APIMONDIA SCANDINAVIA 2025
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PP-326 [Apitherapy]

Identification from a Rwandan propolis of 2,4-Di-*tert*-butyl phenol as an antimicrobial agent against *Cutibacteriumacnes*

Florent Rouvier[1,2], Lydia Abou[3], Emmanuel Wafo[1], Perrine André[4], Julien Cheyrol[4], Mohamed-Mohsen Khacef[4], Claude Nappez[4], Hubert Lepidi[5] and Jean Michel Brunel*[,1]

[1]Aix Marseille Univ, INSERM, SSA, MCT, 13385 Marseille, France. E-mail : rouv.flo@hotmail.fr
[2]Observatoire Francais d'Apidologie (OFA), La Garniere, Route de Mazaugues, 83136 Mazaugues, France
[3] C2VN, Aix Marseille Univ, INSERM 1263, INRAE 1260, 13385 Marseille, France.
[4] CEFOS UAR2020 Aix Marseille Université - Campus Timone - 27 Boulevard Jean Moulin 13005 Marseille.
[4-5] Laboratoire d'Anatomie Pathologique, hôpital de la Timone, 13005 Marseille, France. hubert.lepidi@ap-hm.fr

OFA OBSERVATOIRE FRANÇAIS D'APIDOLOGIE — Bee for life

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- **Propolis**, a resinous substance crafted by honeybees, boasts a rich history in traditional medicine spanning millennia. Derived from tree buds and bark, propolis, with its sticky and gummy consistency, results from the collection of resins by foraging bees, creating a blend with pollen, waxes, and enzymes. Literature sources reveal that propolis is primarily composed of key constituents: plant resins (constituting 50–70%), beeswax (making up 30–50%), essential and aromatic oils (comprising 5–10%), and pollen (contributing 5–10%), in addition to trace quantities of organic compounds and minerals. The exact composition varies based on plant source, bee species, harvesting seasons, and climate changes. This versatile apiary product has demonstrated a spectrum of clinical applications, including antioxidant, anti-cancer, anti-inflammatory, and antimicrobial effects. It is crucial to recognize that propolis is not a singular entity; its composition varies depending on the local environement, and not all types will demonstrate the same effectiveness in treating a specific condition
- **Acne** stands out as the prevailing dermatological condition among humans, primarily affecting approximately 80% of adolescents during the onset of puberty, a period marked by hormonal fluctuations that are uniform across genders. The anaerobic bacillus *Cutibacterium acnes*, recognized as a primary contributor to acne vulgaris, colonizes pilosebaceous follicles, deriving energy from sebum triglyceride hydrolysis and secreting predominantly propionic acid. These by-products can irritate the follicular wall, inducing inflammation that attracts neutrophils to infection or inflammation sites.
- **Antibiotics** play a pivotal role in restraining bacterial growth, with oral antibiotics serving as the primary treatment for moderate acne. Notably, systemic erythromycin and various generations of tetracyclines have demonstrated efficacy in addressing inflammatory acne. However, the prolonged use of topical and oral antibiotics against *C. acnes* has led to a prevalent emergence of resistance, particularly in European Union countries (91% and 92.4% for both antibiotics in Spain, Greece 75.3% and Italy 59.5%)

1 Harvesting Propolis from various collection sites in Rwanda

2 Identification of propolis with best antibacterial properties

Hydroalcoholic Propolis extracts

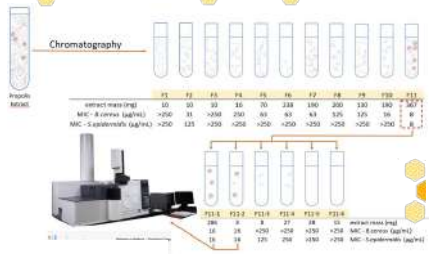
Sample	MIC (µg/mL)						
	E. coli ATCC 25922	*S. aureus* ATCC 25923	*S. epidermidis* CIP 81.55	*B. cereus* ATCC 11778	*C. acnes* DSM 1897	*C. acnes* DSM 31953	*C. acnes* DSM 110516
OFAP 2	>256	>256	256	16	8	16	32
OFAP 6	>256	>256	>256	125	125	64	256
OFAP 7	>256	>256	>256	64	64	32	32
OFAP 9	>256	>256	256	32	32	16	ND
OFAP 10	>256	>256	32	64	16	32	ND
OFAP 11	>256	>256	>256	64	64	64	ND
OFAP 15	>256	64	32	16	16	ND	ND
OFAP 16	>256	>256	>256	32	32	ND	ND
OFAP 18	>256	64	16	16	16	16	16
OFAP 20	>256	>256	>256	64	16	32	>256
OFAP 21	>256	64	64	32	32	32	>256

