Antimicrobial activity of the aqueous extract of pomegranate peel and *Lantana cammara* leaves extracts against *Proteus mirabilis*

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ABSTRACT

To isolate the Proteus, 100 samples of mouth and nose swabs from different animal species were dependent for the current study. The swabs were submitted to routine bacterial procedures isolation, through culturing on nutrient broth, then MacConkey and blood agar, followed by gram stain examination, and fixing the macromorphological characters of colony. Then submitted to further micromorphological examination oxidase, and indole test. A total of *Proteus mirabilis* was 4 out 100 were isolated in pure form nose, in addition to 2 case mixed with *E. coli* from nose. From the mouth 1 case mixed with pseudomonas, and 1 case mixed with Bacilli and Staphylococcus. These isolates were examined for their sensitivity to aqueous extract of Pomegranate peel, *Lantana cammara* leaves using agar well diffusion method. The sensitivity was highest for the pomegranate peel and *Lantana cammara* leaves showed an inhibitory effect.

Key words: *Proteus mirabilis*, Aqueous extracts, pomegranate peel and *Lantana cammara*


INTRODUCTION

*Proteus mirabilis* is a gram negative bacillus, from the *Enterobacteriaceae* family facultative anaerobe with a capacity to ferment sugar to maltose but lacks the ability to ferment lactose sugar. *Proteus mirabilis* too has a swarming phenomena and capacity to self-elongate and display a polysaccharide after in communication through solid surfaces; this allows for connection besides easy motility alongside surfaces. A flagella that allows its movement; this feature not only supports colonization but is also related to its ability to form biofilms and is suggested to contribute to resistance to specific host and antibiotic defenses. [1] In this family its place in the tribe *Proteeeae*, collected with the genera *Providencia* and *Morganella*. *Proteus* bacilli are different from other genera through its ability to swarm thru the surface of the agar of solid media[2]. *Proteus* is usually disseminated in the normal location. It can be establish in contaminated water and in manure and soil, [3]. Plant life are rich source of antibacterial mediators because they yield varied array of bioactive molecules, utmost of which possibly developed as chemical protection alongside infection or predation. [4].Pomegranate belongs to the *Punicaceae* family, has been used in old-style medicine for many cultures, principally in
the Middle East region, and the eatable portions of the pomegranate make up 52% of the fruit’s total weight, including 22% seeds and 78% juice [5]. Non-edible portion termed pomegranate peel extract. Pomegranate became further common due to the attribution of basic physiological possessions, like cardioprotective [8] anticancer [6] anti-proliferative apoptotic [7] anti-hyperlipidemic [9], [10]. It has been demonstrated that pomegranate has many potential effects such as immune modulator stomachic, antifungal and antibacterial activities [11] L. camara has several uses, mainly as a herbal medicine and in some areas as firewood and mulch. It is also used for the treatment of cancers, chicken pox, measles, asthma, ulcers, swellings, eczema, tumors, high blood pressure, bilious fevers, catarrhal infections, tetanus, rheumatism, malaria and atox of abdominal viscera [1]. In some countries, it is planted as a hedge to contain or keep out livestock. Extracts from the lantana leaves exhibit antimicrobial, insecticidal and nematicidal activity and also contain verbascoside, which possesses antimicrobial, immunosuppressive and antitumor activities [2]. Lantanoside, linaroside and camarinic acid have been isolated and are being investigated as potential nematocides. Lantana oil is sometimes used for the treatment of skin itches, antiseptic for wounds, leprosy and scabies L. camara has several uses, mainly as a herbal medicine and in some areas as firewood and mulch. It is also used for the treatment of cancers, chicken pox, measles, asthma, ulcers, swellings, eczema, tumors, high blood pressure, bilious fevers, catarrhal infections, tetanus, rheumatism, malaria and atox of abdominal viscera [1]. In some countries, it is planted as a hedge to contain or keep out livestock. Extracts from the lantana leaves exhibit antimicrobial, insecticidal and nematicidal activity and also contain verbascoside, which possesses antimicrobial, immunosuppressive and antitumor activities [2]. Lantanoside, linaroside and camarinic acid have been isolated and are being investigated as potential nematocides. Lantana oil is sometimes used for the treatment of skin itches, antiseptic for wounds, leprosy and scabies L. camara belong to the family of (Verbenaceae), is public in the field of drugs for its multiple uses. Moreover, it is used to treat chickenpox, cancers, measles, blisters, asthma, eczema, pustules, cancers, high blood pressure, hemorrhagic infections, bile fervor, and tetanus [12]. The extracts from the L. cammara leaves display insecticidal, antimicrobial and nematicidal action, it also comprises verbascoside, which possesses immunomodulatory, anti-tumor and antimicrobial activities [13]. Lantana oil is used occasionally to treat leprosy, itchy skin and scabies, and as a wound disinfectant.

