An indigenous South African plant targeting antibiotic resistance and the pathogenic factors associated with acne vulgaris

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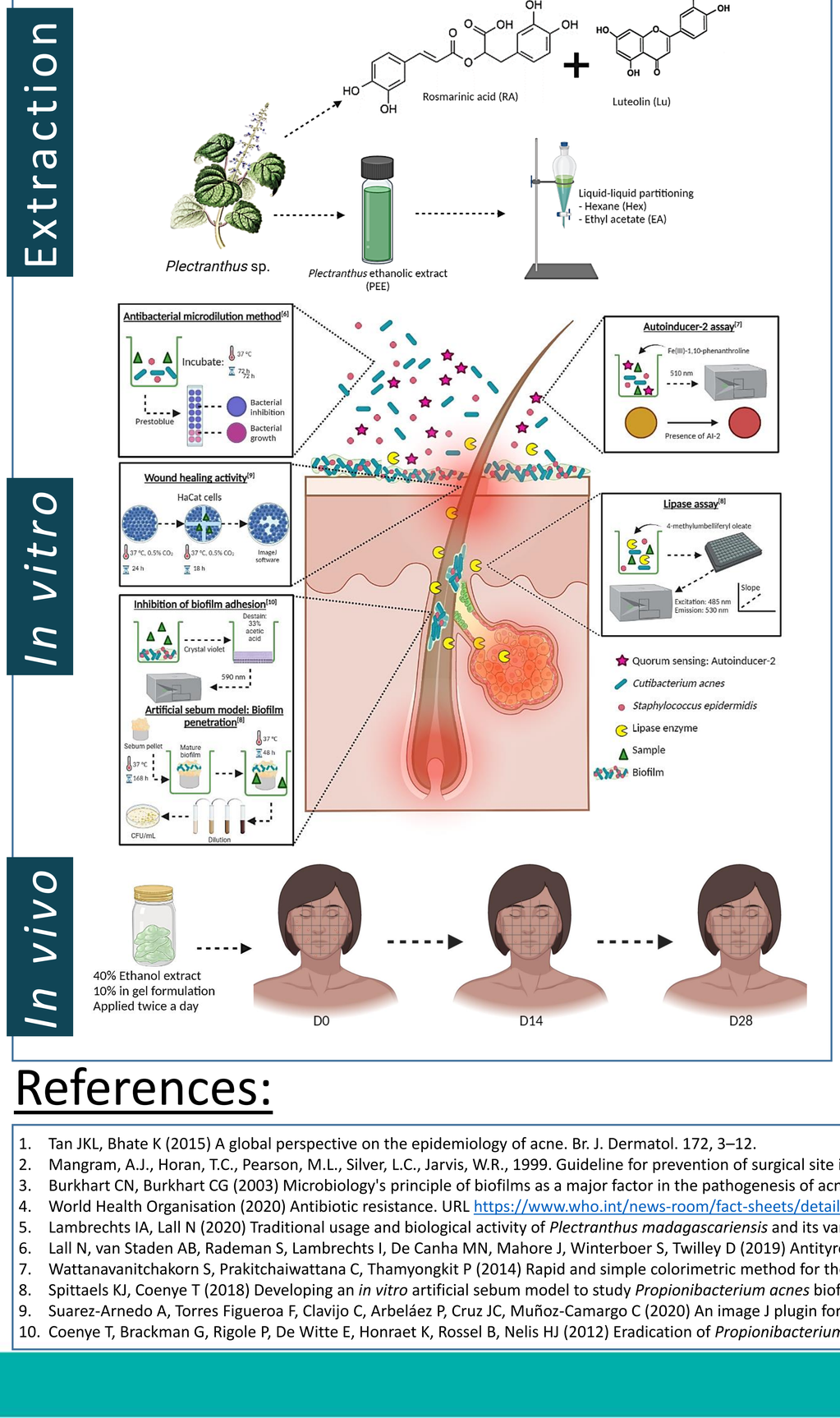
Introduction:

