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Antibacterial activity of some plant extracts on human pathogenic bacteria

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Abstract

Plants have been used since ancient times in folk medicine, involving all medical traditions. Many plants and plant derived antimicrobial components are used in folklore therapeutics for oral hygiene. Salvia officinalis and Aloe vera extracts were assayed for the evaluation of their antimicrobial activity against two pathogenic bacteria that were identified biochemically and by VITEK2 system. The test of antibiotic susceptibility showed the resistance of the two isolates to at least ten antibiotics used in this study. In vitro investigations of nine Egyptian plant extracts showed that ethanolic Aloe vera and ethanolic Salvia officinalis extracts could exhibit an antibacterial activity against human pathogenic isolates and inhibition zones of 16.333±0.58 and 13.0 ±1.0 mm were observed when Salvia officinalis extract applied against the above-mentioned bacteria, while the inhibition zones of 13.333±0.58 and 11.667±0.58 mm were observed by Aloe vera extract.

Keywords: plant, human pathogenic, bacteria.

1. Introduction

There are continuous problems of increasing morbidity and mortality as well as the cost of health care [13]. The need for new antimicrobials to fight resistant pathogens leads to searching for novel drug targets, though much might be gained from targeting resistance mechanisms themselves [7,20,23]. Currently, researchers have examined plants having an extensive variety of secondary compounds that could be potential sources for various antimicrobial agents [17]. These plants contain native bioactive compounds which are the main sources to obtain natural therapeutic agents [8]. Proteus, a rod-shaped Gram-negative bacteria, belongs to the family of (Enterobacteriaceae). Specific tests include positive urease (which is the phenylalanine deaminase test). On the species level, indole is considered reliable, as it is positive for proteus vulgaris but negative for Proteus mirabilis [22]. The prevalence of MDR PM multi-drug resistance Proteus mirabilis strains has been increased in the last few years. Also, the presence of extended spectrum beta-lactamases (ESBL)-coding genes has been found in these organisms [6]. Klebsiella pneumoniae is a gram-negative rod, non motile, encapsulated, lactose-fermenting, and facultative anaerobic [12]. A wide range of infections is due to the presence of Klebsiella pneumoniae, including pneumonia, urinary tract infections, bacteremia, and liver abscesses. Beside susceptible clinical isolates involved in nosocomial infections, multidrug-resistant (MDR) and hypervirulent (hvKP) strains have evolved separately in distinct clonal groups [14]. K. pneumoniae has virulence factors which include capsule, fimbriae, lipopolysaccharides (LPS), siderophores (enterobactin, aerobactin, salmochelin, yersiniabactin), and efflux [19]. Aloe vera is a green plant with dense, fleshy, tapered, spiny, marginated, and dagger-shaped leaves that develop from a short stalk near ground level [1]. It is the most well-known herbal treatment for boosting the immune system of the body [9]. Aloe vera is used as a skin remedy in herbal medicine. The use of Aloe vera can be traced back to ancient times [8]. Salvia officinalis L. is a popular herbal plant that is grown in many parts of the world, but it is native to the Mediterranean. It is grown in a number of countries, primarily for the purpose of extracting dried leaves for use as a raw material in medicine, perfumery, and the food industry [4]. Dry sage (Salvia officinalis) leaves were used in folk medicine for a variety of disorders [13]. Today, sage is also used as a traditional remedy for many diseases [21].

2. Materials and Methods

Nine medicinal plants were used in this study. Fresh leaves and aerial parts of plants were washed using distilled water and were then dried by air at room temperature away from sunlight for six days. Crushing of the dried medicinal plant materials into powder using grinding machine (Siemens-blender). They were then stored in dry bags at room temperature till extraction.

Aqueous extract preparation: 10 grammes of each dried powdered plant material is soaked in 100 ml distilled water in a sterile conical flask for 48 hours with constant shaking. After that, it was filtered through eight layers of muslin cloth and centrifuged for ten minutes at 5000 rpm. Using a Heidolph VE-11 rotaevaporator, the supernatant was collected and concentrated in a vacuum at temperature below 40°C. (16), then stored in labeled sterile bottles in a freezer at 4 ºC until further use [2].