Aim of study was to isolate the Proteus from the natural orifice of the body (Mouth and Nose) of animals and study its sensitivity for aqueous extracts of peel pomegranate and Lantana cammara leaves.

MATERIAL AND METHODS

The study was conducted in Department of Microbiology, College of Veterinary Medicine, University of Diyala, Iraq from November, 2018 – March, 2019.

Methods
The scheme of the study:
Swabs -> cultured on nutrient broth for 24 hours -> cultured on MacConkey agar.
Isolated colonies classified according to their shape, color and morphological characters.
1. Cultured on blood agar swarming appearance
2. cultured on MacConkey agar (Lactose none fermenting)
3. stained and examined microscopically
4. The classifications were according to morphological and biochemical characters.
5. Lactose none fermenting was examined by oxidase test for Proteus
6. Indol test (negative)
7. Sensitivity to plant extract we used brain heart infusion agar for the sensitivity test.

**Bacterial isolation**

**Samples and specimens collection**

On the historical from November, 2018 to March, 2019. The Swabs were collected from nose and mouth from different animal species.

**Cultivation of the sample**

The collected swabs were cultured into nutrient broth and incubated at 37 °C for 5 hr. to rise bacterial level loop full from the incubated broth was distributed over the surface of MacConkey agar then incubated at 37 °C for 24 hr.

**Bacterial diagnosis:**

Isolation of *P. mirabilis* bacteria was done by a surface streak technique on both MacConkey and blood agar by standardized loops and Aerobic incubation by 37°C for 24 hours. Identification of bacteria was completed with biochemical test, namely citrate, indole, catalase, oxidase, urea hydrolysis, lactose fermentation and H2S production.

**API20 system (confirmation test):**

The favorable method used for the documentation of bacterial isolates and study the further biochemical experiments used for bacteria *P. mirabilis* [14].

**Sensitivity:**

McFarland solution was prepared according to [15]. The mix was stirred well and placed in a test tube covered with screws and kept in a dark place at 4 ° C good mixed solution before usage to compare it with bacterial turbidity as it gives a similar turbidity to 0.5 x 10^8 bacteria/ ml.

Sensitivity to plant extract we used brain heart infusion agar for the sensitivity test. We use aqueous extract of the pomegranate peel, and *Lantana cammara* Leaves.

**Crude plants Extract Preparation:**

**Pomegranate Peel Extraction** the preparation steps of the aqueous extract of pomegranate peel which was prepared as follows [16]

1. The peels were cool from native market then washed and left 5 days to get dehydrated beneath sun light. Later that dehydrated peels are cut into lesser parts and grinded using electric grinder till converted well powder.
2. The powder was sieved using a (75 μm × 20 cm) sieve. Weight about 20 g of powder and melted in a suitable quantity of deionized water the mixture was heated till boiling at that point cool for 24hr. at 37c.
3. The mixture was filtered several times to extract the identical product and then heated in the extraction concentration method.
4. The collected extract located in 250 ml volumetric bottle and then diverse concentrations (100, 200, 400, 800 and 1600) ppm were prepared from the standard solution.
**Lantana cammara extraction**

1-The fresh Lantana cammara leaves were collected from gardens from college of veterinary medicine of Diayala University, air dehydrated at 37\(^\circ\)C and crushed into fine particles through using electronic crusher.

