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Antimicrobial activities and mineral profile of selected wild plant *Linum usitatissimum* in Khyber Pakhtunkhwa, Pakistan

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Abstract

The current study was conducted to investigate the possible potential of Linum usitatissimum in the removal of various human ailments and its mineral profile, as widely distributed in different areas of Khyber Pakhtunkhwa, Pakistan. The preliminary analytical investigation is to confirm the antimicrobial significance of L. usitatissimum, due to the presence of certain bioactive compounds. It was observed that L. usitatissimum exhibit broad spectrum activity against pathogenic bacterial strains were Agrobacterium tumefaciens sp., Bacillus subtilis sp., Clavibacterm ichiganensis sp., Escherichia coli sp., Staphylococcus aureus sp. and Xanthomonas campestris sp. While fungal strains including, viz: Aspergillus flavus sp., Aspergillus niger sp., Trichoderma harzianum sp.,Fusarium oxysporum sp., and Paecilomyces sp. Among all the bacterial strains highest inhibitory zone was recorded against B. subtillis (60.71%) followed by S. aureus (48.14%) and E. coli (44.0%) whereas no activity was observed against A. tumefaciens, C. michiganensis and X. compestris. Among all the fungal isolates highest inhibitory zone was observed against T. harzianum (76.0%) as compared to F. oxysporum (65.21%) and A. niger (57.89%) while A. flavus and Paecilomyces sp., showed complete resistance. The results showed that elemental composition of L. usitatissimum contain significant amount of carbon (63.39%) followed by oxygen (33.90%), silicon (0.45%), potassium (0.44%), calcium (0.36%), sodium (0.30%), magnesium (0.25%), sulfur (0.24%), phosphorus (0.21%), chlorine (1.9%), iron (0.18%) and aluminum (0.15%). It was concluded from the results the said plant exhibits promising antimicrobial activity and possess sufficient amount of minerals therefore it should be exploited in traditional health care medicine for the treatment of various ailments.

Keywords: Antimicrobial, Mineral profile, *Linum usitatissimum*, Swat, Human ailments

Introduction

Plants are the lungs of environment and play important role in human well-being. Plants display various natural exercises because of the occurrence of secondary metabolites (Chowdhury et al., 2008) that are in charge of its conventional use in medicine (Mikail, 2010). World Health Organization projected that 80% of the total populace rely on regular medicines for meetings their essential social insurance needs which means two third of the world's (6.1 billion) individuals depend on the healing power of plant based materials for some reason-accessibility, moderateness, security or their faith in conventional cures. Medical profit of food such as; wild edible plants, coniferous forest plants, wild strawberry plants, wild mushrooms and edible weeds etc, have been explored for thousands of years. Numerous nations of the world have conventional medicine as a wellspring of emergency treatment. The presence and utilization of plants to treat human sicknesses, is as old as man (Sangwan et al., 2010). Therapeutic plants are good wellsprings of antimicrobial medications (Bakht et al., 2011).

Common name of *L. ustitatissimum is* Flaxseed and its botanical classification is; *Class Magnoliopsida – Dicotyledons*, belongs to *family Linaceae, Genus*: *Linum L., Species: Linum usitatissimum L.* flaxseed, linseed, Alsi or teesi (Punjabi, Gujarati and Hindi). Major flax producing countries are Russia, Poland, France, Spain, Greece, Italy, Croatia, Egypt, Syria and Lebanon while it is also a common plant in India, Pakistan, Bangladesh and Srilanka (Vetrichelvan et al., 2000). Cultivated Flax plants grow to 1.2m (3ft 11in) tall, with slim stems. The leaves are glaucous green, slim lancelate, 20-40 mm long and 3mm wide. The flowers are immaculate light blue, 15–25mm distance across, with five petals. The natural product is round, dry case 5-9mm distance across, containing a few gleaming cocoa seeds molded like an apple, 4–7mm long. Develop flaxseed is elliptical and straightened, including an incipient organism with two cotyledons encompassed by a

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thin endosperm and a smooth, frequently shiny yellow to dark brown seed coat (Narender *et al*., 2016).

