



Harnessing South African Biodiversity for Cosmeceutical Development – *Greyia radlkoferi* a Novel Botanical Active for Even Skin Tone

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Presentation Layout

- Introduction
- *In vitro* results
- *In vivo* (clinical trial) results
- Preparing for commercialisation
- What's new?
- Closing remarks



Introduction

- South African biodiversity
 - Approximately 21 539 species
 - 60.50% - endemic
- Ethnobotanical heritage
- WHO: Developing countries – 70% to 95%
- Plant-based – prominent in rural areas
- Treatment of skin conditions



Greyia species

- *Greyia* genus – 3 species
 - *Greyia flanaganii* - Rare
 - *Greyia radlkoferi* - LC
 - *Greyia sutherlandii* – LC
- Recorded uses:
 - Wood – utensils and fencing
 - Horticulturally
 - GF – Xhosa tribes: kraals
 - Ward off sickness and prevent theft of livestock
- **No** recorded literature for **medicinal uses**

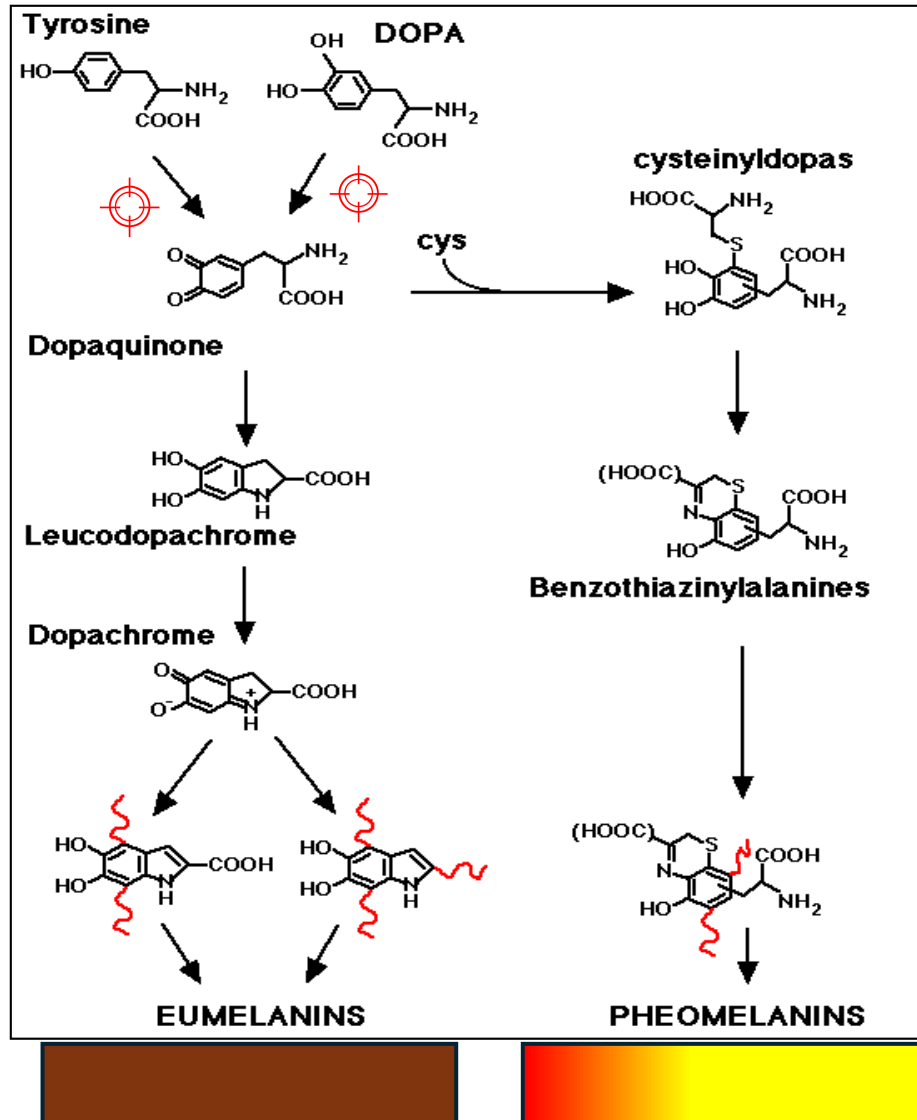


Skin hyperpigmentation

- Condition – excess production of the melanin pigment
- Risk factors
 - Excess UV exposure (sun spots/solar lentigo)
 - Inflammation – post-inflammatory pigmentation (acne)
 - Topical corticosteroids (melasma)
 - Hormonal responses (melasma)
- SA Market: \$7.40 M (2023) → \$10.8 M (by 2030)



