57	---
Salidroside	3.940	1.27	102
Rosarin	2.322	0.75	93
Rosavin	4.765	1.54	
Herbacetin	0.294	0.09	---

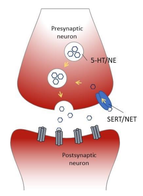
Discussion

- *Rhodiola rosea* shows potent antioxidant activity and no cytotoxicity at predicted physiological doses in both cell models. Increase in resazurin reduction will be further investigated via flow cytometry and light microscopy to establish whether the increase is caused by cell number or cell cycle modulation.
- Initial data suggest that *Rhodiola* modulates neurotransmission via reuptake inhibition – further experiments are required.
- HPLC method developed shows suitability for qualitative and quantitative analysis of bioactive constituents in commercial extracts. Optimisation and analysis of other commercial samples will be completed.

Future Directions

Phase 1. Safety:

- Completion of cytotoxicity/neuroprotective effects study.



Phase 2. Neuromodulation:

- Completion of investigation of drug effects on SERT and NET dependent uptake.

Phase 3. Market comparison:

- Evaluation of OTC Rhodiola formulations available on Irish market via HPLC.

Phase 4. Neuroimmunomodulation

- Collaboration with immunology research group WIT.
- Further antioxidant assays
- Investigation of pro and anti-inflammatory factors at protein and DNA level

Conclusion

Carefully selected bioactive constituents are showing promise. We are beginning to understand their mechanism of action in terms of antidepressant and anxiolytic efficacy. Further work will reveal whether one or more of these compounds have potential as an individual commercial entity for the treatment of depression and anxiety.

Acknowledgements

- T-REx-293 SERT cells were a gift from Prof. Jana Haase, Conway Institute, UCD.
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