Flax plant is cultivating and its all parts are using for several disease treatment. The seeds for sustenance and the generation of linseed oil (another name for flax and is a decent preserver of wood and also good for our bodies) and the stems for instance can be utilized to create material fiber for garments and different items. The oil industry (petroleum) removed some of the needs of flax and so its production took a decline especially after the II World War. Commonly cultivated flax plants are named *Linum usitatissimum.* Flaxseed oil is a good source of the omega-3 unsaturated fatty linolenic acid with typical levels of 55% in the oil, making it perfect for paints, varnishes, and inks because of its quick polymerization properties. The average production of flaxseed worldwide between 2007 and 2011 was 1,862,449 tons (FAOSTAT, 2013).

It contains physiologically essential compounds, i.e. lignin, unsaturated fatty acids, flavonoids, saponins, tannins, retinol, Beta-carotene, vitamin B and some essential minerals like magnesium and manganese. Flax lignin is very effective against microbes like bacteria and fungi (Hall *et al*., 2011). Increasing interest in edible oil sources with noteworthy rates of omega-3 unsaturated fats is bringing about utilization of flaxseed as useful nourishment. Flaxseed is additionally added to animal feed to enhance animal regenerative execution and health (Heimbach, 2009). Entire flaxseed is broadly acknowledged as solid nourishment that has anticancer action. Controlled exploratory eating methodologies have exhibited various helpful impacts of Flaxseed utilization (Clark *et al*., 1995). Dietary flaxseed flour diminishes epithelial cell expansion and atomic variations in female rodent mammary organs. This discovering demonstrates that flaxseed may diminish the advancement rate of mammary development (Serraino and Thompson, 1991).Also, it has been found that flaxseed lignin decreases mammary tumor advancement in the later periods of cancer-causing agents. It has been shown that flax serves as purgative, gastric and disinfectant. It is also helpful in relieving bone disorder and intestinal problem has additionally been related to changes in blood lipids.

Flaxseeds (powder extract) were effective in inhibiting bacterial growth and fungal growth. This is aimed to assess the action of Flaxseeds powder remove against the microscopic organisms and parasites of clinical pertinence: *Bacillus subtilis, Agrobacterium tumefaciens, Staphylococcus aureus, Escherichia coli, Clavibacterm ichiganensis, Xanthomonas compestris* is known to be reasons for anti-toxin resistant. *Aspergillus flavus, Aspergillus niger, Trichoderma harzianum, Paecilomyces species* and *Fusarium oxysporum,* known to be reasons for antibiotic-resistant infections. The agar diffusion method was performed and indicated the bacteriostatic or bactericidal action and fungi static or fungicidal action. It is proposed that concentrates got from Flax seeds may be the viable wellspring of antibacterial mixes and the promising other option to anti-microbial treatment.

Khyber Pakhtunkhwa-Pakistan is pleased with a great deal of wild plants. As per recent estimates, around 6000 plants are by and large created in various parts of Pakistan. The greater part of them is open in Khyber Pakhtunkhwa and bordering areas. Medicinal plants are growing bundle in the wild in Swat, Malakand and Chitral. Area Swat contains around 1550 taxa of blossoming plants and 55 Pteridophytes. Data gathered would be valuable for the preservation of wild therapeutic plants of various zones (Nimri *et al*., 1999).

A lot of research work has been done on the usage, properties, characteristics and sources of above plant species, especially total phenolic compounds, vitamins, flavonoids and other micronutrients. This has led to a common use, this plant as medicine. Finally, it is concluded that *L. usitatissimum* can be used as a medicinal plant. The current study is designed with the objective (1) to analyze the antimicrobial activities (both antibacterial and antifungal activities) of *L. usitatissimum*of clinical relevance and (2) to identify the mineral profile of *L.usitatissimum* (Figure 01)

