EVALUATION THE ANTIBACTERIAL ACTIVITY OF LAWSONIA INERMIS : IN VITRO STUDY

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Abstract
Aqueous and alcoholic extracts of lawsonia inermis were tested for their antimicrobial activity. Four types of bacteria were selected these are Staphylococcus aureus; Escherichia coli; Enterococcus faecalis and Pseudomonas aureginosa. Minimal Inhibitory Concentration (MIC) were determined for both aqueous and alcoholic extracts; the aqueous extracts was more potent than alcoholic extract regarding MIC 8-64 mg/ml and 32-64 mg/ml for aqueous and alcoholic extracts respectively. All the selected bacteria showed clear sensitivity for those extracts except the Escherichia coli showed more and greater. The results revealed that aqueous extract of lawsonia inermis is more potent than alcoholic extract in their antibacterial activity and produced bacteriostatic effects so in this study we recommended the use of this plant for superficial skin infections caused by S. aureus; P. aeruginosa and E. faecalis.

تقييم تأثير المضاد البكتيري البكتيري لنبات الحناء: دراسة مختبرية

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الخلاصة
المستخلص المائي والكحولي لنبات اللويستينيا (الحناء) قد تم اختباره لمعرفة التأثير المضاد البكتيري للبكتيريا، اربع أنواع من البكتيريا قد تم اختبارها وكانت (Staphylococcus aureus; Escherichia coli; Enterococcus faecalis and Pseudomonas aureginosa) قد تم تحديد المستخلص الكحولي والمائي وأظهرت الدراسة أن المستخلص المائي أقوى من المستخلص الكحولي معتمدين التركز الإقليمي للمثبت للكبكتيريا حيث كان 8-16 ملمغم/مل و 32-64 ملمغم/مل للمستخلص المائي والكحولي حسب الترتيب. كلا البكتيريا المختارة اظهرت حساسية لمستخلص هذه البكتيريا ماعدا Escherichia coli) قد أظهرت مقاومة كبيرة. اظهرت نتائج هذه الدراسة إن المستخلص المائي للحناء (Escherichia coli) من المستخلص الكحولي بالنسبة للتركز الإقليمي للمثبت للكبكتيريا لذلك نوصي في هذا الدراسة باستخدام الحناء Staphylococcus aureus; Escherichia coli; Enterococcus faecalis and Pseudomonas aureginosa لالتهابات الجلدية السطحية والتي تسبب بهذه البكتيريا.
Introduction

Many of today's current drugs have their origin in traditional plants medicine and the therapeutics efficacies of many indigenous plants for several disorders have been described by practionars of traditional herbal medicine.[1] Natural products are a significant sources of synthetics and traditional medicine and are still the primary health care system being sources of many life sustaining metabolite; the research is still on plants to be used in healing [2].This part due to the growing problem of worldwide antibacterial resistances; so isolation of antimicrobial agents less susceptible to regulartory antibiotics and recovery of resistant isolate during antibacterial therapy is increasing through the world. [3]

The measures of combat this increasing rate of resistance is to have continious investigation into new safe; and effective antimicrobials as alternative agents to substitutes with less effective one .Because of this research is being carried out to investigate ethnobotanical use of plants prevailing among native people. The lawsonia inermis (henna) is the chief constituents dependable for dying properties of the plant; the dried powder leave of henna containe about 0.5-1.5% lawssone; traditionally used to produce colorfast orange;red and brown dye.[4]The lawssone; is a primary concentrated in the leaf; and is in the highest level in the petiole of the leaf.Products sold as black henna or neutral henna are not made from henna ;may derived from indigo (indigofera tinctoria) and may containe unlisted dyes and chemicals[5]

The United State Food and Drug Administration (FDA) has not approved henna for direct application to the skin[6]. Many reports about medical and cosmetic usage of lawsonia inermis has been published in literatures; antitumor; antioxidants and antituberculous effects of lawsonia are prominent in these reports.[7] also lawsonia inermis produce serious oxidative stress and induction of glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency in children and decreasing effects of glutathione level due to the oxidative stress.[8]

Lawnssia inermis produce diverse antimicrobial activity against Bacillus cereus; Bacillus anthracis and Proteus vulgaris also produce fungistatic effects [9].

Material and methods Plants extraction

Plants used in the present study for the evaluation of its antibacterial activity were the commercial lawsonia inermis leaves (Lythraea). The dried leaves parts of the plants are grained into fine powder and sieved then 1g of powder dissolved in 10ml of solvent which were distilled water and 95% ethanol respectively .The mixtures were filtered by whatman filter paper NO.1.

Determination of MIC

The Minimal Inhibitory Concentration(MIC) values were determined by microdilution assay. This experiments was performed by method of Sahin et al[10]. The MIC was defined as the the lowest concentration of the compound to inhibit the growth of bacteria. A double dilution of each extract in Muller -Hinton agar were made till reach the concentration of 8-64mg/ml;and loopful of each bacteria was inoculated separately into tubes containing nutrient broth and incubated at 37C for overnight; dilution of broth culture was made up to 100 fold with nutrient broth; the results were followed both end for visible growth the tubes containing no extracts were included as control . The selected bacteria were S.aureus;E.faecalis ;P.aeruginosa and E.coli. These bacteria collected from patients ;each bacterial sample were incubated in the nutrient broth at 37C for 18hrs.;and then subcultured for 24hrs. The lowest concentration of extracts that failed to show the macroscopic growth(turbidity) regarded as MIC of those extracts. The MIC determinations was performed in duplicate for each organism;and the experiments was repeated when necessary.