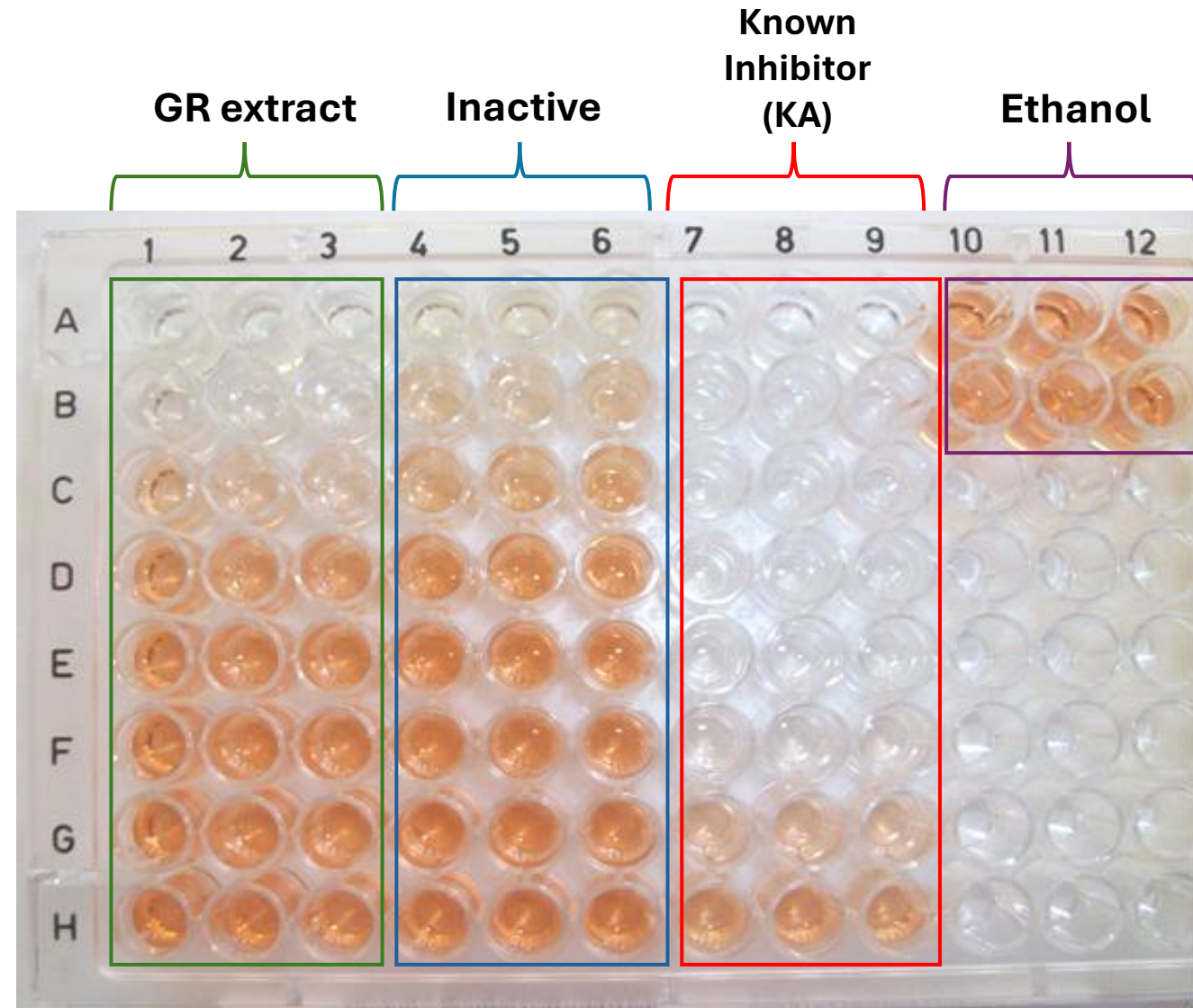
Targeting melanin biosynthesis



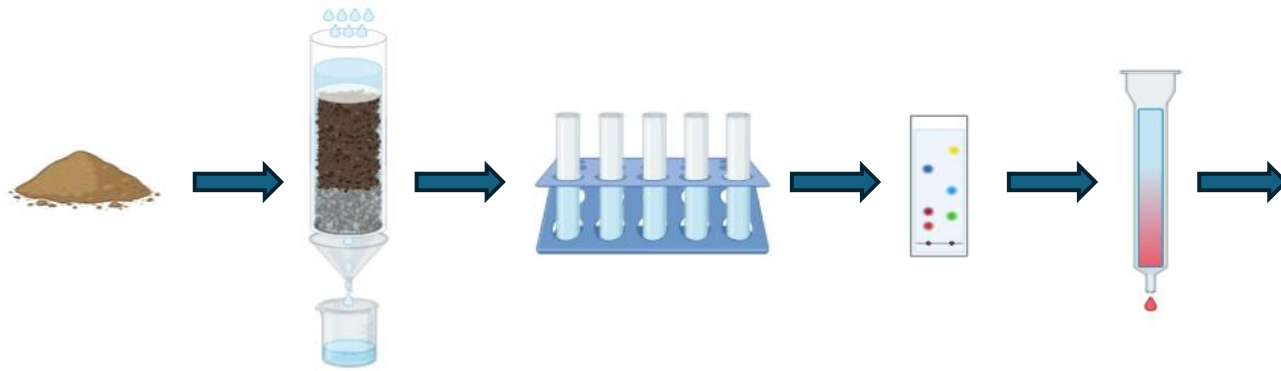
Tyrosinase enzyme

In vitro (lab) – inhibition of tyrosinase activity

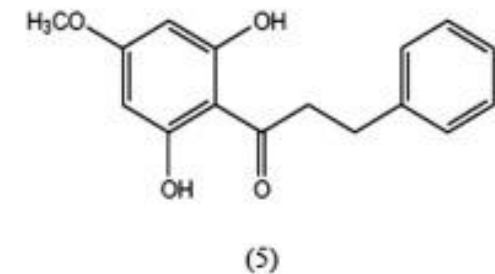
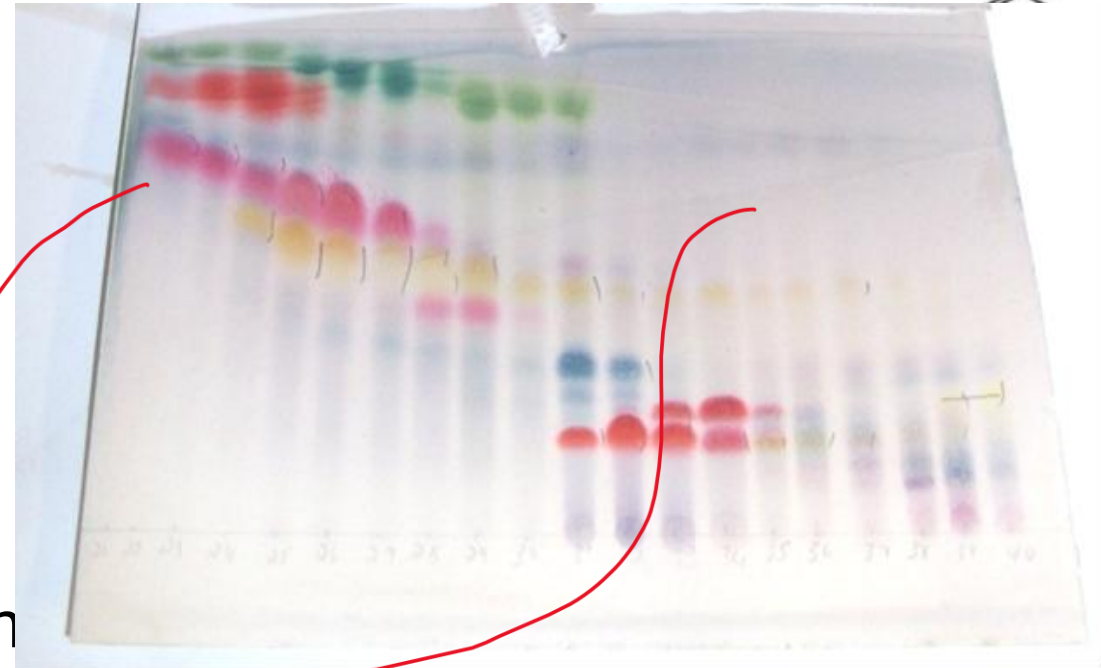
- GR EtOH extract – tyrosinase enzyme inhibition
- Brown colour = enzyme activity
- Clear colour = enzyme inhibition
- GR IC₅₀: 17.96 µg/mL
- Kojic acid IC₅₀: 3.78 µg/mL
- Lower the value – the higher the activity



Identification of bioactive compounds

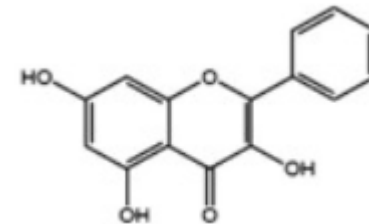
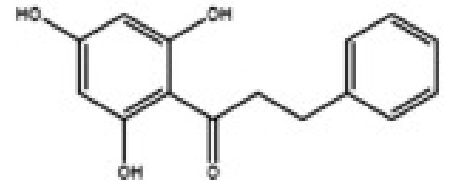


- 5 compounds identified
- C1 – 2',4',6'-trihydroxydihydrochalcon
- C2 – 3,5,7-trihydroxyflavone