2-A amount of 50g of fine particles was heated in distilled water for 15-20 minutes at 80\(^\circ\)C, and filtered via cloth mesh, after that concentrated by rotatory evaporator and set aside in a freezer up to usage [17].

3-different concentrations (100, 200, 400, 800 and 1600) were prepared as of the standard solution and used as the test extracts for antimicrobial activity assay

**Antimicrobial Activity:**

Antimicrobial activities of diverse extracts were assessed by the agar well diffusion technique [18] adapted by [19]. 20ml of purified Muller Hinton Agar was dispensed into sterilized petri plate, after solidification, 100 μl of freshly cultured pathogens were flushed onto particular plates. The wells were cut over the agar plates using sterile gel puncher at numerous concentration (100, 200, 400,800 and 1600) of all plant extract were added to the wells. The plates were incubated for 24 hours at 37\(^\circ\)C. Then the diameter of the retarded areas around each well was measured in mm and documented.

**Determination of Minimum Inhibitory Concentration**

Determination of the MIC of the extract was a 16- hours culture was diluted through sterilized physiological saline solution (0.9% w/v sodium chloride) by reference to the 0.5 McFarland turbidometry to accomplishment the inoculum about equivalent to \(10^8\) CFU/ ml) [20]. In the tube dilution test, typical bacterial suspension and diverse concentration of extracts, (100, 200, 400, 800 and 1600 mg/ ml) were additional to tubes having 2 ml broth. All tubes were cultivated by 37 \(^\circ\)C for 24 hours. The first tube of the sequences through no sign of clear growing was considered as the MIC. This procedure was performed three times [21]. The values are statistically analyzed according to [22].

**RESULTS**

**Isolation of Bacterial spp.**

The results of current study displayed that from 100 swabs collected from mucocutaneous origin of mouth and nostrils of different animal origin, submitted to the study. A total of *Proteus mirabilis* was 4 out 100 were isolated in pure form, in addition to 2 case mixed with *E. coli* from( nose).

From mouth 1 case mixed with *pseudomonas*, and 1 case with *Bacilli* and *Staph*, (Tables 4-1).

**Table- 4-1-Mixed isolates**

<table>
<thead>
<tr>
<th>origin</th>
<th>Sp.</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouth</td>
<td>Prot. Pseudomonas</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Staph Prot. Bacilli</td>
<td>1</td>
</tr>
<tr>
<td>Nose</td>
<td><em>Proteus mirabilis</em></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td><em>Prot. E. coli</em></td>
<td>2</td>
</tr>
</tbody>
</table>
Identification of *Proteus mirabilis*

**Microscopic examination**

Microscopic examination of the results showed that the bacterial cells isolate are negative to Gram stain, red color coccobacilli, variable in length, frequently occurred singly or in short chains and non-spores forming.

**Cultural characteristic**

Developing colonies are single pale colonies on MacConkyagar are medium in size and the edges smooth and no ferment sugar lactose as well as the smell of bacterial growth which is similar to smell of fish rotting and appeared ripple movement or swarming on the blood agar, which is the recipe initial diagnostic for this bacteria. as in (figure 4-1).

![Figure 4-1: Swarming of *P. mirabilis* on blood agar](image)

**API 20 test result**

Bacterial isolates were tested by API 20 E for confirmation the results are shown as in the figure (4-2)

![Figure (4-2): API 20 E test results for *Proteus mirabilis*](image)

**Sensitivity to aqueous extracts:**

The results of sensitivity showed that the pomegranate peel extract exhibit a significant effect as the sensitivity was high and dose dependent. The highest inhibitory zones appear at 1600 mg / ml. *L. cammara* leaves extract did not show any significant inhibition and the bacteria was insensitive to the extract fig(4-3)
Figure (4-3) showed the sensitivity of pomegranate peel extract and insensitivity *Lantana cammara* leaves extract by well agar diffusion method.

