Insights on the anti-allergic constituents of *Bischofia javanica*: network pharmacology and bioactivity-guided fractionation

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Introduction.

- *Bischofia javanica (*BJ*)* Blume (phyllanthaceae), is found in Southeast Asia, such as India, Thailand, and Taiwan.
- Traditionally, leaf extract treats cancerous wounds, **diarrhea, stomach ulcers, and eczema**.
- Our study aimed to evaluate BJ **anti-allergic activity** and to isolate major components.

Material and methods.

- The 10 kg dried leaves extracted with *n*-hexane (BJ-H), ethyl acetate (BJ-E), dichloromethane (BJ-D), methanol (BJ-M), acetone/water (BJ-A/W).
- **Antiallergic effects** were expressed as level of mast cell β-hexosaminidase degranulation induced by calcium ionophore **A23187 in RBL-2H3 cells** [1]. Cytotoxicity of the extracts screened by MTT assay [1].
- Major compounds isolated and identified through **1D, 2D NMR** and literature comparison.
- Hub targets and pathways identified by **network pharmacology and KEGG pathway** enrichment analysis.

Isolation scheme

BJ 10kg dried leaves

Continuous extraction method (Maceration)

BJ-H BJ-D BJ-E BJ-M BJ-A/W

MPLC

BJLERD1 Squalene

BJLERD7 β-Sitosterol

BJLERD25

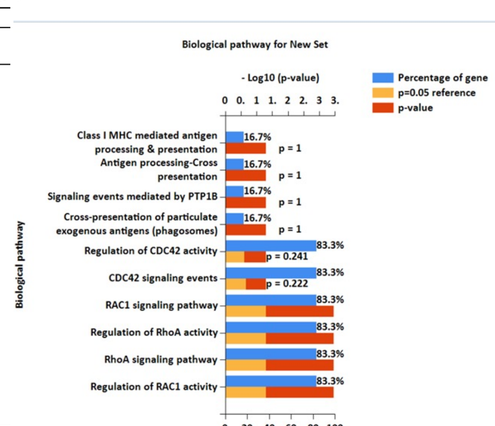
Results and discussion.

- **Anti-allergic effect:** Mid-polar fractions inhibited mast cell degranulation **by 70%** at 100 µg/mL. MTT assay revealed extracts were non-toxic at 100 µg/mL. After screening, active fractions were selected for purification.
- **Isolation: squalene and β-sitosterol** were isolated from active fractions.
- **Network pharmacology:** NCF1 (Neutrophil cytosolic factor 1), CYBB (Superoxide oxidase CybB), or NOX4 (NADPH oxidase 4) were identified as important targets. Enrichment results further underscores that Class I MHC-mediated antigen presentation mediated by PTP1B are critical pathways for the anti-allergic properties of BJ.
- The identified component β-sitosterol was reported to reduce cholesterol, treat heart diseases and rheumatoid arthritis. It has antiproliferative effect on mast cells [2]. Squalene is used in skin care products for its anti-inflammatory properties.

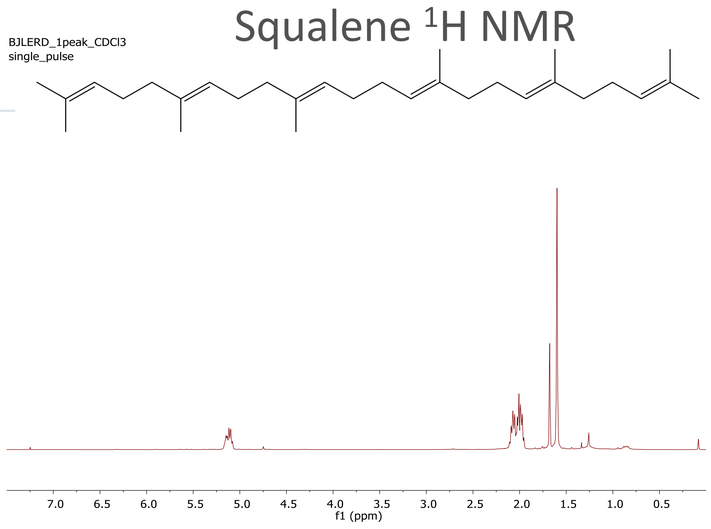
Anti-allergic effects of *Bischofia javanica* extracts and fractions. Network pharmacology results.

Sample	Viability, RBL-2H3	A23187-induced β-hexosaminidase release	
	% viability at 100 µg/mL	% inhibition at conc (µg/mL) 10	100
BJ Hex (1)	>90	—	—
BJ 75% MeOH (1)	>90	—	—
BJ BuOH	>90	—	—
BJ Water	>90	—	—
BJ Residue (1)[b]	>90	—	—
BJ Hex (2)	>90	—	—
BJ 75% MeOH (2)	>90	—	—
BJ Residue (2)	>90	—	—
BJL Hex	>90	—	—
BJL EA Hex	>90	—	—
BJL EA 75% MeOH	>90	—	—
BJL EA Res	>90	—	71.0
BJL DCM	>90	—	70.0
BJL Ace	>90	—	—
BJL MeOH	>90	—	—
BJL Ace : H_2O Mix	>90	—	—
BJL Ace : H_2O Res	>90	—	—
BJL Ace : H_2O Clear	>90	—	—
BJL MeOH ppt	>90	—	—

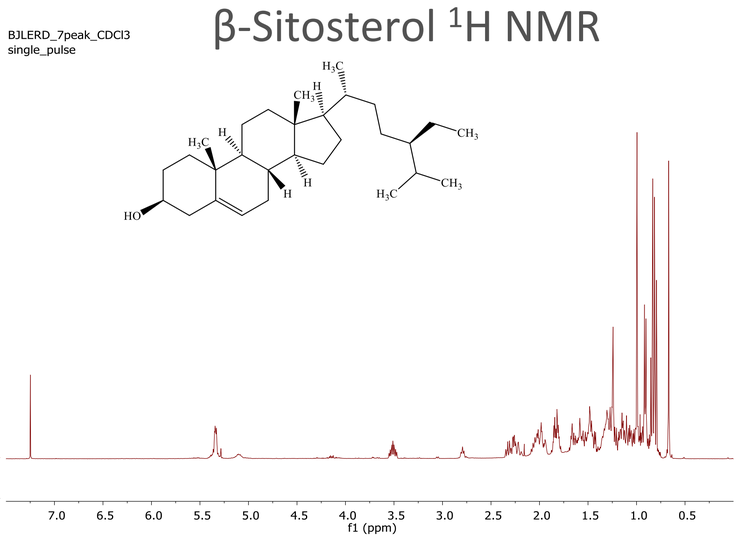
[a]The cytotoxicity of samples to RBL-2H3 was evaluated using MTT viability assay and the toxic concentrations are labelled as 'TOXIC' (viability less than 80%). The inhibition of degranulation was assessed by A23187-induced β-hexosaminidase release in RBL-2H3 cells; percent inhibition; results are presented as average (n = 2); compared with the control value (A23187 only). — not active (insignificant inhibition of degranulation, below 20%).



Squalene ^{1}H NMR



β-Sitosterol ^{1}H NMR



Conclusion. Anti-allergic effects of the BJ leaves were revealed for the first time. The two major components isolated and identified as **squalene and β-sitosterol**. Network pharmacology indicated potential mechanisms.

References:

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2. Han NR, Kim HM, Jeong HJ. The potential anti-proliferative effect of β-sitosterol on human mast cell line-1 cells. Canadian Journal of Physiology and Pharmacology. 2015;93(11):979-83.

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