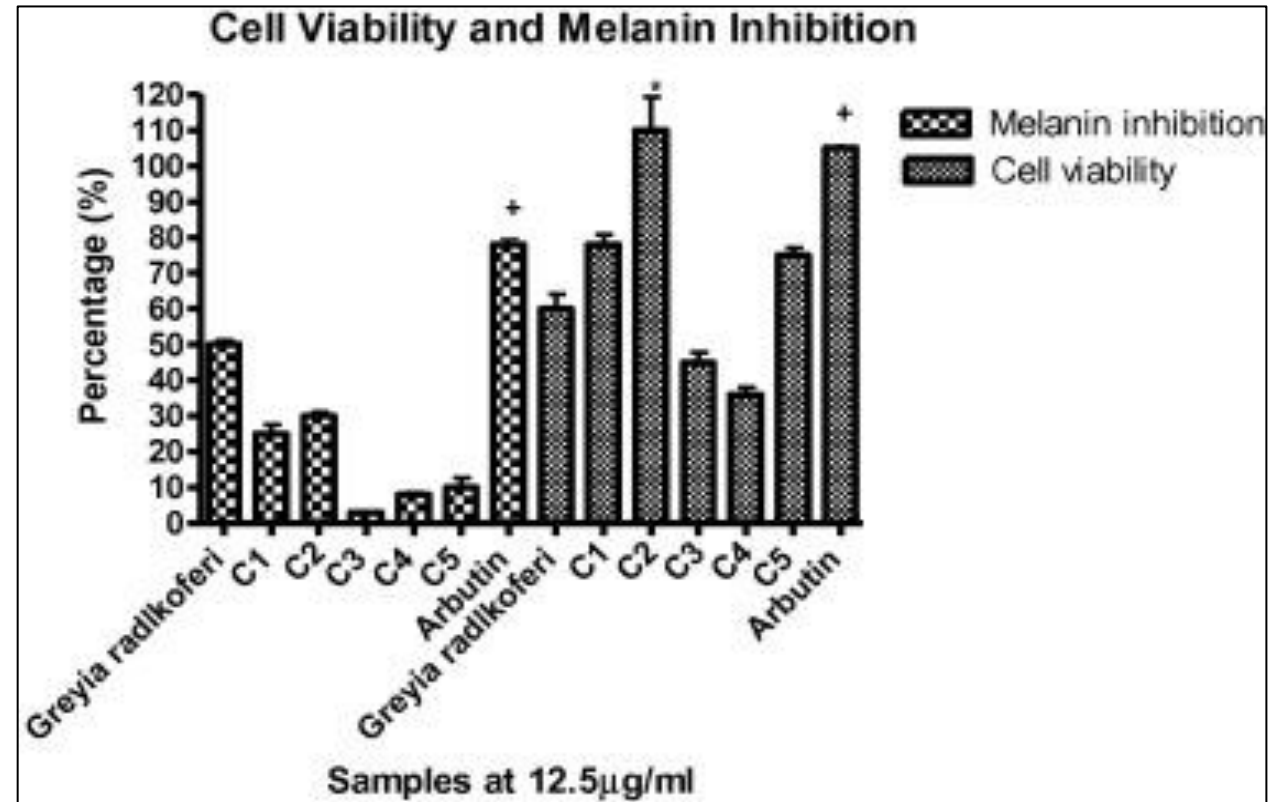
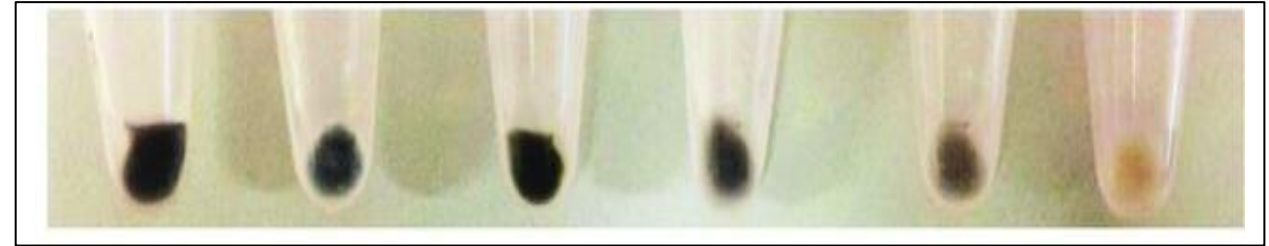
In vitro (lab) – inhibition of tyrosinase activity

- GR EtOH extract IC₅₀: 17.96 µg/mL
- C1 (2',4',6'-trihydroxydihydrochalcone) IC₅₀: 17.70 µg/mL
- C2 (3,5,7-trihydroxyflavone) IC₅₀: 113.60 µg/mL
- Kojic acid IC₅₀ : 3.78 µg/mL



In vitro results (in the lab)

- Inhibition of melanin production (cell-based model)
- B16-F10 melanocyte cells (melanin producing)
- GR extract – Inhibited 50%
- C1 – Inhibited 25%
- C2 – Inhibited 30%
- Arbutin – Inhibited 78%