Results

The antimicrobial activity of lawsonia inermis is regarding the aqueous and alcoholic extracts showed in table (1).

<table>
<thead>
<tr>
<th>Bacterial types</th>
<th>8mg/ml</th>
<th>16mg/ml</th>
<th>32mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aqueous</td>
<td>alcoholic</td>
<td>aqueous</td>
</tr>
<tr>
<td>S.aureus</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>E.fecalis</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>P.auregenouosa</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>E.coli</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Results of this study showed that MIC of alcoholic extracts was weaker than aqueous; and all bacterial that are choosen are susceptible except; E.coli which is resistance for both
aqueous and alcoholic extracts of lawsonia inermis. The MIC of aqueous and alcoholic extracts showed in table (2).

<table>
<thead>
<tr>
<th>Bacterial types</th>
<th>MIC mg/ml aqueous</th>
<th>MIC mg/ml alcoholic</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.aureus</td>
<td>8-64mg/ml</td>
<td>32-64mg/ml</td>
</tr>
<tr>
<td>E.fecalis</td>
<td>8-64mg/ml</td>
<td>32-64mg/ml</td>
</tr>
<tr>
<td>P.aureginosa</td>
<td>8-64mg/ml</td>
<td>16-64mg/ml</td>
</tr>
<tr>
<td>E.coli</td>
<td>&gt;64mg/ml</td>
<td>&gt;64mg/ml</td>
</tr>
</tbody>
</table>

Discussion

In the present study; Lawsonia inermis extracts were examined for antimicrobial activity in opposition to S.aureus; E. fecalis; P. aeruginosa and E. coli. The results showed that lawsonia inermis extracts could effectively inhibit the growth of these bacteria except E. coli. Among individuals extracts the aqueous one produced more antimicrobial activity than alcoholic extracts which showed by lower MIC for aqueous 8-64mg/ml but alcoholic extracts produce this effects at 32-64mg/ml; the MIC values of aqueous extracts against these organisms were equal to 8-64mg/ml but the alcoholic extracts effects ranged between 16-64mg/ml and 32-64mg/ml.

The antimicrobial activity of lawsonia inermis is first tested by Mallekzadeh 1968 study that showed that lawsonia inermis produced antibacterial effects against P. vulgaris and B. anthracis and no activity against E. coli which supported by our study, the antimicrobial substances in lasonia inermis are highly soluble in water; partially soluble in 70% ethyl alcohol and heat soluble [12]. The chemical constituents of active material in the lawsonia inermis that have antimicrobial activity were gallic acid; lawsone; hennatanic acid; and mucilage [13]. Moreover; Bet-a-smith and Swain pointed that polyphenolic and tannins compound are defined as having molecular weight between 300-500 and beside giving usual phenolic reaction; they have special properties such as ability to precipitate the gelatin and other microbial proteins [14,15]. The present study showed that lawsonia inermis extracts were proficient of inhibiting the growth of microorganism that are involved in causing wound and burn infections.

Bonj 2006 study showed that henna extracts are superior to silver nitrate in treatment of burn infection due to hypokalemia and hponatremia as side effects of prolonged uses of silver nitrate [16].

The Scientific Committee on Cosmetic Products and Non-Food Products Consumers (SCCNFP) adopted in 2005; an opinion (sccnfp/os/foline) which state that lawsonia inermis; a natural products in hair dyes production has genotoxic/mutagenic potential in vitro and in vivo and therefore no safe threshold for lawsonia inermis can be established [17]. Add to this; lawsone from lawsonia inermis possesses antimycobacterial activity therefore; lawsone (2-hydroxynaphthoquinone) which is known to be the major bioactive constituents of this herb [18]. Also; lawsonia inermis bioactive substances played as immunostimulant action by promotion T-lymphocyte proliferation response; and it is found that the immunostimulant activity of the total methanolic extracts of henna leaves is greater than that of individual solvent fraction at same concentration [19]. Recently; apinin and apigenin compound of lawsonia inermis exhibited a free radical scavenging activity comparable with that of ascorbic acid; these substances showed a greater antioxidant activity than standard ascorbic acid and prevent the phospholipid membrane peroxidation and protect immunocompromised cells from free radical damage [20,21].

Antimicrobial activity of lawsonia inermis may be direct due to active substance like lawsone or indirect due to apinin that cause immunostimulation and immunomodulatory effects; therefore further in vivo studies will be intended at isolation and identify other substances responsible for the antimicrobial activity of plant extracts which may be further exploited in herbal formulations.

Conclusion and recommendations

1- Aqueous extract of lawsonia inermis is more potent than alcoholic; S. aureus; E. fecalis; and P. aeruginosa are susceptible to lawsonia inermis extracts; but the E. coli was resistance for lawsonia inermis effects.
2- Determination the lawsonia chromatography for selection the biological active substances in the lawsonia inermis extracts.
3- Evaluation the antileishmania activity of lawsonia.
4- Specify the mechanism of action of lawsonia extract.
5- In vivo antibacterial activity of lawsonia and their toxic effects.

References
7. Sharma V. 1990. Tuberculostatic activity of henna (Lawsonia inermis) Linn. Tubercule; 71(4):293-95