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Technological University of the Shannon: Midlands Midwest
Ollscoil Teicneolaíochta na Sionainne: Lár Tíre Iarthar Láir

TUS Research

Monoamine modulators of herbal origin – *Rhodiola rosea* L.

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Introduction

Rhodiola rosea L. is a traditional herbal medicine used to relieve symptoms of fatigue, enhance mental performance and increase resistance to stress whilst promoting antidepressant, anxiolytic and neuroprotective effects[1-6]. Rhodiola's reported activity on mood and cognition might be effective in the treatment of mild to moderate depression as suggested by numerous studies and clinical trials. Furthermore, subjective user reports indicate reduced side effects when compared to conventional pharmacotherapies. Its adaptogenic efficacy is believed to be associated with biogenic monoamine and opioid synthesis, transport and receptor activity[1-6]. The phytochemical composition of Rhodiola is diverse, however, commercial extracts are typically standardised to salidroside and rosavins only[1].

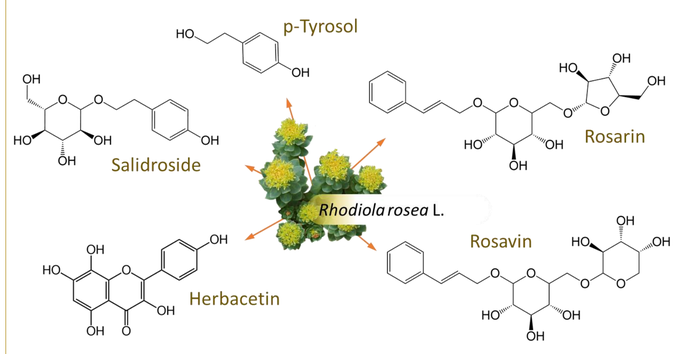


Figure 1. *Rhodiola rosea L. and its active compounds Tyrosol, Salidroside, Rosavin, Rosarin and Herbacetin[3,4]*

Aims and Objectives

To test antidepressant efficacy of selected bioactive constituents and commercial herbal extracts of medicinal plant Rhodiola rosea in two *in vitro* neuronal cell models.

Methods

Antidepressant activity tested via investigation of the effect on biogenic amine transporters (NET and SERT) in two in vitro neuronal cell models:

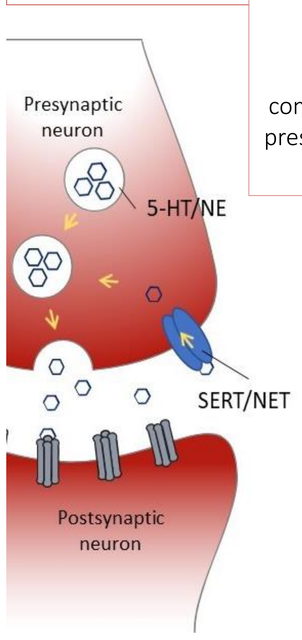
- **SH-SY5Y**: Human neuroblastoma with catecholaminergic phenotype. Cell model for efficacy testing on noradrenaline transporter (NET).
- **T-REx-293 SERT**: Human embryonic kidney, expressing serotonin transporter (SERT) under tetracycline operator[7]. 24 hour prior drug exposure, T-REx cells were treated with 5 ng mL-1 tetracycline for optimal SERT expression.

Effects on NET and SERT specific uptake assessed via radiolabelled substrate assay and scintillation counting.

Cells treated with individual bioactive constituents or commercial Rhodiola extract in the presence of radiolabeled [^{3}H]MPP$^+$.

Non-specific uptake measured in the presence of nisoxetine or paroxetine for NET and SERT respectively.

Membrane integrity post extract exposure measured by Neutral Red Release assay.



Results

1. Rhodiola's main bioactive constituents inhibit NET dependent [^{3}H]MPP+ uptake in SH-SY5Y cells but do not affect SERT in T-REx-293 cells at high (>100µM) concentrations.