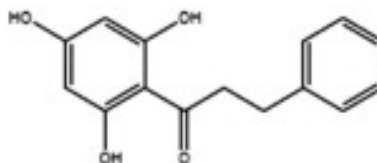
Molecular docking

- Computational technique
- Interaction between compounds and tyrosinase enzyme

- Goodness of fit score (GOF) – higher shows better fit in AS

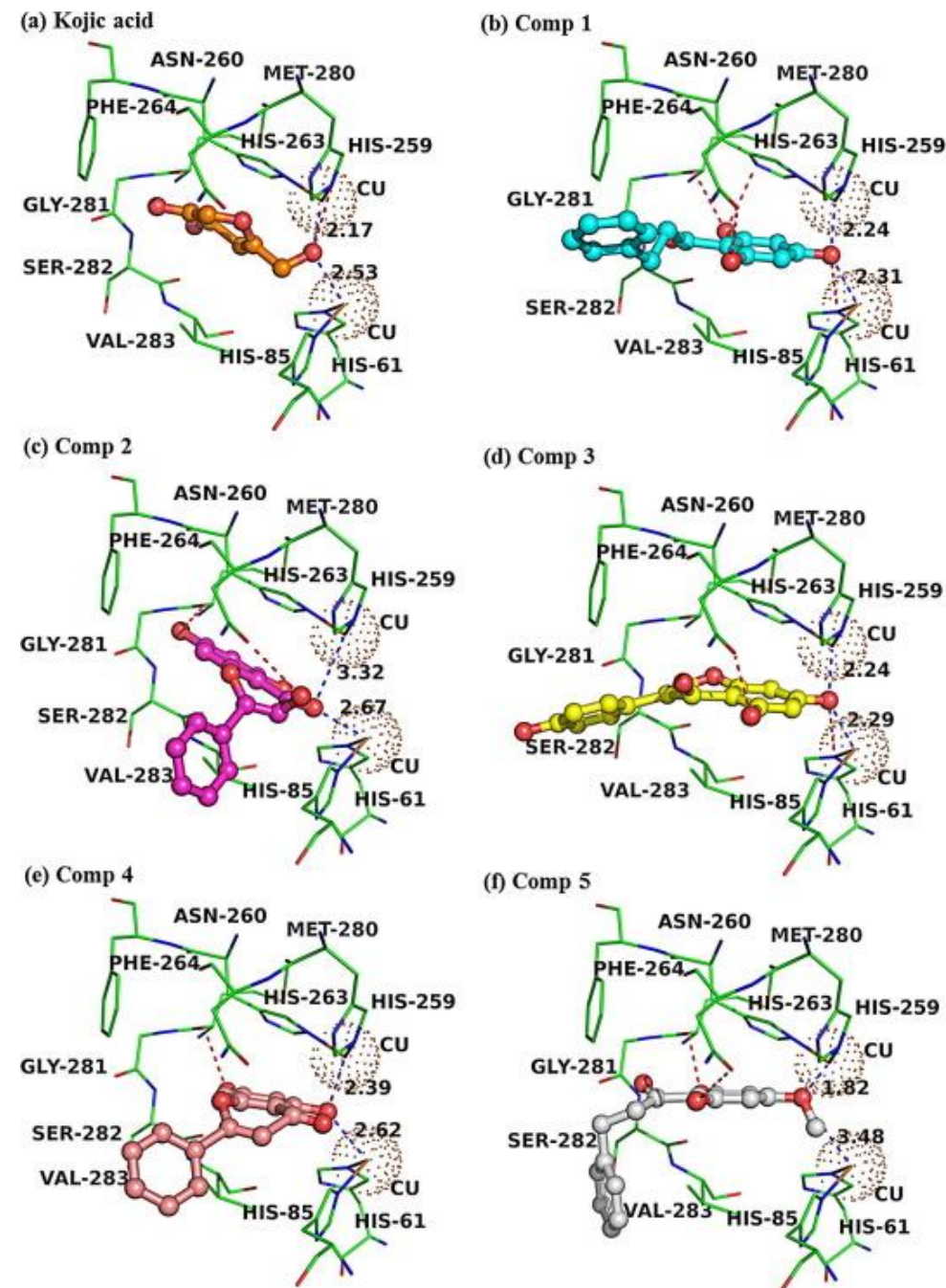
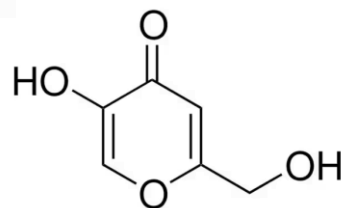
• C1