Ethanolic extract preparation: The plant powder was prepared as described in the previous section. Approximately, 10 grammes of each dried powdered plant material were soaked in 80 percent ethanol in a sterile conical flask for 48 hours with a continuous shaking for hydro-alcoholic extraction. The supernatant was collected and centrifuged at 5000 rpm for 10 minutes after filtration through 8 layers of muslin cloth

and was then concentrated in a vacuum below 40 ºC using Heidolph VE-11 rotaevaporator to make the final volume half of the original volume (stock solutions) [16], and was then stored in labeled sterile bottles in a freezer at 4 ºC until further use [2].

Tested microorganisms: Two bacterial isolates Proteus mirabilis and Klebsiella pneumoniae were isolated from pus and sputum specimens obtained from Microbiology and Immunology Department, Benha University Hospital. These isolates were purified by growing on nutrient agar medium identified depending on cultural, morphological and biochemical analysis.

Antibiotic susceptibility testing

The antibiotics used for testing the sensitivity of the isolated strains using disk diffusion method were Amikacin (AK) (30µg), cefoxitin(fox) (30µg), ceftizidime (CAZ) (30µg), ceftriaxone (CRO) (30µg), Tobramycin (TOB) (10µg), Norfloxacin (NOR) (10µg), levofloxacin (LEV) (5µg), Ampicillin/sulbactam (SAM) (30µg), cefuroxime (CXM) (30µg), trimethoprim+sulfamethoxazole (SXT) (25µg), Azetronem (ATM) (30µg), cephalexin (CL) (10µg), Cefaclor (CEC) (10µg), cefoperazone (CEP) (30µg) [5].

Plant extracts activity assay using well diffusion method:

One ml from an inoculum suspension was placed in each sterile petridish, then about 20 ml from autoclaved nutrient agar were added and left to solidify. Sterile metallic poorer was used 6 mm diameter holes were drilled in the seeded agar. Every well on the seeded medium received a 50 ml aliquot of crude extract from each plant. The plates were incubated for 24 hours at 37 degrees Celsius, and the inhibition zones were measured in millimetres (mm) [8].

Statistical Analysis:

One-way ANOVA was carried out using the statistical analysis system (SAS/STAT ® 9.1) according to the software procedure's guide (SAS,2004)

Citation: SAS Institute Inc.2004.SAS/STAT®9.1 User's Guide. Cary, NC:SAS Institute Inc**.**

3. Results and discussion

Two bacterial isolates (4010S; 210P) were isolated from sputum, and pus specimens were obtained from Microbiology and Immunology Department, Benha University Hospital. These isolates were purified by growing on nutrient agar medium. Isolate No (4010S) showed Large, greyish and highly mucoid colonies, gram negative rods and non-motile. Isolate No (210P) gave out an odour described as fishy, non-lactose fermenting colonies, gram negative rods and motile.

Antibiotic susceptibility testing

Qualitative results from the antibiograms (Table1) showed that the two bacterial isolates were resistant to at least ten antibiotics like Cefoxitin, Ceftizidime, Ceftriaxone, Cephalexin, Azetronem, Norfloxacin, Levofloxacin, Trimethoprim+ Sulfamethoxazole, Ampicillin/Sulbactam, and Cefuroxime. Yet, the two isolates were susceptible to Amikacin and Cefoperazone.. The current problem of widespread antibiotic resistance, especially the threat posed by difficult-to-treat multidrug resistant species, is the result of decades of often ineffective antimicrobial use [13]. Increased morbidity and mortality, as well as the cost of health care, are both issues that come with it [13]. The current emphasis on discovering novel drug targets is driven by the need for new antimicrobials to fight multiple-resistant pathogens, though more could be gained by targeting resistance mechanisms themselves [7, 20, 23]. As a result, there is a growing interest in the discovery of new antimicrobial agents for the treatment of bacterial infections. Amikacin was the most important antibiotic for clinical bacterial isolates (10). Aminoglycosides (AK and CN) are the only bactericidal ribosome-targeting antibiotics. This is due to their mechanism of action in causing mRNA mis-reading during translation [3].

Antibiotic rotation and restricted use of Ceftazidime and Ciprofloxacin caused a decrease in the number of cases of VAP associated with resistant gram-negative bacilli and a rise in the number of meticillin-sensitive S. aureus [11].

