South African Journal of Botany 102 (2016) 46–49

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Antimicrobial and antioxidant efficacy of *Citrus limon* L. peel extracts used for skin diseases by Xhosa tribe of Amathole District, Eastern Cape, South Africa

W.M. Otang [1], A.J. Afolayan *

Department of Botany, MPED Research Centre, University of Fort Hare, Private Bag X1314, Alice 5700, South Africa

ARTICLE INFO

Article history:
Received 14 May 2015
Received in revised form 22 June 2015
Accepted 3 August 2015
Available online 5 September 2015

Edited by J Van Staden

Keywords:
Antimicrobial
Antioxidant
Efficacy
Citrus limon
Skin diseases

ABSTRACT

Skin diseases such as dermatitis, prurigo, and scabies present a major health concern in the Eastern Cape, South Africa, where there is a scarcity of dermatologists, compounded by the fact that most dermatologists are centered near urban areas and are not accessible to 70% of the rural population. Hence, many people still depend to a large extent on traditional herbs such as *Citrus limon* for the treatment of skin diseases. The aim of this study was therefore to screen the acetone and ethanol extracts of *C. limon* for its antioxidant potential and antimicrobial efficacy agents against a panel of microbes implicated in skin diseases. The highest antibacterial activity was obtained with the acetone extract of *C. limon* against *Enterococcus faecalis* and *Bacillus subtilis*, and the most susceptible bacteria based on the overall mean inhibition diameters were the gram-negative *Salmonella typhimurium, Shigella sonnei* and the gram-positive *E. faecalis* and *B. subtilis*. Both extracts were active against *Candida glabrata*. The DPPH scavenging activity of the acetone extract was not significantly different from those of vitamin C and rutin. Nitric oxide scavenging activity was lowest in the ethanol extract of *C. limon*. The reducing ability of both plant extracts was significantly lower than that of vitamin C and rutin. The fact that both extracts of *C. limon* exhibited a broad spectrum of antibacterial activity and comparable efficacy to the synthetic antioxidants highlights the medicinal value of *C. limon* as a potential source for drug development amidst the obvious dearth of effective and safe antibacterial drugs, and also validates the ethnotherapeutic claim of the plant.

1. Introduction

The skin, the largest human body organ, provides a major interface between the environment and the body and is constantly exposed to an array of chemical and physical environmental pollutants (Bickers and Athar, 2006). The use of medicinal plants in the treatment of skin diseases had been practiced by the indigenous people of the Eastern Cape Province (South Africa) since many decades. Many health care centers in the Eastern Cape are often run by clinical officers or nurses (rather than physicians) who act as the primary care workers but have very limited training in diagnosing dermatologic conditions (Hay and Marks, 2004). Hence, skin diseases such as dermatitis, prurigo, scabies, and popular urticaria are either untreated or over-treated with strong topical steroids or antibiotics which have been found to cause considerable disability (Njoronge and Bussmann, 2007).

Skin diseases can be caused by a variety of microbes, the commonest bacterial agents being *Staphylococcus aureus*, *Streptococcus pyogenes*, *Clostridium perfringes, Pseudomonas aeruginosa,* and the bacteriodes group (Kohen, 1999). *S. aureus* and *S. pyogenes* are the most common organisms that cause various cutaneous infections such as cellulites, erysipelas, impetigo, folliculitis, furuncle, carbuncle, and abscess. Infection by *P. aeruginosa* is usually characterized by extensive folliculitis and hot tub rash, while *Proteus mirabilis* was reported to cause skin abscesses in axilla and acute cellulites with black discoloration (Nesy and Mathew, 2014). Common fungal infections of the skin include athlete's foot and ringworm caused by *Trichophyton rubrum* and candidiasis caused by *Candida albicans* that infects the skin, mouth (oral), vagina (vaginal), and digestive tract (gastrointestinal candidiasis) (Nesy and Mathew, 2014).

Although numerous topical and oral drugs are available to treat skin diseases, resistant strains to common antibiotics such as erythromycin, kanamycin, neomycin, or tetracycline have increased in the past years (Ördögh et al., 2010). The problem of resistance, environmental degradation, and pollution associated with the irrational use of orthodox antibiotics has necessitated renewed interest in plants as sources of effective and safer alternatives in the management of human infections (Kohen, 1999). Coupled to this, some synthetic antioxidants such as butylhydroxyanisole and butylhydroxytoluene need to be replaced with natural antioxidants due to their potential health risks and toxicity (Li et al., 2008).

* Corresponding author. Tel.: +27 406022323, tel.: +27 406022321; fax: +27 86 628 2295, fax: +27 866282409.
E-mail addresses: wotang-mbeng@ufh.ac.za (W.M. Otang), aafolayan@ufh.ac.za (A.J. Afolayan).
[1] Tel.: +27 33257274; fax: +27 866282409.

http://dx.doi.org/10.1016/j.sajb.2015.08.005
0254-6299/

Citrus limon (Rutaceae), commonly known as lemon, is an important medicinal plant, used mainly for its alkaloids, which are having anticancer activities, and the antibacterial potential of the crude extracts against clinically significant bacterial strains has been reported (Kawaii et al., 2000). The plant is a potential source of vitamin C, and the oil is used in various preparations to reduce skin itching, for skin nourishment, and the pulp left after extraction of the juice is reported to be used for the treatment of pimples and wrinkles and to soften facial skin (Otang et al., 2015). These facts have inspired the screening of *C. limon* for its antioxidant potential and antimicrobial efficacy agents against a panel of microbes implicated in skin diseases.

