Antimicrobial potentials and phytochemical analysis of desert cotton (*A. Javanica*) and flax (*L. Ustitatissimum)*

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Abstract: The present study reveals antimicrobial potentials and phytochemical analysis of *A. javanica* and *L. ustitatissimum*. Phytochemical analysis indicated that the tested plants contained a substantial amount of flavonoids, terpenoids and steroids while saponins and tannins were absent in *L. ustitatissimum*, however, tannins were present in *A. javanica*. *L. ustitatissimum* contained maximum total phenolic content of 166.36mg/g in methylated spirit fraction while its ethyl acetate fraction contained highest quantity of flavonoids 27.6mg/g in case of *Aerva javanica*. Antimicrobial potentials of the subject plants revealed that *L. ustitatissimum* had maximum antibacterial activity (MIC=4.33µg/ml) while *A. javanica* was most effective against fungal strains (MIC=2.66µg/ml).

Keywords: Phytochemicals, antimicrobial, flavonoid, terpenoid, steroid, saponin, *Aerva javanica, L. ustitatissimum*.

INTRODUCTION

Medicinal plants are good sources of antimicrobial drugs (Bakht *et al.,* 2011 a, b, c, d, 2012; 2013 a, b; 2014a, b and c; 2015; Nasir *et al*., 2015; Wajid *et al*., 2015; Yasmin *et al*., 2015; Rohma *et al*., 2015; Nisar *et al*., 2015). These medicinal plants play a very important role in the treatment of certain ailments where conventional cure system is not yet discovered. A significant portion of research studies had underscored the need for appropriate therapeutic investigation of these native plants. Advances in phytochemical and medicinal chemistry and the discovery of modern technology have shown the curative investigations of these plants. These medicinal plants contain many active bio-compounds such as antioxidants, polyphenols, terpenoids, flavonoids, steroids, tannins and other biomolecules like protein, fatty acids and fiber. It has been established long ago that these bio-compounds present in plants not only protect them but are also physiologically vital and play different various in living bodies by curing different kind of diseases (Wang *et al.,* 2008).

Linseed or flax botanically known as *Linum ustitatissimum* belongs to family Linaceae. 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. Flax fiber has the potential to treat heart related diseases (Hall *et al.,* 2011). It contains physiologically essential compounds i.e. lignins, 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. Further research has discovered that flax is active against breast and prostate cancers. Flax has been found to relieve swelling and oxidative tissue damage (Ravi *et al.,* 2009). It has been shown that flax serves as purgative, gastric and disinfectant. It is also helpful in relieving bone disorder and intestinal problems.

Desert cotton is botanically known as *Aerva javanica* belongs to the family Amaranthaceae and is locally known as "Sparai" whereas its English name is desert cotton. *A. javanica* is found in sandy, calcareous soils in semi-arid and arid regions of Africa (Egypt, Libya, Kenya, Somalia, Nigeria and Sudan) and Asia and sub-continent (Afghanistan, India, Pakistan and Sri Lanka). There are about 25 species of the genus *Aerva* found in Pakistan and India. The plant contains many important bio molecules such as alkaloids, tannins, saponins, sulphates, flavonoids, lipids and carbohydrates. The plant is active against helmintis, used as anti-inflammatory agent (Vertichelvan *et al.,* 2000), helpful in diabetes, cough and infected lesions (Vertichelvan and Jegadeesan, 2002). It is also used to treat urinary disorders, respiratory complications, nasal hemorrhage and cracks (Waikar *et al*., 2007).

Escherichia coli are generally considered a parasitic microbe that is mostly found in the intestines of living organism and causes many infections. It is an active agents of many diseases including urinary tract infections, Pneumonia, kidney malfunctioning and food poisoning (Maddapa, 2011). Similarly, *Pseudomonas aeruginosa* is a parasite of animals and human origin that affects vital body organs like lungs, kidney and urinary bladder (Ryan and Ray, 2004). *Salmonella typhi* is another strain of bacteria that causes typhoid, and affects various organs

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including liver, spleen and bone marrow. It is also severely persuade systemic infections and obstructs the functioning of immune system of human and animals. Likewise, *Bacillus subtilis* is somewhat a beneficial bacterium, which is associated with preventing many plant diseases and fungal infections. *Staphylococcus aureus* feeds on human respiratory tract and skin and causes diseases like Pneumonia, pimples, bone fractures meningitis and food poisoning (Srivivan and Reddy, 2008).