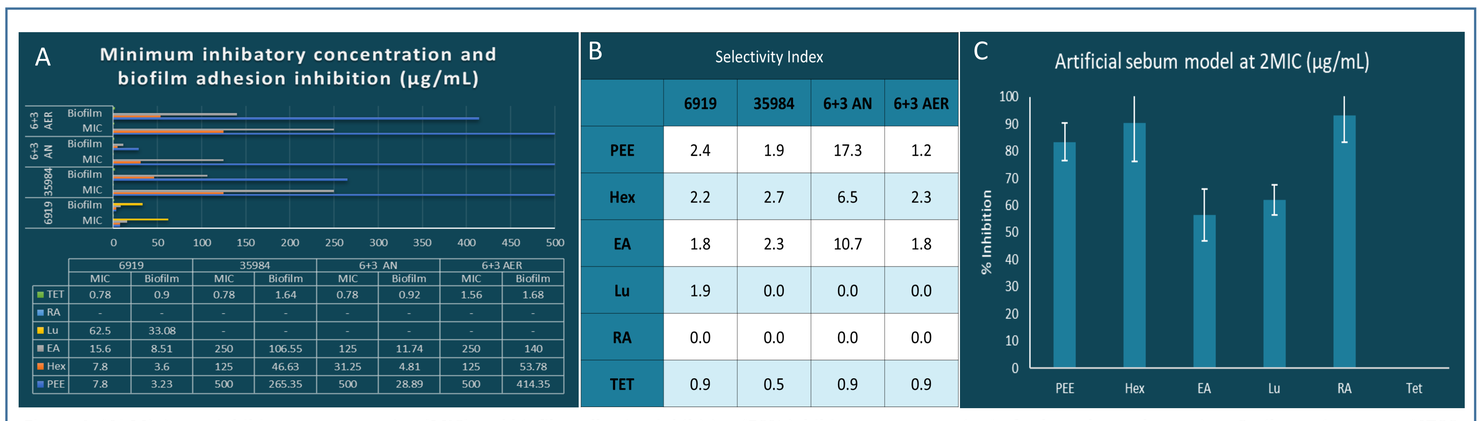
Acne vulgaris affects 9.4% of the world's population, making it the eighth-most predominant disease worldwide. Acne vulgaris is a disease related to the skin's pilosebaceous unit that includes the hair shaft, hair follicle, and the sebaceous gland that produces sebum. Acne-causing bacteria proliferate in the sebum releasing lipase enzymes that results in an inflammatory response[1]. It is hypothesized that *Cutibacterium acnes* and *Staphylococcus epidermis* have a multispecies virulence effect in acne vulgaris and surgical wound infections, relating to 77% of surgical deaths[2]. It has been confirmed that both *C. acnes* and *S. epidermidis* are under quorum sensing control through autoinducer-2 (AI-2) release. Bacteria communicate by releasing AI-2 signalling molecules that regulate gene expression once a certain threshold is reached. Quorum sensing regulates and coordinates the release of virulence factors such as lipase enzymes that elicit an inflammatory response and the formation of biofilms that contribute to antibiotic resistance observed for pathogenic strains of *C. acnes* and *S. epidermidis* in these maladies[3]. The World Health Organisation has recognized antibiotic resistance as a threat to global health and the world economy[4]. Therefore, antibiotic resistance towards acne vulgaris and wounds have become a concern to researchers and physicians worldwide.

An endemic South African *Plectranthus* sp. traditionally used by the Zulu and Xhosa communities to treat skin-related maladies such as scabies and wounds was selected for further investigation targeting acne vulgaris and wounds. Rosmarinic acid (RA) and Luteolin (Lu) have been identified as major compounds present in the species[5]. This is a first-time report on the quorum sensing relationship of *C. acnes* and *S. epidermidis*, an endemic *Plectranthus* sp., and its compounds targeting antibiotic resistance associated with acne vulgaris and wounds.

