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ANTIBACTERIAL ACTIVITY OF *PHYLLANTUS EMBLICA, CORIANDRUM SATIVUM, CULINARIS MEDIC, LAWSONIA ALBA* AND *CUCUMIS SATIVUS*

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Abstract: Present study deals with the demonstration of the antibacterial activity of very common medicinal plants of Pakistani origin i.e., *Phyllantus emblica, Coriandrum sativum, Culinaris medic, Lawsonia alba* and *Cucumis sativus*. The extracts were prepared in crude form by the use of hydro-alcoholic solution and were screened for antibacterial activity against various bacterial species by disk diffusion method. Assay was performed using clinical isolates of *B. cereus, S. aureus, P. aeruginosa* and *E. coli*. Crude extract of *Phyllantus emblica* fruit exhibited strong activity against standard cultures of all studied bacteria. *Lawsonia alba* showed good activity against standard cultures of all the used microorganisms. *Coriandrum sativum* was effective only against *Bacillus cereus*, while *Cucumis sativus* and *Culinaris medic* showed poor activity against *Pseudomonas aeruginosa* only. Hence, *Phyllantus emblica* exhibited strong antibacterial activity against a wide range of bacteria it means that *Phyllantus emblica* extract contains some compounds which have broad spectrum of bactericidal activity.

Keywords: antibatcerial activity, disk diffusion approach, *Phyllantus emblica, Coriandrum sativum, Culinaris medic, Lawsonia alba, Cucumis sativus*

Medicinal herbs possess chemical constituents that have biological activity like volatile oils, coumarins and alkaloids etc. These plants also include additional chemicals, which may work to check unwanted side-effects of the major biologically active compounds or to help in the assimilation of the major compounds. Poppy exudate, for instance, includes chemicals besides morphine, which render the subject to fewer side effects as compared to morphine administered alone. Saponins, which are found in extracts from the leaf of *Convallaria majalis*, help the cardio-active heterosides in penetration to the blood more readily. There is an interest to use medicinal herbs for therapeutic purposes (1) owing to greater knowledge of the limited capability of synthetic pharmaceuticals for the treatment of main diseases as well as the necessity for the discovery of novel chemicals from the plant kingdom. Medicinal plants are considered to be the fundamental source of comprehension regarding modern medicines. Rich natural sources provide fundamental molecular and biologically active structures for the purpose of synthesis. This rapidly increasing global attention in herbal medicines validates many conventional claims concerning the worth of natural products in health care (2). The comparatively lesser occurrence of adverse events related to herbal preparations as compared to present conventional pharmaceutical products, along with their low expenditure, is encouraging equally the consumers and public health care institutes to reflect on herbal preparations as alternative source to synthetic drugs (3). *Phyllantus emblica* belongs to family Euphorbiaceae. All parts of the plant are used, thus presenting the herbalist with tremendous worth for money. The ripen fruit is commonly used in fresh form, but dried fruit is also used. This fruit is considered a very rich source of vitamin C as mentioned in a majority of references if not nearly in all; this is perhaps not the case (4). Minerals and vitamins found in this fruit include Ca^{2+}, P, Fe^{2+}, carotene, vitamin B_1, vitamin B_2, and niacin. The seeds of the

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Indian gooseberry possess a fixed oil, which is known as phosphatides, and an essential oil. The leaves, bark and fruit of this tree possess an appreciable amount of tannins. The roots possess ellagic acid and lupeol and bark contains leucodelphinidin. The seeds yield 16% brownish-yellow color fixed oil. It also contains some fatty acids namely; linolenic acid (8.8%), linoleic acid (44.0%), oleic acid (28.4%), stearic acid (2.15%), palmitic acid (3%) and myristic acid (1%) (4). *Coriandrum sativum* belongs to Umbelliferae family. Dried whole coriander seeds are used for medicinal purpose.

The seeds of this plant contain: approximately 2–2.6% essential oil which in turn consist of up to 55-74% of linalool, while the remaining portion includes other monoterpenes (5). *Lens culinaris* Medik. belongs to Fabaceae family. Its principal constituent is lentil which is considered for having high level of protein content (approximately 30%). The main protein present, is globulin. Concentration of protein of lentils reportedly is in the range of 22–34.6%, and 100 gram of dried seeds contain 340–346 calories, 12% moisture, 20.2 gram protein, 0.6 gram fats, 65 gram total carbohydrates, about 4 gram fibers, 2.1 gram ash, 68 mg Ca^{2+}, 325 mg P, 7.0 mg Fe^{2+}, 29 mg Na^{+}, 780 mg K^{+}, 0.46 mg vitamin B_1, 0.33 mg vitamin B_2 and 1.3 mg niacin (6). *Lawsonia alba* belongs to Lythraceae family. A brown substance has been found in it, which is of a resinoid fracture, having the characteristic chemical properties of tannins, and hence named as hennotannic acid. Henna also contains mannite, mucilage, gallic acid, and naphthoquinone (7). *Cucumis sativus* belongs to Cucurbitaceae family. Its fruit contains D-glucose (0.11–0.98%), saccharose (0.05–0.13%), fixed oil (0.11–90.98%). Seed contains 25% fixed oil (known as Gurken oil), which consists of oleic acid (58%), linolic acid (3.7%) phytine and lecithin. The leaves of this plant contain urea and alkaloid that is known as hyposanthine (8). In this research work, a total of 5 medicinal plants, namely *Phyllantus emblica, Coriandrum sativum, Lens culinaris* Medik., *Lawsonia alba and Cucumis sativus* were selected and were undergone screening for their capability of having potential antibacterial activity against standard cultures and clinical isolates as well as to compare the activity of these crude extracts with antibiotic available.