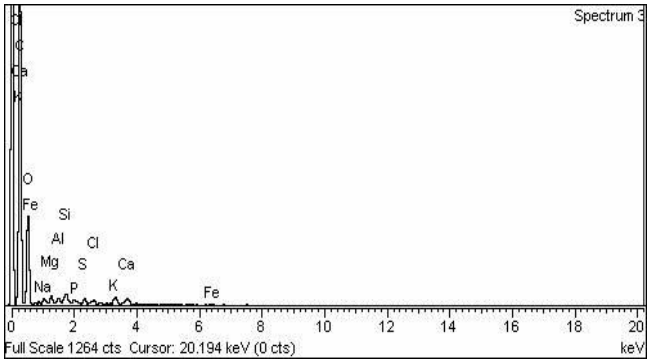

Figure 1: Mineral Profile of *L.usitatissimum*

Materials and Methods

Study area Swat

The valley of Swat is arranged in the remote Hindu-Kush Mountains of Pakistan. The valley of Swat is a champion among the most pleasant spots of sub-territory and is known as the Switzerland of the sub-landmass (Qasim*et al*., 2011). Swat is arranged in the northwest corner of Pakistan. It lies from 34° 34' to 35° 55' north

latitudes and 72° 08' to 72° 50' east longitudes. It is limited by Chitral and Ghizer on the north, Indus Kohistan and Shangla on the east, Buner and Malakand secured zone on the south and Dir on the west. The total domain of the Swat is 5337 Km^2 and masses of around 1.3 million. Swat is a bit of the Malakand Division (Khan *et al*., 2013).

In the present study, it is aimed to investigate the antimicrobial potential and the mineral profile of important wild plant *L. usitatissimum* used against various common diseases. The antimicrobial investigation was evaluated in the department of Agricultural Chemistry, the University of Agriculture Peshawar while the mineral profile was carried out at Centralized Resource Laboratory (CRL), Department of Physics, University of Peshawar, using Energy Depressive X-Rays spectrometer (EDX).

Collection of plant sample

The *Linum usitatissimum* specie was collected at its mature stage from the Swat area. The wild plant was identified by eminent plant taxonomists and voucher specimen was deposited in the Herbarium at university of Peshawar.

Selection of bacterial and fungal strains

The test bacteria used were *Agrobacterium tumefaciens, Bacillussubtilis, Clavibacterm ichiganensis, Escherichia coli, Staphylococcu saureus, Xanthomonas campestris*. Likewise the plant extract was also tested against pathogenic fungal strains viz: *Aspergillus flavus, Aspergillus niger, Trichoderma harzianum sp, Fusarium oxysporum sp and Paecilomyces* species, respectively.

Preparation of plant extract

The seeds of *Linum usitatissimum* were dried and washed at room temperature and afterward pounded into coarse powder utilizing a processor. The powder test sample (100g) was then dipped in methanol (200mL) for a few days. The methanol extract was dried on rotatory evaporator. The dried crude methanol extract was then kept in vials and stored at 4 ^{o}C for further analysis (Hallund *et al*., 2006).

Media used

Nutrient agar (2.8 g/100ml) and nutrient broth (1.3 g/100ml) was used for bacterial culture and Potato Dextrose Agar (2.0 g/100ml technical agar, 2.0 g/100 ml dextrose, potato 20.0 g/100ml) and Potato Dextrose broth (2.0 g/100 ml dextrose, potato 20.0 g/100ml) was used for fugal standardization of fungal cultures. The bacterial and fungal cultures were inoculated and kept at 37°C and 28°C overnight in shaker incubator at 150rpm.

Preparation of bacterial and fungal suspension

Overnight microbial cultures nutrient broth and potato dextrose broth of selected microbial isolates were mixed with physiological saline and turbidity was corrected by adding sterile saline until a McFarland 0.5 $BaSO_4$ turbidity standard 10^8 Colony Forming Unit (CFU) per ml was achieved. These inocula were used for seeding nutrient agar (Baker*et al*., 1983).