- GOF = 55.58
- His60, Asn260, His263, Met280 and **Cu²⁺ ions**



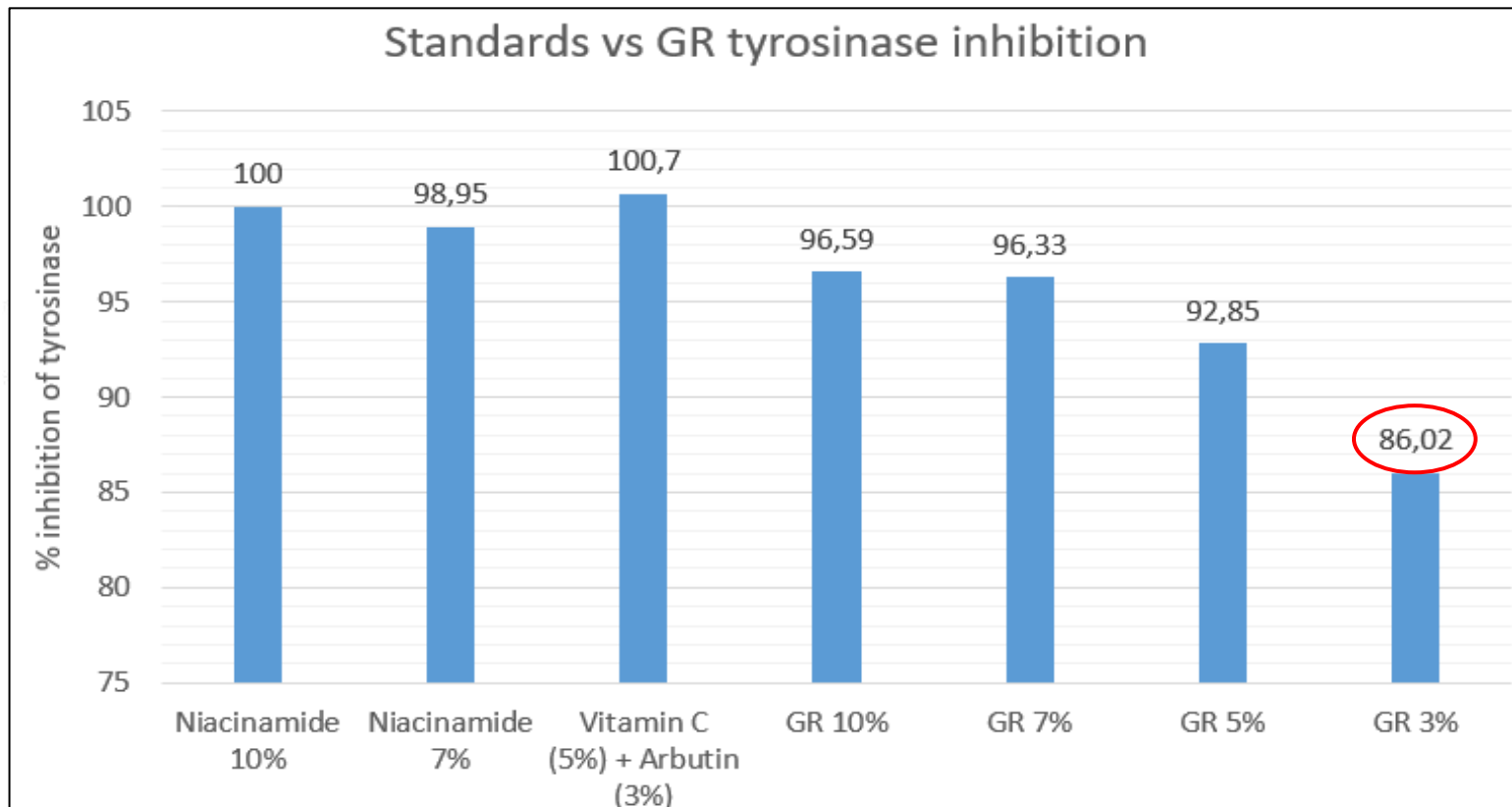
• Kojic acid

- GOF = 47.95
- His259 and **Cu²⁺ ions**



Preparing for commercialisation:

- Compared several concentrations against niacinamide and arbutin-Vit C combination



In vitro mutagenicity/genotoxicity

- Check whether GR EtOH extract had any potential mutagenic effects (cause damage to DNA)

Strain: TA 100 (Base-pair substitutions – changes in nucleotides)			
Sample	Concentration		
	5 mg/mL	0.5 mg/mL	0.05 mg/mL
GR EtOH extract	83.67 ± 1.53	101.33 ± 2.08	109.67 ± 6.03
Blank (solvent control)	112.40 ± 3.65		
4-nitroquinolone-1-oxide	438.67 ± 9.07		

