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# In-vitro evaluation of certain Egyptian traditional medicinal plants against *Propionibacterium acnes*

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ABSTRACT

Many plant species are reported in Egyptian traditional medicine for the treatment of different types of skin infections. The present study was designed to screen the methanolic extracts of twenty five Egyptian plants used in traditional medicine for the treatment of skin infections for their antibacterial activities against *Propionibacterium acnes*, and to isolate the bioactive compound(s) from the most active extract. The results showed that *Myrtus communis*, *Curcuma longa* and *Myristica fragrans* exhibited inhibition on *P. acnes* growth in which *M. communis* demonstrated the highest antibacterial effect with an MIC of 7.8 μg/ml. A phytochemical analysis of the extract of *M. communis* leads to the isolation of eight compounds viz., 5-acetoxy-4-hydroxy-4-isobutyl-2,2,6,6-tetramethylcyclohexan-1,3-dione (**1**), β-sitosterol (**2**), isomyrtucommulone-B (**3**), endoperoxide-G-3-hormone (**4**), gallic acid (**5**), myricetin-3-*O*-α-L-rhamnoside (**6**), myricetin-3-*O*-β-D-glucoside (**7**), and myricetin-3-*O*-β-D-galactoside-6″-*O*-gallate (**8**). Compounds **1** and **3** showed strong inhibition against *P. acnes* with MIC values of 6.3 and 0.8 μg/ml, respectively. This is the first report on the isolation of compound **3** from *M. communis* and the validation in the use of Egyptian herbal medicines against *P. acnes*.



## 1. Introduction

The skin organ remains one of the most important immunity barriers to protect humans against external harsh conditions. In general, skin diseases account for approximately 34% of all the diseases encountered worldwide (Abbasi et al., 2010). These affect people of all ages and constitute a major medical concern. Skin diseases are a recognized major health burden in both developed and developing countries.

Acne vulgaris (acne) is one of the most commonly encountered skin diseases and usually affects nearly everybody during their lifetime (Scheinfeld, 2007). Acne, an inflammatory disease affecting oil glands and hair follicle areas (Leyden, 1997), is caused by the gram-positive bacterium *Propionibacterium acnes* which is an obligate anaerobic that possesses the capability to metabolize sebaceous triglycerides into fatty acids inside sebaceous glands. Furthermore, the resulting increase in sebum production causes a thickening of the epidermis at the outlet of pilosebaceous unit resulting in an obstruction to the outwards flow of sebum leading to the development of a comedo (Chomnawang et al., 2005; Coenye et al., 2012). The usual therapies employed in treatment of acne all have various side effects. Topical antibiotics for instance lead to dryness, redness, irritation of the skin and hypo-pigmentation, whereas oral antibiotics can cause gastro-intestinal disorders and as a consequence, increase the risk of venous thromboembolism (Arican et al., 2005; Shaw and Kennedy, 2007).

In view of an ever increasing resistance to existing anti-microbial agents and high cost of treatment (Hamburger and Hostettmann, 1991; Ghosh et al., 2011), studies to discover and develop an effective natural treatment for acne, well tolerated by the patients, pose both an exciting and challenging undertaking. Bioactive natural crude extracts derived from traditional herbal medicine can be considered to be a prolific source for new therapies. Therefore, researchers are increasingly turning their attention to folk medicine looking for new leads to develop better drugs effective in the treatment of acne.

Folk medicine in Egypt has a long history of development since it is derived from many different cultures. However old pharaonic in conjunction with relatively "recent" Arabic Unani medicine, is recognized as the most well-documented of the traditional medicines and still represents the major and most important source of all formulations in the current herbal market. The easily recognized skin diseases are well documented in old Arabic literature in which literally hundreds of

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formulations have been described for treatment of different skin pathologies.

Books written by famous scholars such as Al-Antaqi (Al-Antaqi, 1935), Al-Turkimany (Al-Turkimany, 1993), Ibn Sina (Avicenna, 1993), and Ibn Al-Bitar (Ibn Al-Bitar, 2001), represent the main references in herbal shops (known as Attarin), and were employed in this study as the major source of traditional information related to herbal medicines used for the treatment of different skin infections.