Trichophyton longifusus causes many diseases primarily in human that are athlete's foot, ringworm, jack itch and infections related to skin, hair and scalp. *Candida glaberata* hinders the function of urinary bladders and disturb blood circulation. The fungus is commonly found in persons having HIV virus. Similarly, *Fusarium solani* is primarily a pathogen of plants especially crops in which they cause many diseases like root rot of pea, fruit rot of *Cucurbita* species, foot rot of bean etc. It also causes skin infections in human. *Aspergillus flavus* infect plants, animals and human. It affects the growth of many important agricultural crops while in mammals they have been reported to damage liver functioning. *Candida albicans* is a fungal pathogen of animals and human where it causes many severe disease including urogenital infections, gastrointestinal dysfunction, impairing immune system, diabetes, respiratory and skin diseases.

MATERIALS AND METHODS

Plant collection

Flax (*L. ustitatissimum*) seeds were purchased from the local market of Peshawar Khyber Pukhtunkhwa Pakistan. Similarly, plants of *A. javanica* were collected from the desert regions of Bannu and Dera Ismail Khan, Khyber Pukunkhwa.

Sampling

The seeds of flax and aerial parts of the *A. javanica* were washed with distilled water to remove dust and other adhering materials. The plant samples were dried in the shade at room temperature for seven days till complete dryness. The dried plant materials were grinded by tissue homogenizer to fine powder (Infinigen™ Tissue Mixer Mill, ACT Gene. The powdered materials were kept in plastic bags, sealed and stored at 4ºC in the refrigerator until used.

Preparation of crude extract

The powdered plant samples were macerated in four liters of methanol (Sigma-Aldrich) and kept at room temperature for 7 days. The solution was stirred several times during this period and filtered (What man ™ What man UK). Two liters of fresh methanol was added to the remaining sample material and filtered again through What man filter paper and this process was repeated three times. Methanol was separated by rotary evaporator (Rotavapor R-R 210/R215; BUCHIL Labortechnik AG) at 45°C under vacuum pressure and a semi-solid extract was obtained (crude extract). The collected extract was mixed with known amount of water and partitioned with different organic solvents (Methanol, ethanol, DCM, ethyl acetate and hexane) in order of increasing polarity using separating funnel. All the fractions thus obtained were dried by rotary evaporator. The crude fractions were stored at 4°C in refrigerated until analyzed.

Phyto-chemical analysis

Determination of tannins

Crude extract of the subject plants (0.5g) were boiled in 20ml of water, allowed to cool and filtered. One percent of ferric chloride solution was added and observed for blue black or brownish green color. The appearance of the required color was the indication of the presence of tannins (Trease and Evans, 1989).

Determination of saponins

For the determination of saponins, 2 grams of samples of the plants under test was boiled in 20ml of distilled water in water bath for half an hour, allowed to cool and filtered. Ten ml of the filtrate was mixed with 5ml of distilled water. The mixture was constantly shaken until a persistent froth appeared. For confirmation, few drops of olive oil were added to froth and shaking was continued till the formation of emulsion (Safowara, 1993).

Determination of flavonoids

Similarly, for the flavonoids determination, crude extract of the subject plants were prepared and filtered as discussed previously and 5ml of the dilute ammonia solution was mixed with each crude extract. Afterwards, known amount of H_2SO_4 (Conc) was added to the mixture. The presence of flavonoids was confirmed by the appearance of yellow color in each plant extract (Sofowara, 1993).

Determination of terpenoids

For the identification of terpenoids, a mixture containing 5ml of each plant sample and 2ml of chloroform was prepared. Three ml of H_2SO_4 (Conc) was added to the mixture for the formation of a layer. The presence of terpenoid was established by the appearance of reddish brown color (Harborne, 1973).

