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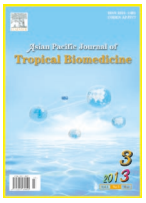

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Activity-guided isolation and identification of anti-staphylococcal components from *Senecio tenuifolius* Burm. F. leaf extracts

Manjunath Manubolu[1*], Lavanya Goodla[2], Sivajyothi Ravilla[3], Vijayasarathi Reddy Obulum[3]

[1]*Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, P.O. Box 7070, Uppsala, Sweden*
[2]*Natural Product Research Center, Faculty of Science, Prince of Songkla University, Hat Yai, Songkla, Thailand-90112*
[3]*Department of Biochemistry, School of Biological Sciences, Sri Venkateshwara University, Tirupati, A.P., India*

PEER REVIEW

Peer reviewer
Dr. Suryakiran Navath, Department of Chemistry & BioChemistry University of Arizona Tucson, AZ 85721 USA.
Tel: 15208343766
E-mail: snavath@email.arizona.edu

Comments
This is a good study in which the authors evaluated the antimicrobial properties of *S. tenuifolius*, which are useful in treating skin diseases. The results are interesting and may be used as an effective topical application medicine against drug-resistant *S. aureus* skin infections.
(Details on Page 195)

ABSTRACT

Objective: To investigate activity-guided isolation and identification of anti-*Staphylococcus aures* components from *Senecio tenuifolius* Burm. F. (*S. tenuifolius*). **Methods:** Hexane, chloroform, ethyl acetate, methanol and aqueous extracts of *S. tenuifolius* were prepared by soxilation for antimicrobial activity against one registered *Staphylococcus aureus* (*S. aureus*) (ATCC No: 25923) and two clinical isolates, methicillin resistant and methicillin sensitive *S. aureus*. NCCL standard methods were followed for antibacterial activity. GC-MS was performed to identify the chemical composition of bio active fraction. **Results:** Among all solvent extracts, methanol extract significantly reduced the growth of *S. aureus* (ATCC No: 25923), methicillin resistant and methicillin sensitive *S. aureus* with the best zone of inhibition at 16.23, 14.06 and 15.23 mm and minimum inhibition concentration (MIC) values at 426.16, 683.22 and 512.12 μg/mL, respectively. In order to detect the active component in methanol extract, it was further purified by column chromatography, which yielded four fractions (St1, St2, St3, and St4). Among these four fractions, St3 was effective against the tested strains of *S. aures*, with the best zone of inhibition at 15.09, 13.25 and 14.12 mm and with best MIC values at 88.16, 128.11 and 116.12 μg/mL, respectively. Effective fraction partially purified from *S. tenuifolius* (St3) yielded MIC′s that were at least 20 fold less when compared to crude extract. GC-MS analysis of St3 revealed the presence of 3-[methyl-6,7-dihydro benzofuran-4 (5H)-one], 1,2-benzenedicarboxylic acid, hydroquinone, methyl ester and 3 unknown compounds. **Conclusions:** The study provides scientific evidence for traditional and folklore medicinal use of *S. tenuifolius* in skin infections treatment.

KEYWORDS
Senecio tenuifolius Burm. F., *Staphylococcus aureus*, Chemical composition, GC-MS, Skin infection, Topical application

1. Introduction

During the early period of the 20th century, fewer than 45% of people lived to the age of 65. Until the mid-20th century, infectious diseases were the leading cause of death. Despite Alexander Fleming′s serendipitous discovery in 1928 of the first bactericidal antibiotic, it was not until the early 1940s that penicillin was actually produced and used to treat infectious diseases including infections caused by *Staphylococcus aureus* (*S. aureus*). Just a decade later, a resistant strain of *S. aureus* emerged. It was resistant not only to penicillin, but the new antibiotic arsenal as well as erythromycin, streptomycin, and tetracycline. It was in 1955 when modern medicine was unable to effectively treat the new strain. Faced with this challenge, scientists and health care professionals continued to work collaboratively to control the transmission of the resistant *Staphylococcus* strain and find a cure. By 1960, methicillin was the newest and the most effective weapons against *S. aureus*. In the late 1970s, hospitals in Eastern Australia found the first outbreaks of methicillin-resistant *S. aureus* (MRSA).

*Corresponding author: Dr. Manubolu Manjunath, Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, P.O. Box 7070, Uppsala, Sweden.
Tel: +46-733466638, +46-08672123
Fax: 018-55 07 62
E-mail: manubolu.manjunath@slu.se
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By the 1980s, MRSA had emerged in various places throughout the world[1].