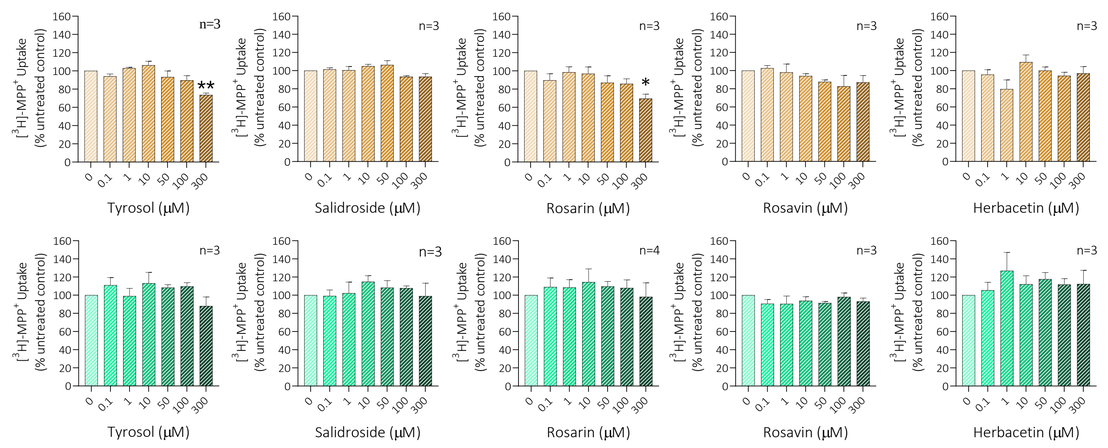


Figure 2. Drug effects on NET (TOP) and SERT (BOTTOM) dependent uptake of [^{3}H]MPP$^+$ in SH-SY5Y and T-REx-293 SERT cells. Data representative of average percentage control of n independent experiments performed in triplicate ±SEM. One-way ANOVA with Tukey post hoc (vs untreated control: *P<0.05, **P<0.01, ***P<0.001). Tyrosol (73.6 ±2%) and rosarin (69.5 ±4.5%) shows significant inhibition of NET dependent MPP+ uptake in SH-SY5Y. No inhibition was noted at SERT.

2. Commercial Rhodiola extract inhibits NET [^{3}H]MPP+ uptake in a competitive manner.

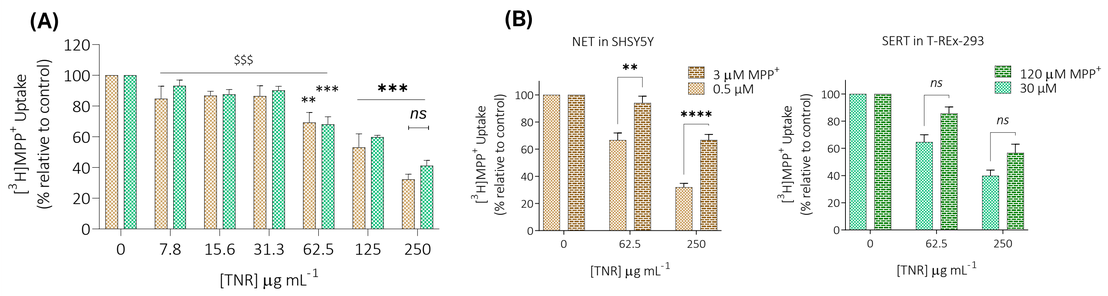


Figure 3. Dose dependent (A) competitive inhibition (B) of NET and SERT [^{3}H]MPP$^+$ dependent uptake by a commercial Rhodiola extract. Data representative of the mean of at least three independent experiments performed in triplicate ±SEM. Two-way ANOVA with Tukey post hoc was used to discover significant differences between treatments (vs untreated control: *P<0.05, **P<0.01, ***P<0.001; vs 250 µg mL^{-1}: $P<0.05, $$P<0.01, $$$P<0.001). Rhodiola extract inhibits NET (250 µg mL^{-1}: 33 c ±4%) and SERT (250 µg mL^{-1}: 47 ±2%) [^{3}H]MPP$^+$ dependent uptake in a dose dependent manner (A). The upward shift of uptake with addition of the substrate (B) at NET, suggests a competitive mode of inhibition of this transporter. Moderate, albeit not significant (P>0.05) difference was observed at SERT.