This paper reports the screening of twenty five Egyptian medicinal plants for their antibacterial properties against *P. acnes*. In addition, the most active extract was subjected to phytochemical studies to identify the antibacterial compound(s).

## 2. Materials and methods

### 2.1. General

Methanol, ethanol, ethyl acetate, dichloromethane, hexane (technical grade), sulphuric acid and acetic acid were secured from Al-Gamhouria (Cairo, Egypt). Sephadex LH-20 and, vanillin were secured from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Silica gel 60 (0.040–0.063 mm), pre-coated plates of silica gel 60 $F_{254}$, deuterated chloroform and DMSO-*d6* were supplied by Merck (Germany). Chemical profiles of the fractions were identified based on the colour produced after viewing under UV and then spraying with the detecting reagent (vanillin/sulphuric acid) followed by heating.

### 2.2. NMR analyses

$^{1}H$ and $^{13}C$ NMR spectra were recorded at 25 °C, using deuterated chloroform or DMSO-*d6* on a Bruker Avance or Varian INOVA-400 NMR spectrometer ($^{1}H$ 400 MHz, $^{13}C$ 100 MHz). Chemical shifts of $^{1}H$ ($\delta_H$) and $^{13}C$ ($\delta_C$) are reported in parts per million (ppm) relative to internal reference ($CDCl_3$; 7.25/77.0; DMSO-*d6*; 2.50/39.51).

### 2.3. Preparation of inoculum of P. acnes

*P. acnes*, NRRL (B-4224), was obtained from the Agricultural Research Service (NRRL, Peoria, IL, USA). The organism was incubated in Mueller Hinton broth (Mast Group Ltd., Merseyside, U.K.) at 37 °C for 72 h under anaerobic conditions (Kumar et al., 2007) before the assay.

### 2.4. Collection of plant materials and preparation of total extracts

#### 2.4.1. Collection and identification of plant materials

In this study we reviewed the information described by some scholars like Al-Antaqi (Al-Antaqi, 1935), Al-Turkimany (Al-Turkimany, 1993), Ibn Sina (Avicenna, 1993), Ibn Al-Bitar (Ibn Al-Bitar, 2001) and Batanouny, (Batanouny, 1994) for the treatment of skin infection. The parts of the plants that could potentially exhibit antimicrobial activity as reported by traditional herbalists were selected for the study (Table 1). Plant material was collected during 2011 of March to May from either their natural habitats, herbal shops or the local market (Table 1). Voucher specimens of the plants were kindly identified by Prof Ibrahim El-Garf and deposited in the herbarium of National Research Centre, Egypt. The plant names have been verified with www.theplantlist.org (accessed in 08/10/2015).

#### 2.4.2. Preparation of crude extracts

Dry plant material (100–150 g) was blended and extracted with methanol (10 ml/g) at room temperature (25 °C) for 48 h. The methanol extract was evaporated till dryness on a rotary evaporator at a bath temperature of 45 °C. The dried crude extracts were kept at −5 °C until evaluated.

### 2.5. Phytochemical studies on Myrtus communis

#### 2.5.1. Bioassay-guide isolation of bioactive compounds

Fresh plant material (950 g) was collected from the El-Orman botanical garden in September 2013 and was air dried at room temperature, blended and extracted with methanol at room temperature (25 °C) for 48 h. The methanol extract was evaporated to a residue on a rotary evaporator at 45 °C bath temperature to yield 45.0 g of a residue (4.7%, fresh weight). The total extract was chromatographed on a silica gel column (35 × 10 cm) and eluted with a mixture of increasing polarity of hexane (Hex):ethyl acetate (EtOAc) from 100:0 to 0:100 followed by 100% methanol (MeOH). In total (41) fractions (500 ml each) were collected and fractions with a similar thin layer chromatographic (TLC) profile were combined which resulted in 23 main fractions (MF). All 23 main fractions were tested for antibacterial activity against *P. acnes*. Fractions 6, 7, 8, 14 and 18 showed the highest inhibitory activity.