Antibacterial activity of some plant extracts against the human pathogenic bacteria.

The antimicrobial activity of nine medicinal plant extracts was examined against the isolated bacteria, with the findings summarised in Table 2. Only Aloe vera and Salvia officinalis extracts were successful against the human pathogenic bacteria.

Table (1) Antibiotic susceptibility pattern of bacterial isolates against fourteen antibiotics**.**

Bacteria	Tob	Ak	CRo	Cip	Cxm	Caz	Sam	Sxt	Lev	Nor	Atm	Cl	CeP	Fox
Klebsiella pneumonia4010s	I	S	R	I	R	R	R	R	R	R	R	R	R	R
Proteus mirabilis 210p	S	S	R	I	R	R	R	R	R	R	R	R	R	R

Denotes for Resistant (R), Intermediate (I) and Susceptible (S).

Amikacin (AK 30 µg), Cefoxitin (fox 30 µg), Ceftizidime (CAZ 30 µg), Ceftriaxone (CRO 30 µg), Tobramycin (TOB10 µg), Norfloxacin (NOR10 µg), Levofloxacin (LEV10 µg), Ampicillin/ Sulbactam (SAM 30 µg), Cefuroxime (CXM 30 µg), Trimethoprim+ Sulfamethoxazole (SXT 25 µg), Azetronem (ATM 30 µg), Cephalexin (CL10 µg), Cefoperazone (CEP 30 µg).

Table (2) Antibacterial activity of medicinal plant extracts against pathogenic bacteria Klebsiella pneumoniae (4010S). Each value is the mean of three readings (mm) ± standard deviation(SD).

Plant extract	Mean diameter of inhibition zone(mm),original diameter (5mm)
	Klebsiella pneumoniae (4010S)
Commiphora myrrha	R
Aloe vera	13.333±0.58^{e}
Salvia officinalis	16.333±0.58 ^{c}
Moringa oleifera (leaf)	R
Artemisia	R
Senegalia senegal	R
Cinnamomum camphora	R
Matricaria chamomilla	R
Piper cubeba	R

*Means having different letters in each column are significantly different (P<0.05)

Table (3) Antibacterial activity of medicinal plant extracts against pathogenic bacteria *Proteus mirabilis (210P)*. Each value is the mean of three readings (mm) ± standard deviation(SD).

Plant extract	Mean diameter of inhibition zone(mm),original diameter (5mm)
	Proteus mirabilis (210P)
Commiphora myrrha	R
Aloe vera	11.667±0.58^{e}
Salvia officinalis	13.0 ±1.0 ^{e}
Moringa oleifera (leaf)	R
Artemisia	R
Senegalia senegal	R
Cinnamomum camphora	R
Matricaria chamomilla	R
Piper cubeba	R

*Means having different letters in each column are significantly different (P<0.05).

4. Conclusion

This study describes the isolation of four human pathogenic bacteria; Klebsiella pneumoniae 4010S, Proteus mirabilis 210P from Egypt. When the antibacterial properties of certain medicinal plant extracts were tested against isolated bacteria, it was discovered that extracts of Salvia officinalis and Aloe vera were rich sources of essential compounds with numerous therapeutic applications. Both are considered viable alternatives to antibiotics in the treatment of nosocomial pathogenic bacteria. Currently, we're looking into the efficacy of specific types of nanoparticles and phage therapy as new approaches to treat human pathogenic bacteria.

Reference

[1] A.O.Adesuyi, O.A.Awosanya , and F.B.Adaramola. Nutritional and phytochemical screening of A. barbedensis. Curr. Res. J. Biol. Sci.vol.4(1),pp.4–9,2011.

[2] K.R.Aneja, R.Joshi, and C.Sharma. Antimicrobial efficacy of fruit extracts of two Piper species against selected bacterial and oral fungal pathogens. Braz J Oral Sci.vol. (4)9,pp.p 422-426,2009.

[3] N.M.Atef, S.M.Shanab, and S.I.Negm. Evaluation of antimicrobial activity of some plant extracts against antibiotic susceptible and resistant bacterial strains causingwound infection. Bulletin of the National Research Centre.vol. 43,pp.144. https://doi.org/10.1186/s42269-019-0184-9,2019.