Determination of steroids

Approximately 20g of each crude plant sample was soaked in ethanol and boiled for 10 minutes. The extract was filtered and the ethanol fraction was separated and the remained crude solid sample was dissolved in 3ml chloroform followed by the addition of acetic anhydride (4.5 ml) and sulfuric acid (0.5ml). Steroids presence was confirmed by change of color of crude extract from violet to green (Sofowara, 1993).

Determination of total polyphenol content

FCR (Folin-Ciocalteu reagent) was used for the determination of total phenolic content in the crude fractions of both subject plants with Gallic acid as a reference for comparison. Hundred micro liter of each crude fraction was mixed with 900µl of water followed by the addition of FCR (500µl). $Na_2CO_3.10H_2O$ solution (20%) was made from this solution and 1.5ml was separated and subsequently poured into above mixture. The whole mixture was heated for 2 hours and absorbance was measured by spectrophotometer at 765nm. Total polyphenols were evaluated by comparing with Gallic acid as standard.

Determination of total flavonoid content

The total flavonoid content in the subject plant was estimated by colorimetric method using quercitin as a standard. One milliliter of crude fraction of each plant sample was mixed with 4ml of distilled water and 300µl each of $NaNO_2$ and $AlCl_3$. The mixture was warmed for about 5 minutes and NaOH was added so that the volume of the mixture became 10ml. Absorbance was measured by spectrophotometer and total flavones were represented as quercitin equivalents (QE) in mg/g of dry crude extract.

Disc diffusion susceptibility method

The antibacterial activity of different solvent extracted samples and of *L. ustitatissimum* and *A. javanica* was carried by disc diffusion assay as described in Bauer *et al.* (1966) and antifungal activity by Ramdas *et al.* (1998). Different antibiotics (Ciprofloxacin at 50µg concentrations for Gram-positive and Gram-negative bacteria; 50µg Amphotercin B for fungus were aseptically placed over the seeded agar plates. The plates were incubated at 37ºC for 24 hours and the diameter of the inhibition zones

Minimum inhibitory concentration (MIC) measurements

Minimum inhibitory concentration (MIC) was measured according to Khan *et al*. (2007). Briefly, the plant crude extract was dissolved in 2ml distilled water and added with 2 drops tween-80 for complete dissolution. The suspension of each test organisms was prepared by approximately 10^7 per ml and 1 drop of this suspension was added to each broth dilution. After 18-24h incubation at 37°C, the tubes were examined for the growth. The MIC of the extract was taken as the lowest concentration that showed no growth. Growth was observed in those tubes where the concentration of the extract was below the inhibitory level and the broth medium was observed turbid (cloudy). Distilled water with 2 drops of tween-80 and Ciprofloxacin and Amphotercin B were used as negative and positive control, respectively.

Microorganisms tested

The selected bacterial strains for the current study were *Bacilus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherchia coli* and *Salmonella typhi*. The fungal strains for the current investigation were *Trichophyton longifusus*, *Aspergillus flavus*, *Fusarium solani*, *Candida glaberata* and *Candida albicans*.

STATISTICAL ANALYSIS

The experiment was repeated in triplicate and MSTAT computer software was used for the analysis of the data. Standard deviation was calculated for each sample (Steel *et al.,* 1997).

Table 1: Phytochemical analysis of crude aqueous methanolic (15%) extracts of *A. javanica* and *L. usitatissimum*

Chemical compound	*Aerva javanica*	*Linum usitatissimum*
Flavonoids	++	++
Terpenoids	++	++
Steroids	++	++
Tannins	++	_ _
Saponins	_ _	_ _