Main fraction 6 (MF6, 1.7 g) was chromatographed on silica gel using a Hex/EtOAc (95:5) followed by Sephadex (using 95% aqueous ethanol) to produce **1** (20 mg, 0.002%, fresh wt). MF 7 (1.3 g) was chromatographed on silica gel using a gradient of Hex/EtOAc (0:100–93:7), to produce **2** (50 mg, 0.005%). MF8 (2.4 g) was chromatographed on silica gel using a Hex/EtOAc (95:5) followed by Sephadex (using 90% aqueous ethanol) to produce compounds **3** (20 mg, 0.002%) and **4** (35 mg, 0.0037%). MF14 (2.1 g) was chromatographed on silica gel using a gradient of Hex/DCM (DCM containing 1% MeOH) (50:50) to produce **5** (25 mg, 0.0026%). MF18 (2.5 g) was chromatographed on Sephadex L-H 20 eluting with 100% methanol to yield **6** (15 mg, 0.0016%), **7** (17 mg, 0.0018%) and **8** (10 mg, 0.0011%).

#### 2.5.2. Compound *1*

White amorphous powder. NMR data; $^{1}H$ ($CDCl_3$, 400 MHz), δ 5.18 (s, H-5), 2.08 (s, Me-OAc), 1.70 (m, H-8), 1.61 (dd, 4.2, 17.2 Hz, H-7A), 1.37 (dd, 6.2, 17.2 Hz, H-7B), 1.30, 1.28 (s each, Me's-C2), 1.08, 1.05 (s each, Me's-C6), 0.87, 0.86 (d each, Me's-9, 10); $^{13}C$ ($CDCl_3$, 100 MHz), d, 214.2 (s, C-3), 212.7 (s, C-1), 169.4 (s, CO-Ac), 79.3 (s, C-4), 77.1 (d, C-5), 55.3 (s, C-2), 49.2 (s, C-6), 43.7 (t, C-7), 25.1, 24.5 (q each, Me's-9, 10), 24.4, 23.8 (q each, Me's-C2), 23.7, 21.8(q each, Me's-C-6), 23.4 (d, C-8), 20.8 (s, Me-Ac).

#### 2.5.3. Compound *3*

Yellow amorphous powder. NMR data; $^{1}H$ ($CDCl_3$, 400 MHz), δ 12.48 (1H, s, 2-OH), 8.27 (1H, s, 4-OH) 6.16 (1H, s, H-5), 4.36 (1H, d, $J$ = 3.3 Hz, H-7), 3.91 (1H, hept., $J$ = 6.8 Hz, H-8′), 1.98 (1H, m, H-8), 1.58 (3H, s, H-13′), 1.43 (3H, s, H-11′), 1.41 (3H, s, H-14′), 1.37 (3H, s, H-12′), 1.18 and 1.19 (2 × 3H, d, $J$ = 6.8 Hz, H-9′and H-10′), 0.76 and 0.78 (2 × 3H, d, $J$ = 7.0 Hz, H-9 and H-10); 13C NMR (CDCl3, 100 MHz) δ 212.2 (s, C-4′), 211.2 (s, C-7′), 198.4 (s, C-2′), 168.4 (s, C-6′), 162.1 (s, C-2), 159.0 (s, C-4), 156.4 (s, C-6), 111.8 (s, C-1′), 106.9 (s, C-3), 104.6 (s, C-1), 95.0 (d, C-5), 56.1 (s, C-3′), 47.4 (s, C-5′), 39.8 (d, C-8′), 34.5 (d, C-8), 31.8 (d, C-7), 25.14 (q, C-11′), 25.08 (q, C-13′), 24.6 (q, C-14′), 24.0 (q, C-12′), 19.30 (q, C-10′), 19.28 (q, C-9′), 19.1 (q, C-9), 18.7 (q, C-10).

### 2.6. Determination of inhibitory activity using 96 well plate assay

Antibacterial activity of the different samples against *P. acnes* was evaluated using a 96 well micro-plate assay as previously described by (Mapunya et al., 2011) with few modifications. Briefly, the 72 h culture of the bacterium was dissolved in Mueller Hinton broth and the suspension was adjusted to 0.5 McFarland standard turbidity. All samples were dissolved in dimethylsulphoxide (5% of the final volume) and diluted with Mueller Hinton broth to a concentration of 250 (total extracts), 100 and 10 (fractions; μg/ml) and 50 μg/ml (pure compounds). 100 μl of each sample was pipetted into the 96 well plate, as well as the positive control (100 μg/ml erythromycin), and negative control (5%