Antimicrobial activities of various Rwandan propolis extracts

3 Identification of the antibacterial molecule from OFAP 18, the 2,4-Di-tert-butylphenol

Method :

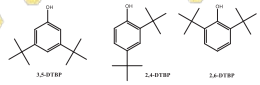
- Submission to flash chromatography of crude 1,8 g powder of OFAP 18
- Antimicrobial activities test of all the fractions
- Determination of the nature of the molecule present in fraction F11.1 by GC-MS



The structure of the molecule was clearly determined as 2,4-di-*tert*-butylphenol (2,4-DTBP) and ^{1}H and ^{13}C NMR spectrometry.

Notably, we also detected the presence of 3,5-Di-*tert*-butylphenol (3,5-DTBP) an isomer of 2,4-DTPB in a 1:10 ratio relative to 2,4-di-*tert*-butylphenol in the analyzed propolis sample.

4 Antimicrobial activities of OFAP18, 2,4-DTBP, 3,5-DTBP and 2,6-DTBP



Structure of Di-tert-butyl phenol identified in propolis OFAP 18 (3,5-DTBP, 2,4-DTBP) and (2,6-DTBP)

The antimicrobial activity tests of the commercial molecules 2,4-DTBP, 3,5-DTBP demonstrated strong antimicrobial activity against *B. cereus* and *C. acnes*, particularly for 2,4-DTBP whereas no activity was encountered by using isomer 2,6-DTBP.

We extend the spectrum of action of 2,4-DTBP on a larger panel of *C. acnes* strains confirming the great activity towards this pathogen with a MIC of 16 µg/mL even against an erythromycin resistant strain.

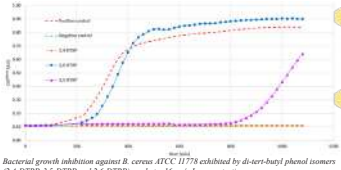
Sample	CMI (µg/mL)				
	E. coli 25922	*S. aureus* 25923	*S. epidermidis* 81.55	*B. cereus* 11778	*C. acnes* 1897
OFAP18	>256	64	16	16	16
2,4-DTBP	>256	16	16	4	16
2,6-DTBP	>256	>256	125	256	>256
3,5-DTBP	>256	32	32	16	64

Antimicrobial activities of OFAP18, 2,4-DTBP, 3,5-DTBP and 2,6-DTBP against a wide range of bacterial strains

Sample	CMI (µg/mL)					
	C. acnes DSM1897	*C. acnes* DSM110512	*C. acnes* 110516 (TRY-R)	*C. acnes* DSM110517	*C. acnes* DSM110528	*C. acnes* DSM A170
2,4-DTBP	16	16	16	16-32	16	16

Antimicrobial activities of 2,4-DTBP against a large panel of C. acnes bacterial strains

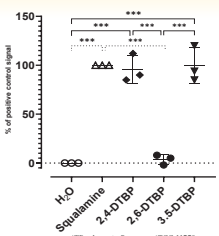
No inhibition of bacterial growth was observed in the presence of 2,6-DTBP at a concentration of 16 µg/mL. In contrast, the growth response to 2,5-DTBP was more nuanced; inhibition was observed during the first 13 hours, followed by a subsequent escape from the action of the compound. Finally, significant growth inhibition was only observed with the 2,4-DTBP isomer used at 16 µg/mL concentration.



Bacterial growth inhibition against B. cereus ATCC 11778 exhibited by di-tert-butyl phenol isomers (2,4-DTBP, 3,5-DTBP and 2,6-DTBP) used at a 16 µg/mL concentration.