Materials & Methods:



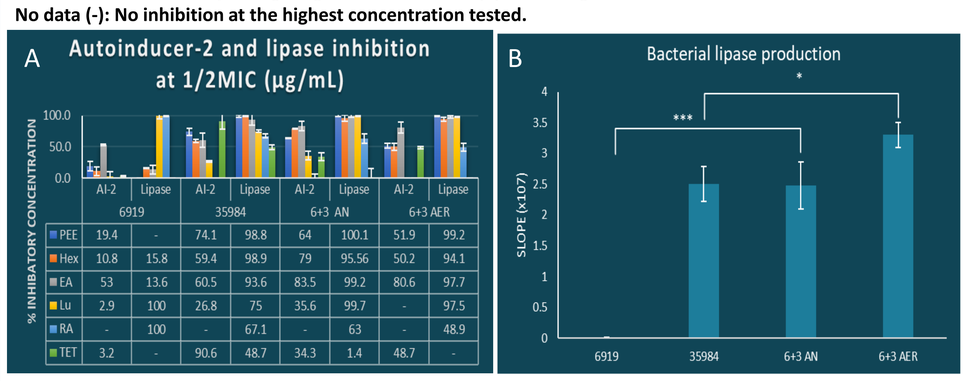
Results & Discussion:



	6919		35984		6+3 AN		6+3 AER	
	MIC	Biofilm	MIC	Biofilm	MIC	Biofilm	MIC	Biofilm
TET	0.78	0.9	0.78	1.64	0.78	0.92	1.56	1.68
RA	-	-	-	-	-	-	-	-
Lu	62.5	33.08	-	-	-	-	-	-
EA	15.6	8.51	250	106.55	125	11.74	250	140
Hex	7.8	3.6	125	46.63	31.25	4.81	125	53.78
PEE	7.8	3.23	500	265.35	500	28.89	500	414.35

	6919	35984	6+3 AN	6+3 AER
PEE	2.4	1.9	17.3	1.2
Hex	2.2	2.7	6.5	2.3
EA	1.8	2.3	10.7	1.8
Lu	1.9	0.0	0.0	0.0
RA	0.0	0.0	0.0	0.0
TET	0.9	0.5	0.9	0.9

Figure 1. (A) Minimum inhibitory concentration (MIC) and biofilm adhesion inhibition (50% inhibitory concentration) of selected samples against *Cutibacterium acnes* (ATCC 6919), *Staphylococcus epidermidis* (ATCC 35984), and a combination of these bacteria grown under aerobic (AER) and anaerobic (AN) conditions. PEE: *Plectranthus* ethanolic extract; Tet: tetracycline, RA: rosmarinic acid; Lu: luteolin; EA: liquid ethyl acetate fraction of PEE; Hex: liquid hexane fraction of PEE. (B) A selectivity index larger than one is an indication of the sample targeting biofilm adhesion. (C) Percentage inhibition of bacteria within the biofilm using the artificial sebum model against *C. acnes* (ATCC 6919). No data (-): No inhibition at the highest concentration tested.



	6919		35984		6+3 AN		6+3 AER	
	AI-2	Lipase	AI-2	Lipase	AI-2	Lipase	AI-2	Lipase
PEE	19.4	-	74.1	98.8	64	100.1	51.9	99.2
Hex	10.8	15.8	59.4	98.9	79	95.56	50.2	94.1
EA	53	13.6	60.5	93.6	83.5	99.2	80.6	97.7
Lu	2.9	100	26.8	75	35.6	99.7	-	97.5
RA	-	100	-	67.1	-	63	-	48.9
TET	3.2	-	90.6	48.7	34.3	1.4	48.7	-