Certain strains of MRSA were found to have the propensity to spread very quickly in hospitals. MRSA infections will transmit from person to person, by direct contact with the skin, clothing, or areas (such as sink, bench, bed, and utensil) that had recent physical contact with a MRSA-infected person[2]. This poses a major threat to public health. MRSA is related to its potential for nosocomial transmission and the limited number of antibiotics are available for its treatment. It has been reported that between the years 1983 and 1994, of 93 new antibacterial agents submitted to analysis by the Food and Drug Administration, six were natural products (teicoplanin, mupirocin, miokamycin, carumonam, isepamicin and RV-11). The screening of plant extracts for antimicrobial activity has shown that higher plants represent a potential source of new anti-infective compounds[3]. Antimicrobial compounds such as benzoin and emetin have been isolated from plants[4]. The systematic screening of antibacterial plant extracts represents continuous efforts to find newer compounds with the potential to act against multi-drug-resistant bacteria[5]. The development of antibiotically resistant strains of microbial pathogens like MRSA, is a growing problem, and it is therefore, extremely important to discover and develop new antimicrobial compounds. One of the measures to minimize the increasing rate of resistance in the long run is to have continuous in-depth investigation for new and effective antimicrobials as alternative agents to substitute the existing ones. Natural resources, especially the plants and microorganisms, are the potent candidates for this purpose[6].

Even though, phytochemicals play a major role as antimicrobial therapeutics, it is important to have awareness of medicinal plants which are poisonous if wrong plant parts or wrong concentrations of its constituents are used. For example, *Chelidonium majus*, which is often prescribed to treat gastric and biliary disorders and as potent antimicrobial agent, can cause cholestatic hepatitis. Some of the poisonous plants such as *Papaver somniferum*, *Datura alba*, *Nerium oleander*, *Strychnos nuxvomica*, *Cleistanthus collinus*, *Cannabis sativa*, *Gloriosa superba*, *Anamirta cocculus*, *Citrulus colocynthis*, *Abrus precatorius*, *Semecarpus anacardium*, *Excoecaria agallocha*, *Digitalis purpurea*, *Aconitum ferrox*, *Croton tiglium* and *Plumbago zeylanica*, which are known to own medicinal properties, are used in the traditional Indian systems of medicine as potent drugs in prescribed doses[7].

Senecio tenuifolius Burm. F. (*S. tenuifolius*) is one of the plants belonging to *Senecio* genus, it is poisonous to livestock, but, the leaves of the plant are used topically as remedy for skin diseases and to reduce swelling and pain according to ethnobotanical information[8,9]. *Senecio* is one of the important genus of the Asteraceae family. The genus *Senecio* has been widely investigated and nearly all species contain pyrrolizidine alkaloids as the most characteristic metabolites, chalcones and flavonoids have also been reported. *Senecio* species were used as food, anti-emetic, anti-inflammatory and also in the treatment of wounds[10]. Hence, the present study aimed to investgate screen and activity guided isolation of active ingredients from *S. tenuifolius* leaves for its anti-staphylococcal activity.

2. Materials and methods

2.1. Source of plant material

S. tenuifolius leaves were collected from Eastern Ghats of Andhra Pradesh, India (Tirumala hills, India) in the month of December 2006 on the basis of ethanobotanical information of traditional Indian herbalists. Identification and authentication were kindly made by Professor Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, India. A classified reference voucher specimen (Voucher No. DOB702) was deposited at Department of Botany, Sri Venkateswara University, Tirupati, India.

2.2. Preparation of the extracts

Collected leaves were washed with distilled water, dried at 37 °C for 72 h, and crushed in a mechanical motor. Powdered sample (100 g) was extracted with 98% hexane (500 mL, w/v), at 69 °C for 10 h using soxhlet extraction apparatus (BOROSIL 3840, India). The leftover powder in thimble was dried and extracted with 99.8% chloroform (450 mL, w/v), at 62 °C for 8 h using soxhlet apparatus. In a similar manner the leftover powder was simultaneously extracted with 99.5% ethylacetate (400 mL, w/v) and 99.9% methanol (400 mL, w/v), at 77 °C and 65 °C for 8 h each, respectively. The whole process was repeated for four times, respective solvent extracts were combined and the solvents were completely evaporated at 65 °C using a rotary vacuum evaporator (BUCHI Rotavapor R-114, Switzerland). Remaining material after solvent extractions, was suspended in 1 L distilled water and boiled for 1 h at 90 °C-95 °C. The supernatant was removed and the extraction was repeated once again. The supernatants thus obtained were combined and filtered through Watmann No. 1 filter paper. The filtrate was concentrated by lyophilization. The extract was designated as aqueous extract[11].

2.3. Preparation of test samples

In the studies of the antimicrobial activity, the soxhlated plant extracts and silica gel G column based fractions were distilled and dissolved in dimethylsulphoxide (DMSO) to obtain the stock concentration of 2 mg/mL and 1 mg/mL, respectively for bioassay. It was established that dilution of DMSO lacked antimicrobial activity against any of the test micro-organisms.

2.4. Test for microorganisms

The soxhlated plant extracts were assayed for antimicrobial activity against one registered bacterial isolate, *S. aureus* (NCIM No: 5021, ATCC No: 25923) which was obtained from the NCIM (NCL, Pune, India). Two clinical isolates, MRSA and methicillin sensitive *S. aureus* (MSSA) were gifted by Dr. P.V.G.K. Sharma, Head, Department of Biotechnology, Sri Venkateswara Institute of Medical Sciences, Tirupati (India).

2.5. Agar well diffusion method

An agar-well diffusion method was employed for determination of antibacterial activities[12]. The extracts