[4] J.Bruneton, "Pharmacognosy, Phytochemistry, Medicinal Plants". Second ed., Lavoisier Publishing, Paris.vol.8,pp.540/544 ,1999.

[5] Clinical and Laboratory Standards Institute (CLSI); formerly NCCLS) Performance standards for antimicrobial susceptibility testing seventeen informational supplement.vol.27. (1), pp.26. 3 ,(2007.

[6] K.Cohen-Nahum, L.saidel-odes, and K. Riesenberg. Urinary Tract Infections Caused by Multi-Drug Resistant Proteus mirabilis: Risk Factors and Clinical Outcomes. Infection.vol.38,pp.41–46,2010.

[7] K.Coleman, M.Athalye, and A.Clancey. Bacterial resistance mechanisms as therapeutic targets. J. Antimicrob. Chemo- ther.vol.33,pp. 1091–1116,1994.

[8] M.M.Cowan. Plant products as antimicrobial agents. Clinical microbiology reviews.vol.12,pp.564-582,1999.

[9] Davis, H. R..(1997) Aloe vera: A scientific approach published by vantage press, New York. 3 – 5.

[10] Y.A.El-Zawahry, F.M.Reda, and W.M.Azazy. Synergistic effect of combination treatment by

certain plant extracts and some antibiotics on the resistance of pathogenic bacteria to some common antibiotics. Life Science Journal.vol.10(4),pp. 3477- 3489,2013.

[11] D.Gruson , G.Hilbert , and F.Vargas. Impact of colony-stimulating factor therapy on clinical outcome and frequency rate of nosocomial infections in intensive care unit neutropenic patients. Crit Care Med .vol. 28,pp. 3155–60,2000.

[12] Y.Guo, Z. Cen, and X.Fang. Whole-genome sequence of Klebsiella pneumonia strain LCT-KP214. J Bacteriol.vol. 194(12),pp.3281. doi: 10.1128/JB.00531-12,2012.

[13] W.C.Hellinger. Confronting the problem of increasing antibiotic resistance. South. Med.vol. J. 93,pp.842–848,2000.

[14] C.Hennequin, and F.Robin. Correlation between antimicrobial resistance and virulence in Klebsiella pneumonia. European J of Clinical Microbiology & Infectious Diseases.vol.35,pp.333–341,2016.

[15] M.Keshavarz, A.Mostafaie, and K.Mansouri. In vitro and ex vivo antiangiogenic activity of Salvia officinalis. Phy- tother Res.vol.24,pp.1526-1531,2010.

[16] P.D.Lokhande, KR.Gawai, and K.M.Kodam. Antibacterial activity of extracts of Piper longum. J Pharmacol Toxicol.vol. 2,pp.574-9,2007.

[17] Y.A.Mahmoud, M.EBRAHIM, and M.M.Aly. Influence of some plant extracts and microbioagents on some physiological traits of faba bean infected with Botrytis fabae. Turkish J of Botany.vol.28,pp.519-528 ,2004.

[18] V.Navarro, M.L.Villarreal, and G.Rojas. Antimicrobial evaluation of some plants used in Mexican traditional medicine for the treatment of infectious diseases. Journal of ethnopharmacology.vol. 53(3),pp.143-147,1996.

[19] R.Podschun, and U.Ullmann. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev .vol.11,pp.589–603. ,1998.

[20] T. E.Renau, S.J. Hecker, and V. J.Lee. Antimicrobial potentiation approaches: targets and inhibitors. Annu. Rep. Med. Chem.vol.33,pp.121–130,1998.

[21] M.R.Rodrigues, L.K.Kanazawa, and das T.L.Neves. Antinociceptive and anti-inflammatory potential of extract and isolated compounds from the leaves of Salvia officinalis in mice. J Ethnopharmacol .vol.139,pp.519-526,2012.

[22] k. Struble. Proteus Infections:Overview Medicine.vol.8,pp.7 ,2009.

[23] G.D.Wright. Resisting resistance: new chemical strategies for battling superbugs. Chem. Biol. 7: R127–R132 wwwcdcgov/ pulsenet/pathogens / shigellahtml. Accessed November .vol.22,pp. 2014 ,2000.