RESULTS

Phytochemical analysis

Preliminary phytochemical analysis of the subject plants revealed that they are good sources of natural products. The results showed that *A. javanica* are moderate sources of tannins, flavonoids, steroids and terpenoids, however, the plant is devoid of saponin content (table 1). Similarly, terpenoids, steriods and flavonoids were also investigated during the preliminary phytochemical screening of *L. ustitatissimum* and it was observed that these phytochemicals were also present in promising amounts in the tested plant. However, this species was also devoid of saponin along with tannin (table 1). The subject plants were also tested for their flavonoid and polyphenolic contents. The results revealed that DCM extract of *A. javanica* contained maximum amount of polyphenol (28.26±0.909mg GAE g^{-1}) followed by ethyl acetate fraction (27.82±3.2676mg GAE) (table 2). The results also showed that methanol fraction contained moderate amount of polyphenols content (18.08±3.038mg GAE g^{-1}) and hexane fraction had the lowest phenolic content (4.1±0.2mg GAE g^{-1}). Similarly, the total flavonoid content was also investigated in *A. javanica* plant using Quercetin as standard. Our results shown in table 2 indicated that maximum amount of total flavonoids was observed in ethyl acetate fraction (27.6±8.39mg Quercetin g^{-1}) followed by methanol fraction (12.96±3.7027 mg Quercetin g^{-1}) whereas low level was found in hexane fraction (12.96±3.7027mg Quercetin g^{-1}). In case of *L. ustitatissimum*, methylated spirit fraction contained maximum total phenolic content (166.35±13.620 mg GAE g^{-1}) followed by methanol fraction (131.51±14.0513

Table 2: The phytochemical constituents (flavonoid and total phenolic content) of *A. javanica*

Plant Extract	Total Phenolic contents	Total Flavonoid content
Methanol	18.08±3.038	12.96±3.7027
Ethyl acetate	27.82±3.2676	27.6±8.3862
Dichloromethane (DCM)	28.26± 0.909	15.20±2.10008
Hexane	4.1±0.2	13.60±3.54

± Represents Standard deviation (S.d)

Table 3: The phytochemical constituents (flavonoid and total phenolic content) of *L. ustitatissimum*

Plant Extract	Total Phenolic contents	Total Flavonoid content
Methanol	131.51±14.0513	18.84±2.3833
Methylated spirit	166.35±13.620	18.16±1.5820
Hexane	114.88±4.5155	16.22±1.857

*Quercetin equivalent mg g^{-1} of extract (Total flavonoid content), Gallic acid equivalent mg g^{-1} of extract (Total polyphenol content)

Table 4: Antibacterial assay at 5mg/ml of different crude fractions of *A. javanica*L (MIC; Average Value ±SD, µg/ml)

Plant Extracts	*E. coli*	*P. aeruginosa*	*S. typhi*	*B. subtilis*	*S. aureus*
Methanol	13.66±1.53	12.24±2.31	8.59±1.32	8.66±1.15	8.66±1.15
Dichloromethane (DCM)	9.66±1.53	13.6±1.53	4.60±1.15	5.66±1.15	12.66±0.57
Ethyl acetate	11.86±2.78	12.66±2.51	6.33±157	6.61±2.57	13.54±1.39
Hexane	10.33±1.53	15.38±1.28	4.33±1.53	5.66±0.57	8.33±1.52

± Represents Standard deviation (S.d)

mg GAE g^{-1}) and hexane fraction (114.88±4.5155mg GAE g^{-1}). Similarly, methanol fraction of *L. ustitatissimum* contained maximum concentration of flavonoid (18.84±2.3833mg Quercetin g^{-1}) followed by methylated spirit fraction (18.16±1.5820mg Querecetin g^{-1}). However, hexane fraction showed lowest value (16.22±1.857mg Quercetin g^{-1}) (table 3).

Antimicrobial activity

The result showed that all crude fractions of *A. javanica* were effective against *E. coli, S. aureus, S. typhi, B. subtilis* and *P. aeruginosa* (table 4). The results also suggested that crude methanolic fraction of *A. javanica* exhibited good activity against *E. coli* (MIC=13.66±1.53) compared with other fractions which revealed moderate activity i.e. hexane (MIC=10.33±1.53), ethyl acetate (MIC=12.23±2.47) and dichloromethane fraction (MIC= 9.66±1.527). Similarly, crude methanolic extract of *A. javanica* was also more effective (MIC=12.24±2.31), against *P. aeruginosa* as compared to ethyl acetate fraction (MIC= 2.66±2.51) and DCM fraction (MIC= 13.66±1.53). Hexane fraction of *A. javanica* had no considerable effect (MIC=15.38±1.28) on growth of *P. aeruginosa*. Crude extracts of hexane from *A. javanica* revealed good activity (MIC=4.33±1.53) against *S. typhi* followed by DCM fraction (MIC=4.60±1.15) and ethyl acetate (MIC=6.33±1.577). Methanolic fraction of *A. javanica* was least effective (MIC=8.59±1.32) against *S. typhi.* Similarly, hexane (MIC=5.66±0.57) and DCM (MIC=5.66±1.15) fractions of the same plant were equally effective against *B. subtilis* followed by methanolic fraction (MIC=8.66±1.15). The data shown in table 4 also showed that hexane fraction of *A. javanica* was effective (MIC=8.33±1.52) against *S. aureus* as compared to methanolic fraction (MIC=8.66±1.15) and DCM (MIC= 12.66±0.57). Ethyl acetate indicated minimum activity against *B. subtilis* (MIC=13.54±1.39).