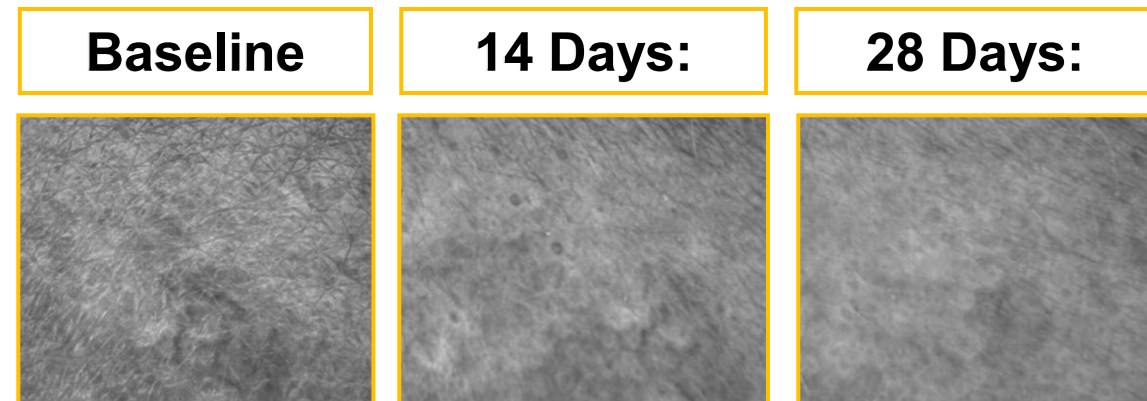
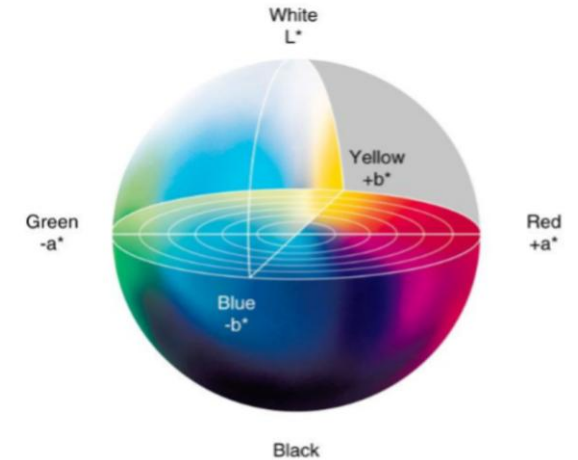
Mutagen – shows a **2-fold increase** in # of revertant **colonies** compared to the **blank** (EtOH)

In vivo safety results (clinical studies)

Sample number	Sample type	Number of subjects with a reaction after 48hrs	Irritancy Potential %	Irritancy
3239	<i>Greyia radlkoferi</i> extract (neat)	2	-34.21	Non-irritant
Positive control	Sodium-lauryl sulfate (1%)	12	100.00	Irritant
Negative control	Demineralised water	6	0.00	Non-irritant

In vivo safety results (clinical studies)

- Efficacy evaluation
 - Skin even tone
 - 20 volunteers (20 – 57 years old)
 - 10 – Fitzpatrick Type II
 - 10 – Fitzpatrick Type IV
- GR extract
 - 3% in carrier formulation
 - Improve skin even tone after 28 days
 - Twice-daily application



Additional testing (batch-to-batch)

- Stability testing (formulation)
- Preservative Challenge Test – Euxyl® PE9010
- Total Yeast and Mold Count (TYMC)
- Heavy Metal Analysis
- **Bothlale Ba Rramutla Farms** (Exhibition Stand)



Intellectual property

- South African Patent
 - 2014/08128
- PCT Patent (international)
 - China
 - European Union (France, Germany, UK)
 - India
 - Malaysia
 - South Korea
 - USA

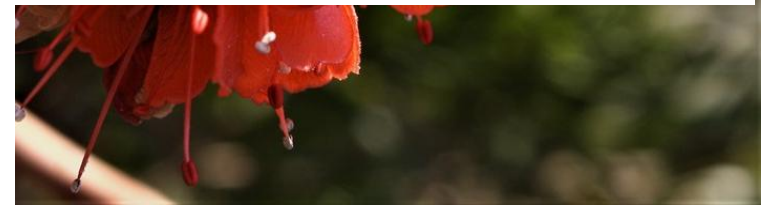


WO2013171720A1
WIPO (PCT)

Extract of *greyia radlkoferi* and use thereof

Abstract

This invention relates to the isolation, and use of a plant extract in the treatment of skin hyper-pigmentation. More particularly, this invention relates to the isolation of a tyrosinase inhibitor in an extract of plant material from the *Greyia radlkoferi* (*G. radlkoferi*) plant, the extract including 5,7-dihydroxyflavone[(2S)-pinocembrin]; 2', 6'-dihydroxy-4'-methoxydihydrochalcone; 2',4',6'-trihydroxyhydrochalcone; 3,5,7-trihydroxyflavone and 4',5'7-thhydroxyisoflavone.



Prototype



What's new?

- Developed a chromatographic method (HPTLC) to quantify bioactive marker compounds
 - QC method for potential manufacturers
 - Confirm batch-to-batch consistency
 - Provide opportunity for the *Greyia radlkoferi* extract to reach global markets(strict regulations – quality/standardisation)