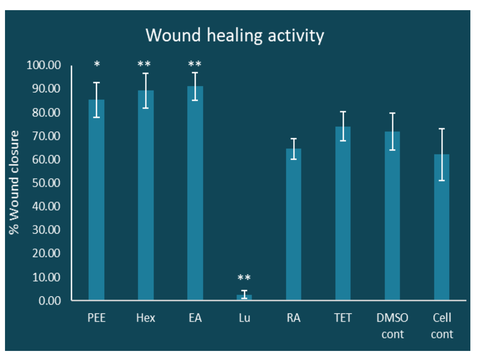


Figure 2. (A) Inhibition of autoinducer-2 (AI-2) and lipase production of selected samples against *Cutibacterium acnes* (ATCC 6919), *Staphylococcus epidermidis* (ATCC 35984), and a combination of these bacteria grown under aerobic (AER) and anaerobic (AN) conditions. PEE: *Plectranthus* ethanolic extract; Tet: tetracycline, RA: rosmarinic acid; Lu: luteolin; EA: liquid ethyl acetate fraction of PEE; Hex: liquid hexane fraction of PEE. (B) Bacterial lipase production of *C.acnes* (ATCC 6919), *S.epidermidis* (ATCC 35984), and a combination of these bacteria grown under aerobic (AER) and anaerobic (AN) conditions. One-way ANOVA Tukey's multiple comparison test * P < 0,05; ** P < 0,01; *** P < 0,001 statistical significance. Significant lipase production was observed for the multispecies system under aerobic conditions. No data (-): No inhibition at the highest concentration tested.

Figure 3. Percentage wound closure compared to the DMSO control. PEE: *Plectranthus* ethanolic extract; Tet: tetracycline, RA: rosmarinic acid; Lu: luteolin; EA: liquid ethyl acetate fraction of PEE; Hex: liquid hexane fraction of PEE. One-way ANOVA Tukey's multiple comparison test * P < 0,05; ** P < 0,01; *** P < 0,001 statistical significance. Significant wound healing activity was observed for PEE, Hex and EA.

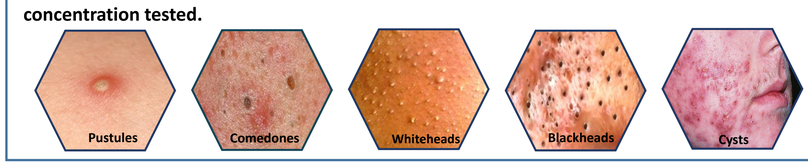


Figure 4. In vivo irritancy and efficacy studies. Neat, the Plectranthus ethanolic extract was confirmed to be a non-irritant. At 10% in a gel formulation, Plectranthus ethanolic extract was confirmed to be effective for treating pustules, comedones, whiteheads, blackheads, and cysts between 14-28 days of consecutive use twice a day.

Conclusions:

Medicinal plants play an integral role in the daily lives of many South Africans[5]. Acne vulgaris and surgical site wound infections are a result of C. acnes and S. epidermidis bacterial infections. However, the relationship between these bacteria in these maladies is not yet fully understood. Cutibacterium acnes and S. epidermidis are under AI-2 quorum sensing control. Quorum sensing is a form of bacterial communication involved in releasing virulent factors such as lipase enzymes and biofilm formation that contribute to inflammation and antibiotic resistance observed in these maladies, respectively[3]. Antibiotic resistance associated with these bacteria is concerning and affects the global population's health [4]. There is a need for new therapies targeting quorum sensing and the effects of this communication mechanism. This is a first-time investigation on the quorum sensing relationship between C. acnes (ATCC 6919) and S. epidermidis (ATCC 35984) and the identified endemic South African Plectranthus sp. and its compounds for the potential treatment of acne vulgaris and wounds. The samples were able to target quorum sensing, biofilm formation, lipase production, and penetrate the biofilm to inhibit the bacteria within, compared to the antibiotic tetracycline. In vivo, the Plectranthus sp. successfully treated mild to severe forms of acne between 14-28 days. Significant wound healing activity was observed for the Plectranthus ethanolic extract and its liquid partitions. The wound healing activity observed for the Plectranthus sp. corresponds to its traditional usage[5]. Promising results were obtained that could give insight into the relationship of C. acnes and S. epidermidis that cause infection. Increased antibiotic resistance towards tetracycline and increased lipase production were observed in a multispecies system of C. acnes and S. epidermidis under aerobic conditions. The combined bacterial effect under aerobic conditions confirms that these acne-causing bacteria could have a synergistic effect in the progression of inflammatory acne and surgical wound infections. This is a first-time report on the synergistic activity of C. acnes and S. epidermidis and an endemic Plectranthus sp. and its compounds for the successful treatment of acne vulgaris and potentially other skin-related maladies.

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