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Bioactivity and application of plant seeds' extracts to fight resistant strains of *Staphylococcus aureus*

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ABSTRACT

Staphylococcus aureus is a highly dangerous pathogen that causes lots of health problems. The resistant strains to methicillin (MRSA) are dangerously health threatens. Nine plant seeds' extracts (*Alium ampeloprasum*, *Allium cepa*, *Brassica juncea*, *Lycium shawii*, *Nigella sativa*, *Ocimum basilicum*, *Peganum harmala*, *Phyllanthus emblica* and *Portulaca oleracea*) were evaluated as microbial inhibitor agents against *S. aureus* isolates, including MRSA strains. The crude extracts of *L. shawii* and *P. emblica* seeds proved to be the most active antimicrobials, against the entire *S. aureus* strains, using both quality and quantity assays for their bactericidal activity. Both *L. shawii* and *P. emblica* seeds contained remarkable amounts from active phytochemicals, alkaloids, phenolic compounds, tannins and flavonoids. *P. emblica* seeds were exceedingly rich sources of phenolic compound and flavonoids. The electron scanning micrographs of *S. aureus* cells, after exposure to plant seeds' extracts, showed that bacterial cells were shrunk and became tiny, diminutive and dehydrated after 3 h and the entire cells were fully lysed, exploded or disrupted after 6 h of exposure to *P. emblica* extract; whereas exposure to *L. shawii* extract derived treated cells to lyse and combine with each other's after 3 h then complete cell wall lysis was observed after 6 h of exposure. The applications of plant seeds' extracts, for textile finishing and ointment formulation, confirmed their efficacy as potent applicable anti-MRSA agents. It may be recommended to apply plant seeds' extract, e.g. *P. emblica* and *L. shawii*, as powerful antibacterial agents for the control of the skin and foodborne pathogenic bacteria, *S. aureus*, and their resistant MRSA strains.

1. Introduction

Plants continued to be marvelous rich sources of medicinal compounds that recurrently helped to save human health since ancient history; plant extracts and their bioactive phytochemical constituents were reported in traditional therapies and folk medicine of 80% from the earth population (Cowan, 1999). Moreover, more than the half of all current modern clinical drugs was derived from natural plant origins (Kirbag et al., 2009). The screening of antimicrobial agents, from plant phytochemicals and derivatives, was repeatedly positioned as the starting point for the discovery of novel antimicrobial drugs (Cederlund and Mårdh, 1993; Tayel et al., 2013a). The wide variety of bioactive phytochemicals in plant derivatives promoted the researchers for investigating more and more pharmaceutical usages from their mostly safe compounds (Aiyegoro and Okoh, 2009).

The emergence of additional bacterial resistance to commonly used antibiotics urged the requisite for novel powerful antimicrobial agents, from non- conventional sources (Cowan, 1999).

These complex factors have obligated researchers to explore innovative antimicrobial agents from all available sources to act as alternative antimicrobial chemotherapeutic compounds; the high production cost of synthetic drugs and their adverse effects, compared with naturally plant derived agents, persuade the direction back to nature (Cowan, 1999; Tayel et al., 2013a).

Staphylococcus aureus is the Gram positive bacteria that mostly involved in food poisoning, wound infections, toxic shock and scalded-skin syndrome, osteomyelitis and endocarditis (Lowy, 1998; Winn et al., 2006), via their production of diverse dangerous toxins; enterotoxin A-E, exfoliated toxins A and B and toxic shock syndrome toxin-1 (TSST-1) (Projan and Novick, 1997). The ingestion of *S. aureus*

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enterotoxin from contaminated food is a very dangerous source of food poisoning worldwide (Howard and Kloos, 1987). *S. aureus* was reported to be the most important pathogen in seafood; it was recorded to contaminate 20% of various fisheries products including whole fish, fish fillets, crab-meat, shellfish-meat and shrimp tails (Ayulo et al., 1994). *S. aureus* was reported to facilitate and increase the susceptibility of fish infection with *Streptococcus agalactiae* that causes the mortality of > 60% from infected fish (Amal et al., 2008).

S. aureus is from the well-established organisms that have the ability to acquire resistance toward many chemical antibiotics; the resistant bacteria to methicillin and its derivatives (MRSA) is considered as the most threatening cause of nosocomial infections. MRSA infections are very challenging to treat because of bacterial resistance to most of clinically usable antibiotics (Brooks et al., 2007). A large number of *S. aureus* isolates was obtained from tilapias fish (*Oreochromis niloticus*); 50% from them were characterized as MRSA strains (Atyah et al., 2010).

The bacterial resistance to antimicrobial is multifactorial, involving the relationship between bacterial cell and antibiotic, environmental factors, the type of antibacterial agent usage and host characteristics (Adwan and Mhanna, 2008).

However, current study was designed to evaluate the effectiveness of many plant seeds' extract as potential antibacterial agents against *S. aureus* and their MRSA strains, and to elucidate their potential antibacterial actions against the microbial strains.