3. Inhibition of MPP+ uptake is not associated with membrane integrity loss.

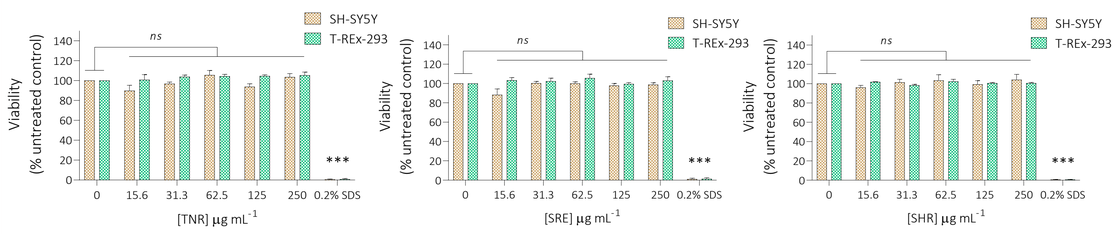


Figure 4. Acute (10 minutes) effects of Rhodiola extracts exposure on membrane integrity assessed via Neutral Red Release assay. Data representative of the mean of three independent experiments performed in triplicate ±SEM. One way ANOVA with Tukey post hoc was used to discover significant differences between treatments vs untreated control (*P<0.05, **P<0.01, ***P<0.001). Data suggests that extract efficacy as shown by reduced intracellular MPP$^+$ is not associated with compromised membrane integrity.

4. Rhodiola's main secondary metabolites do not affect biogenic monoamine transporters.

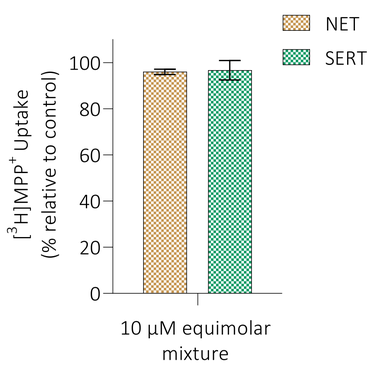


Figure 5. Equimolar (10 µM) mixture of main secondary metabolites does not inhibit NET in SHSY5Y and SERT in T-Rex-293 dependent uptake of [^{3}H]MPP+. Data: mean of n=3 ±SEM. A paired t-test (treatment vs untreated control) did not discover a statistically significant difference (P>0.05) suggesting that the efficacy of the extract is not associated with the additive effect of main constituents.

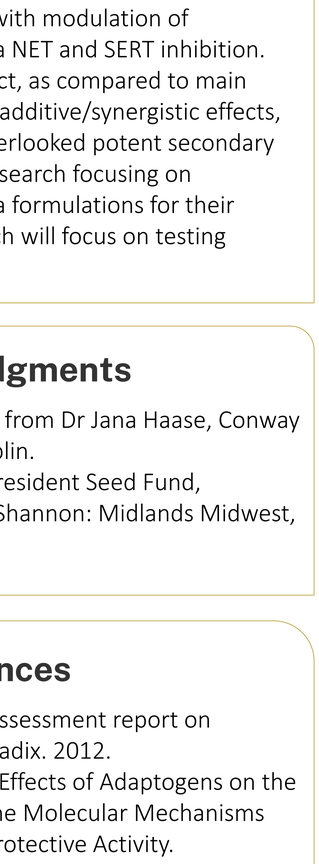
Future Directions

Neuromodulation:
- Investigation of potential additive/synergistic effects.
- Discovery of a novel inhibitor from Rhodiola extract.

Market analysis:
- Investigation into the content of commercially available Rhodiola extracts on Irish market

Neurotoxicity:
- Further investigation of toxicity of commercial Rhodiola extracts.

Neuroinflammation:
- Investigation the effect of Rhodiola on neuroinflammation.



Conclusion

- Results suggest that the reported effect on mood, attention and focus could be associated with modulation of noradrenaline and serotonin via NET and SERT inhibition.
- The higher efficacy of the extract, as compared to main constituents, possibly suggests additive/synergistic effects, or perhaps a presence of an overlooked potent secondary metabolite. There is ongoing research focusing on evaluating commercial Rhodiola formulations for their content and the future approach will focus on testing additional extracts.

Acknowledgments

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