MATERIALS AND METHODS

Plant materials

The plant material used during present study include fruit of *Phyllantus emblica*, leaves of *Lawsonia alba*, seeds of *Cucumis sativus*, fruit of *Coriandrum sativum* and seeds of *Lens culinaris* Medik., which were purchased from the local market and identified.

Medias

The media used for assay included nutrient agar (pH = 7) containing peptone, 5.0 gram NaCl, meat 3.0 gram extract, agar (18 g) and distilled water (1 L). To rehydrate the medium, 36 g of dehydrated medium was dissolved in 1000 mL of cold distilled water. The medium was dissolved completely by heating and pH was adjusted to 7 and sterilized in autoclave for a period of 15 min at pressure 15 psi and temperature 121°C. In order to maintain the bacterial cultures, nutrient agar slants were used. Nutrients agar was prepared according to the same formula. After streaking the culture on nutrient agar

Table 1. Zone of inhibition using standard culture test

Name of the plant	Zone of inhibition (mm)			
	B. cereus	*S. aureus*	*E. coli*	*P. aeruginosa*
Phyllantus emblica	19.66 ± 0.43	26.66 ± 0.72	32 ± 0.51	34.33 ± 0.23
Coriandrum sativum	9.33 ± 0.27	-ve	-ve	Poor growth, no zone of inhibition
Cucumis sativus	-ve	-ve	-ve	Poor growth, no zone of inhibition
Lawsonia alba	16.33 ± 0.29	6.66 ± 0.08	9.33 ± 0.13	18.66 ± 0.25
Lens culinaris	-ve	-ve	-ve	Poor growth, no zone of inhibition

"-ve" = no activity

Table 2. Zone of inhibition of plant extract studied on different clinical isolates

Name of the plant	Zone of inhibition (mm)			
	Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
Phyllantus emblica	22.03 ± 0.10	20.31 ± 0.43	23.68 ± 0.36	20.39 ± 0.17
Corianderum sativum	-ve	-ve	-ve	-ve
Cucumis sativus	-ve	-ve	-ve	-ve
Lawsonia alba	7.10 ± 0.03	7.10 ± 0.03	7.10 ± 0.03	8.67 ± 0.52
Lens culinaris	-ve	-ve	-ve	-ve

"-ve" = no activity

slant, the slants were kept in incubator, maintained at 37°C for overnight and then the slants were transferred to refrigerator on next day.

Preparation of crude extracts

For the purpose of carrying out screening of a given plant material for demonstrating their antibacterial activity, first step is to prepare the crude extract of the plant material. Extraction is treatment of plant or animal tissues with solvent whereby medicinally active principles are dissolved leaving the inert matter undisolved. Presently, we used 50% ethanol/water for preparing our crude extract of plants/herbs. The air dried material was grinded mechanically to powdered form. The ground material was drenched in 50% aqeous solution of ethanol and kept at room temperature for 5–8 days. Then, the extract was subjected to filtration using Whatman filter paper (no. 1) and the resultant filtrate was concentrated in a rotary evaporator, which resulted in the formation of a gummy material. This gummy material was dissolved in a small quantity of distilled water and was employed for the assessment of antibacterial activity (9).

Prepation of fresh culture

At the time of antibacterial assay, fresh culture was made from the stock culture. For this purpose, a loop fully contaminated with culture from nutrient agar slant was inoculated into 5 mL of sterile nutrient broth. The seed broths were inoculated at 37°C ± 1°C for 24 h, then it was compared with 0.5 McFarland tube and if turbidity was exceeded, certain amount of fresh broth was added.

Antibacterial assay

For the assessment of antibacterial activity of crude extracts against a given bacterial culture, the antibacterial assay was performed as follows: (i) Nutrient agar was prepared according to the formula and poured into Petri plates (10 × 10 cm); (ii) after solidification, plates were kept in incubator at 37°C for 24 h to check sterility; (iii) each plate was tagged with the name of various bacterial cultures; (iv) using a sterile cotton swab, a lawn was made on nutrient agar from fresh bacterial culture followed by marking all the plates with the name of respective bacterial culture; (v) all the plates were subjected to drying at 37°C for 30 min with lids partly opened then, the wells were engraved in the inoculums, using a sterile cork borer; (vi) the wells were filled with crude extract of plant material to be tested, after marking them for identification purpose; (vii) all the plates were subjected to incubation at 37°C for a period of 24 h; (viii) on the following day, the zones of inhibition were determined to the nearest "mm" after taking the average of three readings and results are recorded, and (ix) finally, each test was run in duplicate (10).