2. Materials and methods

2.1. Plant seeds

The examined plant seeds, for their antibacterial activity, i.e. *Alium ampeloprasum, Allium cepa, Brassica juncea, Lycium shawii, Nigella sativa, Ocimum basilicum, Peganum harmala, Phyllanthus emblica* and *Portulaca oleracea*, were kindly obtained from the Vegetable and Medicinal Plants Research Centre, Giza, Egypt. Plant seeds were washed with distilled water, dried with hot air at 42 °C in an electrical oven, then ground using electrical grinder to have plant particle sizes of about 40 mesh. Weights of 200 g from each seed powder were extracted using 1 L of solvent, i.e. methanol (70%), for 72 h with occasional shaking at 120 x g. Extracts were then filtered, completely evaporated under reduced pressure at 40 °C, weighted and re-suspended in 20% dimethyl sulfoxide (DMSO) solution to attain concentrations of 100 mg/ml (Tayel et al., 2012). DMSO solution was used as negative control for comparison.

2.2. Isolation of bacterial pathogens

The examined bacterial strains were isolated from skin wound infections in patients at some universities hospital, by the aid of a physician. Samples were aseptically collected using sterile cotton swabs, spread on mannitol salt agar medium (MSA) and incubated overnight at 37 °C, the appeared colonies were then individually isolated, purified and kept in MSA slants. Isolates were then subjected to the described biochemical tests by Cowan and Steel's Manual (Barrow and Feltham, 1993), to confirm their identity. The isolates identification was further confirmed using highly automated VITEK 2 System, produced from bioMérieux Co., France. A reference strain, i.e. *S. aureus* (ATCC 25923), was used for comparison and result confirmation.

2.3. Assays for plants seed extracts' antimicrobial activity

The antibacterial activities of plant seed extracts were evaluated using both qualitative and quantitative assays, i.e. well diffusion method and minimal inhibitory concentration (MIC), respectively.

The well diffusion assay was conducted according to Obeidat et al. (2012); 100 µl of bacterial suspension (~2×10^6 CFU) was uniformly spread onto solidified Nutrient agar (NA) plates for bacterial isolates, allowed to dry for 5 min. then holes of 8 mm diameter were made using a sterile cork borer. Aliquots from each plant seeds' crude extract (50 µl of diluted extracts at 10% w/v) were pipetted into each well and the plates thereafter were incubated at 37 °C for 24–48 h. The appeared zones of bacterial growth inhibition were measured for each extract.

The MIC determination of plant seeds' extracts was performed using the microdilution method described by Tayel et al. (2010), using extracts concentration range of 1–10 mg/ml. Triphenyl tetrazolium chloride (TTC) was used as indicator for confirming the bactericidal activity of plant seed extracts.

2.4. Quantitative determination of some phytochemical constituents

The contents of phytochemical groups in plant seed materials were quantitatively determined as follow:

The total alkaloids contents and total phenolic compounds were measured according to previously described methods (Harborne, 1998); alkaloids contents were expressed as mg/g of the plant sample dry weight, whereas total phenolics were spectrophotometerically estimated using the Folin Ciocalteau's reagent and expressed as mg equivalent of Gallic acid/g plant material.

The total tannins were estimated using the Gravimetric method and their contents were calculated as illustrated by Ali (1991). Flavonoids were determined as mg/g using the described modified aluminum chloride colorimetric method (Chang et al., 2002), with rutin as a standard for comparison.

2.5. Imaging with scanning electron microscopy (SEM)

For the potential explanation of antimicrobial action from the most powerful plant seeds' extracts toward *S. aureus* strains, the bacteria cells were treated with seed extracts and the micrographs were captured using SEM (Jeol JSM-5300), operated at 20 kV and magnification of 20 Kx, after 0, 3 and 6 h from the treatment with extracts. The fixation of bacterial cells was performed using 2% Osmium peroxide (OsO_4), then they were dehydrated at 4 °C using a graded ethanol series. Samples were critical dried using a carbon paste and coated with gold to a thickness 400A° inside a sputter – coating unit (JFC-1100 E). Micrographs capturing was depended on the variation in cells morphology after exposure period to extracts.

2.6. Application of anti-MRSA seed extracts

2.6.1. Fabrication of extracts-treated textiles

Standard and scoured cotton textile (104 g/m^2 plain weave, Style S/400, TESTEX, Germany) were used for impregnating with plant seeds' extracts. The method of "pad-dry-cure" was performed for textile finishing. 1 × 1 cm squares from fabrics were cut and immersed in extracts solution, at their MIC levels, stirred for 2 h at 50 °C, then padded and squeezed using 2 nips and dips to 100% wet pick up. Treated fabric pieces were then dried for 3 min at 75 °C, then cured at 125 °C for 5 min, as modified after Tayel et al. (2013b) and Koh and Hong (2014). The antibacterial evaluation of extract-treated fabrics was conducted using inhibition zone assay on inoculated NA plates with *S. aureus* and MRSA strains.

2.6.2. Extracts topical formulation

The topical formulation of seeds extracts was conducted using an ointment base from soft white paraffin, supplemented with 1% Sodium lauryl sulfate (SD Chemicals, India), plant seeds' extracts were blended with the liquefied ointment base (at 45 °C), to have a concentration of 10 mg/ml from each extract (Gaud and Gupta, 2006). For the antibacterial evaluation, 50 µl from liquefied extract-supplemented ointment were pipetted into 6 mm diameter wells, made in inoculated NA plates with bacterial strains using sterile cork borer, and incubated at