Table 4-2 Sensitivity of *Proteus mirabilis* to aqueous extract of pomegranate peel and *L. cammara* leaves by agar well diffusion method

<table>
<thead>
<tr>
<th>Extract</th>
<th>Conc. mg/ml</th>
<th>Pome. peel</th>
<th>L. leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Am</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aug</td>
<td>None</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>8.0± 0.55Aa</td>
<td>1.0 ±0.2b</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>9.0±0.92Aa</td>
<td>1.5±0.2b</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>11.0± 0.43Ba</td>
<td>1.5±0.4b</td>
<td></td>
</tr>
<tr>
<td>800</td>
<td>11.0± 0.14Ba</td>
<td>2.0±0.3b</td>
<td></td>
</tr>
<tr>
<td>1600</td>
<td>16.33± 0.34Ca</td>
<td>2.0 ±0.5 b</td>
<td></td>
</tr>
</tbody>
</table>

Value are Mean ± SEM; a, b significant when compared between the two extracts; A, B, C, when compared between concentrations at P< 0.05 level.
Minimum inhibitory concentration

Pomegranate peel and *L. cammara* leaves at 800, and 1600 mg / ml concentration

DISCUSSION

Isolated bacteria:
The results of current study showed that from 60 swabs collected from mucocutaneous origin of mouth and nostrils, only (3; 0.05%) *Proteus mirabilis* were isolated in pure form, with 3 isolates were mixed with other bacteria. One of etiological factors of numerous human and animal diseases are *Proteus* sp., since in human it is the etiological agents of wound infections, urinary tract, burn, as well to bacterial chronic and acute otitis media [23] and as a causal of pneumonia and nosocomial infections [24]. *Proteus vulgaris* (4/98; ratio was 4.1% and *Proteus mirabilis* (2/98; ratio was 2.0%) were isolated from (98) patient suffered from wound infection. While from 173 patients suffered from burn complications *Proteus* sp. (21/173; 12.1%) were isolated. Urine samples obtained from patients with urinary tract associated with *Proteus* sp., were isolated from 11/90; 12.2%). From those who suffered from otitis media, *Proteus* sp. (16/50; 32%) was isolated [25]. According to [26] diverse Gram negative bacteria are opportunistic infections in immunocompromised patients. The organisms most often occupied members of the genera *Klebsiella*, *Escherichia*, *Proteus*, *Acinetobacter*, and *Enterobacter*.

Sensitivity to aqueous extracts:
The aqueous extract of pomegranate peel by agar well diffusion method, showed there were bacterial inhibition, of dose dependent type. The highest inhibitory zone was at 1600 mg / ml. and bacterial inhibitory test showed that the level at which there was no bacterial growth. Meanwhile extract of *Lantana cammara* leaves did not show significant inhibitory effect at all concentration. The isolates were resisted to amoxicillin and augmentin as there was no any inhibition. [27] reported that the agar well diffusion technique was further essential to determine the antibacterial properties of together alcoholic and aqueous extracts of cotoneaster sp., in contrast through agar disc technique, the alcoholic extract was further active and exhibited greater antibacterial influence alongside all bacteria *sp*. in comparison by way of aqueous extract as well both aqueous and alcoholic extract were concentration independent [27]. All extract (aqueous and methanol extract) of *Urticadioica* display important antibacterial action against isolated pathogenic bacterial strains of *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *E. coli*, *Klebsiela* and *Proteus*. In aqueous extract, by well method *Proteus* was the the majority sensitive at 200mg /ml merely. Although in disc way aqueous extract show an inhibition to *Pseudomonas*, followed by *Proteus*. Ethanol extract via well technique did not showed any inhibition to *Proteus*, whereas through disc method methanol extract of *urticadioica* show an inhibition to *E. coli*, *Proteus*, *Staphylococcus* and *Klebsiela* [28]. Results of a study done by [29] confirm that the fruit extracts of *M. azedarach* displayed an antibacterial effect. *Bacillus* and *E. coli* were the minimum sensitive bacteria. *Pseudomonas*, *Proteus*, *Staphylococcus*, and *Klebsiella* were sensitive to *M. azedarach* fruit aqueous and ethanol extract. No variances were detected among the well and disc diffusion techniques and among ethanol and aqueous extract.