Crude methanolic extract of *L. ustitatissimum* was the most effective among all fractions of the subject plant against different microbes (*E. coli, P. aeruginosa, B. subtilis and S. aureus*) with maximum activity (MIC= 4.33±0.577), however, the same extract showed negligible activity against *B. subtilis* (table 5). On other hand, methylated spirit fraction of *L. ustitatissimum* was only effective against *S. aeruginosa* (MIC=8.56±2.33) and *E. coli* (MIC=10.26±2.11) while the same extract had no inhibitory activity against *P. aeruginasa, S. typhi* and *B. subtilis.* Likewise, hexane crude extract of *L. ustitatissimum* had maximum activity (MIC=7.33±1.54) against *S. aureus,* followed by *E. coli and B. subtilis* while the same fraction was completely unable to block the activity of *S. typhi* and *P. aeruginosa* (table 5).

Antifungal activity of the subject plants were also investigated using different fungal stains (*Trichophytan longifusus*, *Candida glaberata*, *Fusarium solani*, *Aspergillus flavus* and *Candida albicans*) (tables 6 and 7). Aqueous and ethyl acetate fraction fractions of *A. javanica* showed more activity as compared to other extracts such as n-hexane. Hexane fraction was ineffective to control the growth of all fungus strains

Table 5: Antibacterial assay at 5mg/ml of different crude fractions of *L. ustitatissimum* L (MIC; Average Value ±SD, µg/ml)

Plant Extracts	*E. coli*	*P. aeruginosa*	*S. typh*	*B. subtilis*	*S. aureus*
Methanol	9.55±1.41	5.88±1.81	ND	4.33±0.577	10.66±2.36
Methylated spirit	10.50±2.52	ND	ND	ND	8.56±2.33
Hexane	10.41±1.27	ND	ND	10.26±2.11	7.33±1.154

Table 6: Antifungal assay of different crude fractions of *A. javanica*L (MIC; Average Value ±SD, µg ml^{-1})

Plant Extracts	*Trichophyton longifusus*	*Candida albicans*	*Candida glaberata*	*Aspergillus flavus*	*Fusarium solani*
Hexane	ND	ND	ND	ND	5.38±1.34
DCM	ND	7.42±1.37	6.10±1.52	ND	5.33±1.154
Methanol	8.33±1.52	8.00±2	8.60±1.52	ND	7.54±1.45
Ethyl acetate	ND	6.20±1.52	11.48±1.41	12.39±1	12.81±±0.63

Table 7: Antifungal assay of different crude fractions of *L. ustitatissimum* L (MIC; Average Value ±SD, µg ml^{-1})

Plant Extracts	*Trichophyton longifusus*	*Candida albicans*	*Candida glaberata*	*Aspergillus flavus*	*Fusarium solani*
Methanol	15.33±1.52	9.66±1.527	13.29±1.15	2.66±1.15	ND
Methylated spirit	ND	9.39±1.27	10.49±2.39	3.34±1.25	ND
Hexane	ND	12.66±1.154	ND	4.66±1.15	-

± Represent Standard deviation (S.d); ND = Not detected

except *Fusarium solani* with MIC value of (5.38±1.34). Dicloromethanol fraction showed significant activity (MIC=5.33±1.154) against *Fusarium solani* as compared to *Candida glaberata* (MIC=6.10±1.52) and *Candida albicans* (MIC=7.42±1.37). However, the Dichloromethanol fraction of the same plant species had no activity against *Trchophyton longifusus* and *Aspergillus flavus*. Aqueous methanol fraction of *A. javanica* showed maximum activity against *Fusarium solani* (MIC=7.54±1.45) as compared to other fungal strains. Ethyl acetate fraction also revealed significant anti fungal activity (MIC=6.20±1.52) against *Candida albicans* as compared to other strains, however, the same fraction had negligible activity against *Trchophyton longifusus* (table 6).