5 Mechanism of action 2,4-DTBP: Permeabilizing and disrupting behavior of the outer membrane of Gram-positive B. cereus

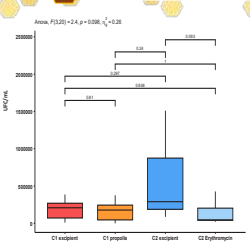
A bioluminescence method was developed involving the detection of the external concentration of ATP which was used as a reporter reflecting the permeabilizing effect of 2,4-DTBP and 3,5-DTBP.



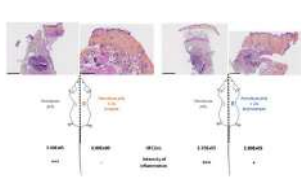
ATP release in B. cereus ATCC 11778

6 Application: Propolis anti-acne ointment

We formulated an ointment using OFAP18 and petroleum jelly (1% propolis or erythromycin) for the treatment of acne in a mouse model. We applied propolis and erythromycin ointments daily for 8 days before injecting the bacterial suspension. We then evaluated the efficacy of propolis and erythromycin ointments against *C. acnes* colonization over the course of 2 days.



Representation of bacterial counts of C. acnes based on the treatments performed on the two cohorts of mice.



Propolis and erythromycin treated mice exhibited lower epidermal inflammation than control ones treated with excipient 2 days after injection with 10^8 CFU/mL of C. acnes DSM1897 suspension.

These data support the effectiveness of the propolis-based cream OFAP18 and, more broadly, the molecule 2,4-DTPB responsible for its antibacterial activity.

ROUVIER *et al*, *Antibiotics* **2024**

PP-326 [Apitherapy]
Identification of 2,4-Di-tert-Butylphenol as an Antimicrobial Agent Against Cutibacterium acnes Bacteria from Rwandan Propolis

Florent ROUVIER[1], Lydia Abou[3], Emmanuel WAFO[2], Perrine ANDRE[4], Julien Cheyrol[4], Mohamed Mohsen Khacef[4], Claude Nappez[4], Hubert Lepidi[5], Jean Michel Brunel[2]
[1]Observatoire Français d'Apidologie (OFA), La Garniere, Route de Mazaugues, 83136 Mazaugues, France
[2]Aix Marseille Université, INSERM, SSA, MCT, 13385 Marseille, France
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[4]CEFOS, UAR2020, Aix Marseille Université, Campus Timone, 27 Boulevard Jean Moulin, 13005 Marseille, France
[5]Laboratoire d'Anatomie Pathologique, Hôpital de la Timone, 13005 Marseille, France

Background/OBJECTIVES:
Acne is the most prevalent dermatological condition among humans, affecting approximately 80% of adolescents during puberty. To date, numerous compounds have been used for acne treatment, including erythromycin ointments and antiseptics, with varying degrees of success. The emergence of erythromycin-resistant C. acnes strains has spurred the search for new antimicrobial agents, particularly from natural sources.

The composition of propolis is highly variable, influenced by factors such as plant sources, bee species, harvesting seasons, and climatic conditions. This diverse apiary product has been shown to possess a wide range of clinical applications, including antioxidant, anticancer, anti-inflammatory, and antimicrobial properties. It is essential to acknowledge that propolis is not a uniform substance; its chemical profile is shaped by local environmental factors, meaning its effectiveness can differ across various conditions. In our study, we collect and analyze Rwandan propolis to evaluate its antibacterial activity and identify the most potent extracts against Cutibacterium acnes.

METHODS: Propolis collected in Rwanda identified as having a antibacterial activity on C. acnes was extracted and fractionated by flash chromatography and tested against C. acnes growth by using NCLSI recommendations.

RESULTS: In our research, we identified a molecule, 2,4-Di-tert-butylphenol (2,4-DTBP) which inhivbited the C. acnes growth at a concentration of 16 µg/mL. Based on these results, we formulated an ointment (1%) using OFAP18 and petroleum jelly for the potential treatment of acne using a mouse model.

CONCLUSIONS: In vitro and in vivo evidence suggests that 2,4-DTBP has anti-inflammatory properties and could effectively manage the overgrowth of C. acnes as well as serve as a potent alternative for the formulation of an active propolis ointment for acne treatment.