Twenty *P. mirabilis* susceptibility to antibiotics was examined by using Kirby- Bauer technique. The results showed that *P. mirabilis isolates* had altered markers of resistance to a number of groups of antibiotics, it involved tetracycline, clarithromycin, ampicillin, erythromycin, and clindamycin 100%,
cefixime, cloxacillin, vancomycin 95% in spite of its act is commonly on gram positive bacteria [30]. Immediately from previous results, it can be seen that P. mirabilis isolates have temperate to little antibiotic resistance. That prevent protein production, such as aminoglycosides (amikacin, streptomycin, gentamycin, tobramycin and kanamycin), Nalidixic acid, and chloramphenicol, so as antibiotics that prevent nucleic acids production such as rifampicin, whereas wholly isolates displayed resistance to clindamycin, erythromycin, tetracycline and clarithromycin [31]. Proteus sp. (Proteus vulgaris and P. mirabilis) showed a sensitivity to Amp., CTX (otitis media), resistant to CX, AMX, CM, L, E (otitis media) [25], referred to multi resistant to antibiotics showed by local isolated strains of E. coli and Proteus mirabilis [32]. The results showed that the resistance of the P. mirabilis to some antibiotics increases with prescription over a period of years due to the incorrect and indiscriminate use of these antibiotics and the increased incidence of proteus infection. On the further hand, this bacteria had capacity to yield beta – lactamases, specially extended spectrum beta- lactamases (ESBLs), as well as, numeral of alterations occur with these kind of enzymes leading to increase resistance to antibiotic beta- lactam, their capacity to transmission genetic elements carrying the genes of these enzymes, and, in addition to additional machines like variation the target location or variation the access to the target place by alteration of penicillin binding proteins (OBPs) [33]. Inhibitory influence of pomegranate is forever attributed to the phenolic content of the fruit and antioxidant activity that depends mainly on the anthocyanin [32]. The maximum antibacterial action was noted alongside Kl. pneumoniae and amongst fungi great action alongside Aspergillus was noted. [34] Commonly antimicrobial special effects can be attributed to the plant's phytochemicals used in our study. Polyphenols and phenols contain phenolic toxicity to microorganisms that includes inhibition of the enzyme by oxidative mixtures, possibly through reaction through sulphhydryl groups or through more unspecified cross-links with proteins. [35]; Quinones, they afford a basis of a constant free radicals besides are also known to difficult irreversibly throughnucleophilic amino acids in protein [36], Often lead to protein disturbance and function impairment, and may also decrease substrates unavailable to microbes; flavonoids, Flavonoids, and flavonols are known to be formed by plants in reply to microbial infections [37] and establish in vitro to be active antimicrobial substances alongside a varied array of microorganisms. Their action is perhaps due to their capacity to complex with extracellular and soluble proteins and to complex with bacterial cell walls, more lipophilic flavonoids may also interrupt microbial membranes [38]. Common L. camara action is related to the bioactive compounds flavones, triterpenoids, alkaloids, tannin, isocatechins isoflavones, flavonoids, and saponins. [39] have reported that the leaves extract of L. camara be active alongside numerous gram negative and gram positive bacteria. The vital oil of L. camara showed noticeable antibacterial action alongside wholly the bacterial strains tested. Gram negative P. aeruginosa and Kl. pneumoniae were not liable to the vital oil at minor concentration [40].

CONCLUSIONS:

From results we can concluded that very few cases (3/60; 0.05%) of Proteus mirabilis were isolated in pure form and 3 mixed with other bacteria. Ethanol: aqueous extracts of Pomegranate peel and Lantana cammara leaves have an inhibitory effect in sensitivity point of views.
REFERENCES