Using the same protocol, antifungal activity of *L. ustitatissimum* was also investigated (table 7). The methanolic extract exhibited significant activity against *Aspergillus flavus* (MIC=2.66±1.15), which was compatible with standard drug values, however, the same extract was comparatively less effective against other fungal strains including *Trichophyton longifusus* (MIC= 15.33±1), *Candida albicans* (MIC=9.66±1.527) and *Candida glaberata* (MIC=13.29±1.15). It was also observed that methanolic fraction of the same plant was ineffective to control the growth of *Fusarium solani*. Similarly, MIC value of methylated spirit fraction was MIC=3.34±1.25 against *Fusarium solani* showing maximum potential to inhibit the growth of *Fusarium solani* as compared to other fungal strains. Hexane fraction of *L. ustitatissimum* was more effective (MIC= 4.66±1.15) against *Aspergillus flavus*, followed by *Candida albicans* (MIC=12.66±1.154) while the same extract had no activity against *Trchophyton longifusus*, *Candida glaberata* and *Fusarium solani* (table 7).

DISCUSSION

Plant based compounds normally known as phytochemicals are very important bioactive molecules, which are widely distributed in plants. The general phytochemical includes essential oils, alkaloids, polyphenols, steroids, terpenoids, glycosides, saponins, flavonoids etc. These bioactive compounds are pharmacologically active against many diseases including jaundice, cough, bronchitis, diarrhea, asthma, heart diseases, brain abnormalities, breast/prostate cancers and diabetic mellitus. In the present study preliminary phytochemical screening of the subject plants revealed that they are good sources of natural products. The results showed that *A. javanica* are moderate sources of tannins, flavonoids, steroids and terpenoids, however, this plant is devoid of saponin content. Yamunadevi *et al*. (2011) reported similar results regarding the qualitative determination of flavonoid, tannins, steroids and terpenoid as main constituents in the subject plant. Different studies confirm that the presence of steroidal compounds is of vital interest in pharmacy due to their relationship with such compounds as sex hormones (Okwu, 2001). Likewise, terpenoids, steriods and flavonoids were also examined during the preliminary

phytochemical analysis of *L. ustitatissimum*. Our results that these phytochemicals were also present in promising amounts in *L. ustitatissimum.* However, this species was devoid of saponin along with tannins. It has been reported that larger amount of flavonoids might be responsible for their healing effect by controlling the growth of different pathogenic micro-organisms (Cowan, 1999).

The bioactivity potential of wild plants is mostly assessed by the quantitative analysis of phyto-nutrients especially polyphenol and falvonids contents. In this regard both the subject plants were screened for their flavonoid and polyphenolic contents. The polyphenol content was determined using the absorbing capacity of the subject plants extracts using Folin-ciocalteu reagent and gallic acid as standard. In the present study four different fractions examined for polyphenol content revealed that DCM extract of *A. javanica* contained highest amount of polyphenol followed by ethyl acetate fraction. The results also revealed that methanol fraction contained moderate amount of polyphenols content and hexane fraction had the lowest phenolic content. Similarly, the total flavonoid content was also investigated in the *A. javanica* plant using Quercetin as standard. Our results indicated that maximum amount of total flavonoids was measured in ethyl acetate fraction followed by methanol fraction and minimum concentration was found in hexane fraction. Similarly, *L. ustitatissimum* was also investigated for total polyphenol and flavonoid contents using the protocol as described earlier. The results showed that methylated spirit fraction contained maximum total phenolic content followed by methanol fraction and hexane fraction. Methanol fraction of from the same plant contained maximum concentration of flavonoid followed by methylated spirit fraction. However, hexane fraction on the other hand showed lowest value.