Statistics

The significance of difference between various parameters was done using SPSS version 15.0, setting the level of significance at 0.05.

RESULTS AND DISCUSSION

In this research work, some selected indigenous medicinal plants namely, *Phyllantus emblica*

Table 3. Zone of inhibition using discs of different antibiotics.

Antibiotics	Zone of inhibition (mm)			
	Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
Piperacillin/ Tazobactum (10 : 01)	10.41 ± 0.23	-ve	11.41 ± 0.13	10.61 ± 0.40
Vancomycin 30 µg	12.53 ± 0.41	16.63 ± 0.26	-ve	14.36 ± 0.53
Cefoxitin 30 µg	-ve	-ve	-ve	-ve
Cefizox 30 µg	-ve	-ve	-ve	-ve
Peflacine 5 µg	14.27 ± 0.19	-ve	5.59 ± 0.09	17.78 ± 0.71

"-ve" = no activity

(AMLA), *Coriandrum sativum* (CORIANDER), *Lens culinaris* Medik. (LANTIL/DHAL), *Lawsonia alba* (HENNA), *Cucumis sativus* (CUCUMBER) were assessed for their potential antibacterial activity, as these plants are known for centuries in the folk medicines for having therapeutic values. The extracts were prepared in crude form by the use of hydro-alcoholic solution (9) and the antibacterial activity was assessed for various pathogenic as well as non-pathogenic bacteria (10). Diffusion method (well method) was employed for assay performance, in which crude extracts were poured in the wells which allowed to diffuse into the solidified agar having bacterial lawn and results were noted after 24 h of incubation at a temperature of 37°C. The antibacterial activity was determined by assessing the zones of inhibition around the wells i.e., the place where growth of bacteria was inhibited due to the bactericidal activity of the plant extracts (11).

Table 1 indicates that all the five medicinal plants were used to determine antibacterial activity against standard culture of *B. cereus, S. aureus, E. coli, and P. aeruginosa*. *Phyllantus emblica* have shown excellent antibacterial activity and *Lawsonia alba* have also shown good activity while remaining three plant material were not very effective. Table 2 indicates that all the five medicinal plants were used to determine antibacterial activity against clinical isolates of of *B. cereus, S. aureus, E. coli, and P. aeruginosa*. *Phyllantus emblica* and *Lawsonia alba* showed significantly higher ($p < 0.05$) antibacterial activity against all bacteria, while the remaining three plants materials were not significantly ($p > 0.05$) effective. Table 3 shows that five different available antibiotics were used to compare the antibacterial activity against standard cultures of *B. cereus, S. aureus, E. coli* and *P. aeruginosa*. Piperacillin/Tazobactum (10 : 01) have shown good activity against all except *Staphylococcus aureus*. Peflacine 5 µg have shown good activity against *B. mirabilis*, *P. aerigonosa* and *E. coli*, while the remaining three antibiotics i.e., vancomycin 30 µg, cefoxitin 30 µg and cefizox 30 µg were uneffective. Among the baterial species used for antibacterial assay, *Bacillus mirabilis* is Gram positive, spore forming, non pathogenic rod shaped organism. *Staphylococcus aureus* is Gram positive cocci which live in bunch forms, cause abscesses, various pathogenic infections such as endocarditis and osteomyelitis, food poisoning and toxic shock syndrome (12).

Among Gram negative bacterial species used in this assay are *Escherichia coli* and *Pseudomonas aeuruginosa*. *E. coli* is the most common cause of UTI's and sepsis. It is also associated with neonatal meningitis and traveler's diarrhea. *Pseudomonas aeruginosa* causes sepsis and UTI's. *Salmonella typhi* is facultative anaerobic, Gram negative bacteria, rods. It is most common cause of typhoid fever, also causes bacteremia, abdominal pain and hepatomegaly (13).

Plants having the capability for potential antimicrobial activity should be screened against a suitable microbial model for the confirmation of antimicrobial activity and ascertaining the related parameters. Various plant extracts have been

screened for their antibacterial activity by many researchers in various countries (10, 13). Much more research has been carried out on ethnomedicinal plants in India. Researchers are taking more and more interest in a lot of traditional natural products. They have suggested that aqueous as well as alcoholic extracts from medicinal plants used in allopathic medicines are potential sources of antiviral, anticancer and antimicrobial agents (9). For the assessment of antimicrobial activity, crude plant extracts are more successful in initial steps than these of pure isolated compounds from natural products.

CONCLUSION

Phyllantus emblica exhibited strong antibacterial activity against a wide range of bacteria what means that it contains compounds which have broad spectrum of bactericidal activity.

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