2. Materials and methods

2.1. Extraction procedure

Fresh lemon fruits used in the study were obtained from the local super market at SPAR in Alice, in 2014. The fruits were surface sterilized with 70% alcohol and rinsed with sterile distilled water. Ground samples of the peels were put into separate conical flasks containing acetone and ethanol and shaken for 24 h on an orbital shaker. After filtering with a Buchner funnel and Whatman No. 1 filter paper, the filtrates were concentrated to dryness under reduced pressure at a maximum of 40 °C using a rotavapor. Aqueous filtrates were freeze-dried. Each extract was re-suspended in the respective solvent of extraction to yield a 20 mg/ml stock solution (Koduru et al., 2006).

2.2. Microorganisms and media

The microbes used in this study were chosen primarily on the basis of their importance as pathogenic microbes that cause human skin disorders. Pure cultures of all experimental bacteria and fungi were American Type Culture Collection (ATCC), obtained from Total Laboratory, South Africa. Antibacterial studies were conducted using six gram-positive bacteria (*S. aureus*, *S. pyogenes*, *Enterococcus faecalis, Bacillus cereus, Bacillus subtilis,* and *Klebsiella pneumoniae*) and five gram-negative bacteria (*Salmonella typhimurium, Escherichia coli, P. aeruginosa, Shigella sonnei,* and *Shigella flexneri*). Antifungal activities were evaluated using *Candida krusei* and *Candida glabrata*.

2.3. Antimicrobial susceptibility assays

As per the recommendations of the manufacturer, nutrient agar and Sabouraud dextrose agar (SDA) media were prepared and poured into sterilized disposable Petri dishes under aseptic conditions. The labeled plates were inoculated with 100 μl of 0.5 Mcfarland solutions of the respective organisms and loaded with extract of *C. limon* (50 mg/ml) into 6 mm wells. Gentamicin and nystatin (25–50 μg/well) were used as positive controls for bacteria and fungi, respectively. Each test was replicated in triplicates. After proper incubation period at 37 °C, zones of inhibition were recorded in millimeters. The microdilution method was employed to determine the minimum inhibitory concentration (MIC) of the plant extracts using 96-well microtitre plates as previously described (Otang et al., 2012). The smallest concentration of the plant extract that was able to kill the microorganisms was considered as the minimum inhibitory concentration (MIC).

2.4. Antioxidant assays

2.4.1. Assay of DPPH scavenging activity

The DPPH radical scavenging activity of the test extracts was examined as previously described (Ebrahimzadeh et al., 2001). Different concentrations (0.025–0.5 μg/ml) of each extract were added, at an equal volume, to a methanolic solution of DPPH (100 μM). The mixture was allowed to react at room temperature in the dark for 30 min. Vitamin C and rutin were used as standard controls. Three replicates were made for each test sample. After 30 min, the absorbance (A) was measured at 518 nm and converted into the percentage antioxidant activity using the following equation: % Scavenged [DPPH] = [(Ao − A1) / Ao] × 100, where Ao was the absorbance of the control and A1 was the absorbance of extract and standard. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals. The IC_{50} values were calculated by linear regression of plots, where the abscissa represented the concentration of the tested plant extracts and the ordinate the average percent of scavenging capacity from three replicates.

2.4.2. Assay of nitric oxide-scavenging activity

This assay was according to the procedure of Ebrahimzadeh et al. (2001). Two milliliters of 10 mM sodium nitroprusside in 0.5 mM phosphate-buffered saline (pH 7.4) was mixed with different concentrations of each extracts dissolved in water and incubated at 25 °C for 2.5 h. After the incubation period, 0.5 ml of Griess reagent was added and the absorbance was read at 540 nm. Inhibition of nitric oxide generation was estimated by comparing absorbance value of controls (vitamin C and rutin) with that of the test.

2.4.3. Reducing power assay

Different amounts of each extracts (0.025–0.05 μg/ml) in distilled water were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml potassium ferricyanide (1% w/v). The resulting mixture was incubated at 50 °C for 20 min, followed by the addition of 2.5 ml of trichloroacetic acid (10% w/v). This was then centrifuged at 3000 rpm for 10 min. The supernatant (2.5 ml) was mixed with an equal volume of distilled water and 0.5 ml of FeCl3 (0.1% w/v), and the absorbance was measured at 700 nm. Vitamin C and rutin were used as positive controls.

2.4.4. Scavenging of hydrogen peroxide

The ability of the extracts to scavenge hydrogen peroxide was determined according to standard procedures (Erukainure et al., 2011). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations of the plant extracts (0.025–0.05 μg/ ml) in distilled water were added to hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extracts and standard compounds was calculated as follows: % Scavenged [H2O2] = [(Ao − A_1) / Ao] × 100, where Ao was the absorbance of the control and A_1 was the absorbance in the presence of the sample of extract and standard.

3. Results and discussion

3.1. Antimicrobial assays

The result of the agar well diffusion assay and the MICs of the extracts are summarized in Table 1. The highest antibacterial activity was obtained with the acetone extract of *C. limon* against *E. faecalis* and *B. subtilis* with inhibition zone diameters of 23 and 20 mm, which were not significantly different ($P < 0.05$) from the positive control.

The most susceptible bacteria based on the overall mean inhibition diameters of both extracts were the gram-negative *S. typhimurium* and *S. sonnei* and the gram positive *E. faecalis* and *B. subtilis.* The lowest MIC (0.01 mg/ml) was recorded on the acetone extract against *E. faecalis.* Both extracts were active against *C. glabrata.* The antimicrobial activity observed in this study is very interesting since it suggests a different mechanism of action of *C. limon* extracts than that of currently used antibiotics and further highlights its therapeutic value as an antimicrobial agent against multi-drug resistant strains.

Fungal infections of the skin, also known as 'mycoses' are generally mild. Superficial fungal infections affect outer layers of the skin, nails,