Well diffusion method

Antimicrobial vulnerability testing was done using the well spread technique to perceive the proximity of antagonistic to bacterial or against parasitic activities of the plant tests (Perez *et al*.,1990). The bacterial and fungal cultures (50µL) were seeded with the help of micropipette over the suitable medium as expressed already. The plates were allowed to dry for 15 minutes before use in the test. Wells were then made and a pipette was utilized to place 30 µl of the unrefined concentrate of the said plant was connected into each well. A similar concentrate was utilized on each plate; with a sum of two plates utilized for each concentrate including two wells for the positive and negative control. Ciprofloxacin was used as positive control against bacterial strains while Itraconazole and Fluconazole were exploited against fungal cultures. The pure dimethyl sulfoxide (DMSO) was utilized as negative control for both bacterial and fungal contagious strains individually. The bacterial plates were incubated at the 37 ^{o}C for 24hours whereas fungal plates were incubated at 28 ^{o}C for seven days after which they were analyzed for restraint zones. A caliper was utilized to quantify the inhibition zones. The zone of inhibition was noted in mm and expressed in percentage by the equation 1:

$$PI = \frac{PE}{S} \times 100 \qquad \textbf{(1)}$$

Where PI represents the percent inhibition, PE represent plant extract (mm) and S represent standard (mm).

For mineral analysis, the following materials are used

Scanning electron microscope (SEM)
Energy -dispersive X-ray spectroscopy (EDX)

Preparation of plant sample for analysis

The seeds of *L. usitatissimum* were washed and dried at room temperature and after that compressed into a coarse powder utilizing a processor. The Sample was mounted rigidly on a specimen holder called a specimen stub. The stubs were used which were made of conductive materials

such as aluminum, brass or copper. The stubs were coated with conductive tapes on both sides. The sample was mold on the conductor taps to stick. Then stub was put into the scanning electron microscope, which was connected to EDX and it was an analytical technique used for the elemental analysis or chemical characterization of a sample (Bibi et al. 2006). The EDX analysis identified the elemental composition of the sample.

Table 1: Inhibitory zone of crude methanol extract (MeOH) of *L. usitatissimum* against pathogenic bacterial strains

Bacterial strain	MeOH (mm)	MeOH (%)	Ciprofloxacin (mm)	DMSO
A. tumefaciens	00	00	26	NA
B. subtilis	17	60.71	28	NA
C. michiganensis	00	00	27	NA
E. coli	11	44.0	25	NA
S. aureus	13	48.14	27	NA
X. compestris	00	00	26	NA

Results and Discussion

Antibacterial activity

The results given in Table 1 divulged that crude methanol extract of *L. usitatissimum* showed notable bactericidal activity against the tested strains. Among all the bacterial strains highest inhibitory zone was recorded against *B. subtillis* (60.71%) followed by *S. aureus* (48.14%) and *E. coli* (44.0%) whereas no activity was observed against *A. tumefaciens, C. michiganensis* and *X. compestris*. The standard drug ciprofloxacin showed complete inhibition of all the fungal isolates whereas dimethyl sulfoxide (DMSO) exhibits no activity. It was concluded from the results that the said plant extract contains bioactive compound which are responsible for growth inhibition of the tested bacterial strains. Bakht *et al*. (2011), Kaithwas and Majumdar (2010), (2011) and Panda (2014) reported notable antibacterial activity of different fraction of *L. usitatissimum* which are in line with the present findings. The results also find supportive evidence from the study of Shad *et al*. (2016) who observed that the methanol extract of the said plant showed appreciable bactericidal activity against *S. aureus, B. subtilis, P. aeruginosa, and E. coli*, respectively.

Antifungal activity

The results of fungicidal activity of crude methanol extract of the subject plant depicted in Table 2 revealed that among all the fungal isolates highest inhibitory zone was observed against *T. harzianum* (76.0%) as compared to *F. oxysporum* (65.21%) and *A. niger* (57.89%) while *A. flavus*and *Paecilomyces sp.,* showed complete resistance. The antifungal drug itraconazole was tested against *A. flavus* and *A. niger* while fluconazole was used against *F. oxysporum, Paecilomyces spp.,* and *T. harzianum*. All the fungal strains were susceptible to the standard drug showing significant inhibitory zone while the dimethyl sulfoxide (DMSO) exhibits no fungicidal activity. These results agree with Dalleau *et al*. (2008), Pemmaraju *et al*. (2013) and Zore *et al*. (2011), who revealed that terpenoids isolated from subject plant showed excellent activity against *Candida spp.* These results were also similar to Shad *et al*. (2016) who examined that methanol extract of the subject plant fraction have a considerable inhibitory zone against *A. flavus, C. glaberata, C. albicans, and* T. *longifusus,* respectively.