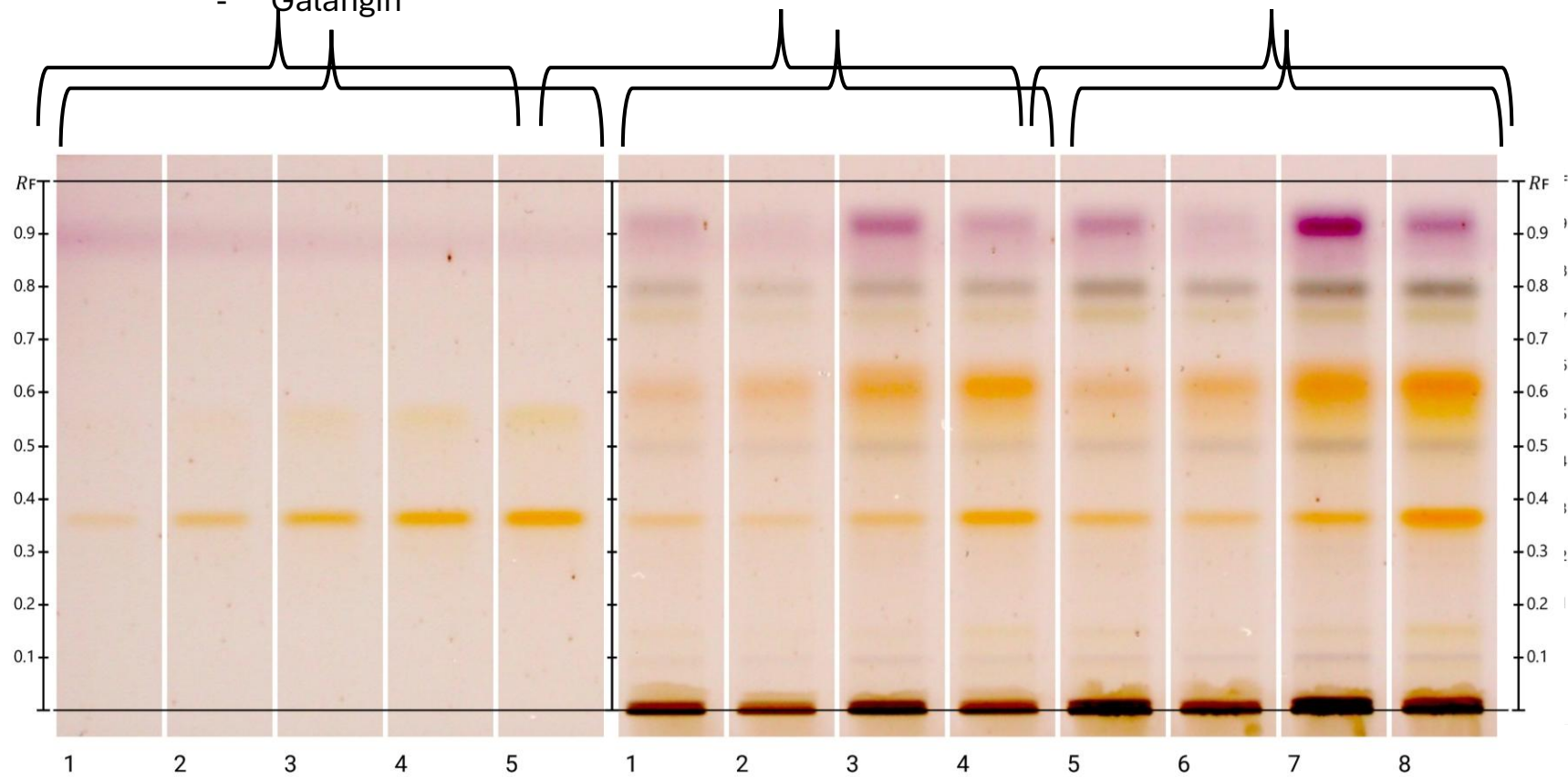
World Leader in Planar Chromatography



Standards:
Standards:
- Chalcone
- Chalcone
- Galangin

GF extracts:
GF extracts:
5.0 μ L
2.5 μ L

GS extracts:
GS extracts:
5.0 μ L
5.0 μ L



Regulations - Bioprospecting, Access and Benefit Sharing (BABS)

- Discovery Phase Notification (BABS/001611N)
- Bioprospecting Permit (BP 201713)
 - Material Transfer Agreements
 - Benefit Sharing Agreement – Ndabakazi Community (Eastern Cape)
 - Valid until November 2029



environmental affairs

Department:
Environmental Affairs
REPUBLIC OF SOUTH AFRICA

BIOPROSPECTING PERMIT

(Issued in terms of Regulations 33 (1) of the Bioprospecting, Access and Benefit Sharing Amendment Regulations of 2015 made under the provisions of the National Environmental Management: Biodiversity Act, 2004 (Act No. 10 of 2004))

Permit number: BP

G.P.-S. 09/15
201713

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Concluding remarks

- South Africa's biodiversity – source of new cosmeceutical active ingredients
 - Collaboration between IKH, researchers and industry is key
- Scientific validation (evidence-based) active ingredients - SA global stance
 - Pre-clinical and clinical S & E data



“We can live without every other science but we cannot live without plants.

They are our food, our lungs, our strengths, our relaxation, our pleasure, our future and our hope.

Our moment of wisdom is when we are able to recognize this fact.”

- Andi Mellis



science, technology
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Department:
Science, Technology and Innovation
REPUBLIC OF SOUTH AFRICA



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