Table2 Inhibitory zone of crude methanol extract (MeOH) of *L. ustitassimum* against pathogenic fungal strains

Fungal strain	MeOH (mm)	MeOH (%)	Fluconazole (mm)	Itraconazole (mm)	DMSO
A. flavus	00	00	NT	21	NA
A. niger	11	57.89	NT	19	NA
F. oxysporum	15	65.21	23	NT	NA
Paecilomycessp	00	00	22	NT	NA
T. harzianum	17	76.0	25	NT	NA

Elemental composition of *L. usitatissimum*

The results of elemental composition of *L. usitatissimum* of the subject plant depicted in Table 3 revealed that *L. usitatissimum* contain significant amounts of carbon (63.39%) followed by Oxygen (33.90%), silicon (0.45%), potassium (0.44%), calcium (0.36%), sodium (0.30%), magnesium (0.25%), sulfur (0.24%), phosphorus (0.21%), chlorine (1.9%), iron (0.18%) and aluminum (0.15%). Flaxseeds are a source of numerous minerals and vitamins as magnesium, phosphorus, and calcium. It is of incredible significance, being that a 30g part of the seed constitutes 7% to 30% of the Recommended Dietary Allowances (RDAs) for these minerals (Singh *et al*., 2011). The current results find supportive evidence from the study of Khan *et al*. (2010) who observed appreciable amount of calcium, magnesium, potassium, sodium, chlorine, phosphorus, copper, iron, manganese and zinc in different varieties of the said plant. These findings are at par with the results of (Narina *et al*.,2013) who examined the elemental

composition of three different cultivars of *L. usitatissimum*. Singh et al. (2011) investigated the elemental composition of the said plants which are in agreement with present research data. These elements exhibit paramount in various physiological functions of the body.

They are fundamental constituents of skeletal structures, for example, teeth, and bones, keep up osmotic weight and in this way direct the trading of water and solutes inside the creature body, fill in as auxiliary constituents of delicate tissues, help in the transmission of nerve driving forces and muscle constriction, manages corrosive base harmony of the body and consequently keep up the pH of the blood and different liquids, fill in as imperative segments of numerous compounds, vitamins, hormones, and respiratory shades or as a cofactor in digestion system (Youdim et al., 2008). Macro minerals, specifically calcium, sodium, magnesium, and potassium are responsible for the development, maintenance; blood clotting and heart beat regulation; biochemical reactions in the body; prevention of muscle cramps; and transmission of energy and electricity. It was concluded from the results that *L. usitatissimum* are is the major source of minerals that protect our body from different damages because these minerals are the integral parts of many enzymes.

Table 3: Elemental composition of *L. usitatissimum* through Energy-dispersive X-ray spectroscopy (EDX)

Element	Weight (%)
Carbon	63.39
Oxygen	33.90
Sodium	0.30
Magnesium	0.25
Aluminum	0.15
Silicon	0.45
Phosphorus	0.21
Sulfur	0.24
Potassium	0.44
Calcium	0.36
Iron	0.18
Chlorine	0.19

Conclusion

It is concluded that linseed is an antimicrobial agent. Its extracts are responsible for antibacterial and antifungal activity. These extracts exhibit a maximum zone of inhibition against different bacterial and fungal strain. The presence of these appreciable amounts of minerals like calcium, magnesium, potassium, sodium, chlorine, phosphorus, copper, iron, manganese and carbon are responsible for the heartbeat regulation, biochemical reactions in the body, prevention of muscle cramps and blood clotting. It is also the major source of minerals that protect the body from different damages because these minerals are the integral parts of many enzymes.

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