Antimicrobial activity

Antimicrobial potentials of the subject plants were also tested using various crude fractions (methanol, ethyl acetate, n-hexane, DCM (dichloromethane) and methylated spirit by disc diffusion assay. The crude extracts of both the plants were tested against five each different bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Bacillus subtilis*) and fungal strains (*Candida albicans*, *Candida glaberata*, *Trchophytan longifusus*, *Fusarium solani* and *Aspergillus flavus*). Minimum inhibitory concentrations (MICs) were evaluated by agar dilution methods using ciproflaxacin and amphotericin B as standard drugs. The data indicated that both the plants under investigation had moderate antibacterial activity against the selected strains of bacteria. Furthermore, it was also observed that various crude fractions of *A. javanica* exhibited more antibacterial activity against selected bacterial strains as compared to *L. ustitatissimum*. The data revealed that all crude fractions of *A. javanica* showed activity against *E. coli, S. aureus, S. typhi, B. subtilis* and *P. aerogenosa*. The results also suggested that crude methanolic fraction of *A. javanica* exhibited good activity against *E. coli* compared to the other fractions which showed moderate activity. Similarly, crude methanolic extract of *A. javanica* was also more effective against *P. aeruginosa* as compared to ethyl acetate fraction and DCM fraction. Hexane fraction of *A. javanica* had considerable effect (MIC=15.38±1.28) on growth of *P. aeruginosa*. Crude extracts of hexane from the same plant revealed good activity against *S. typhi* followed by DCM fraction and ethyl acetate. Methanolic fraction of *A. javanica* was least effective against *S. typhi.* Similarly, hexane and DCM fractions from the same plant were equally effective against *B. subtilis* followed by methanolic fraction. The results also revealed that hexane fraction of *A. javanica* revealed maximum activity against *S. aureus* as compared to methanolic fraction and DCM. Ethyl acetate on the other hand indicated minimum activity against *B. subtilis*. These results agree with Chowdhury *et al*. (2002) and Srinivas and Reddy (2012).

L. ustitatissimum was also screened for its antibacterial activity against different microbial strains. Crude methanolic extract of *L. ustitatissimum* was the most effective among all fractions of the subject plant against different microbes (*E. coli, P. aeruginosa, B. subtilis and S. aureus*) with maximum activity, however, the same fraction showed negligible activity against *B. subtilis.* On other hand, methylated spirit fraction of *L. ustitatissimum* was only effective against *S. aeruginosa* and *E. coli* and the same extract had no inhibitory activity against *P. aeruginosa, S. typhi* and *B. subtilis.* Similarly, hexane crude extract of *L. ustitatissimum* showed maximum activity against *S. aureus,* followed by *E. coli and B. subtilis* while the same fraction was completely unable to inhibit the growth of *S. typhi* and *P. aeruginosa*. From these results it can be concluded that both plants had moderate antibacterial activity, however, crude fractions of *A. javanica* were more effective against selected bacterial strains as compared to *L. ustitatissimum* fractions. Similar results are also reported by Kaithwas, Majumdar (2010), Kaithwas *et al*. (2011) and Panda (2014). Bakht *et al.* (2011b) reported antibacterial activity of different solvent extracted samples from the seeds of *L. ustitatissimum*.

Antifungal activity of the subject plants were also investigated using different fungal stains (*Trichophytan longifusus*, *Candida glaberata*, *Fusarium solani*, *Aspergillus flavus* and *Candida albicans*). It was observed that different crude fractions of *L. ustitatissimum* were more effective than *A. javanica*to reduce the activity of different strains of fungus. Aqueous and ethyl acetate fractions of *A. javanica* showed more activity as compared to other extracts such as n-hexane. Hexane fraction was ineffective to control the growth of all fungus

strains except *Fusarium solani*. Dichloromethane fraction revealed good activity against *Fusarium solani* as compared to *Candida glaberata* and *Candida albicans*. However, the dichloromethane fraction of the same plant species revealed no activity against *Trichophyton longifusus* and *Aspergillus flavus*. Aqueous methanol fraction of *A. javanica* showed maximum activity against *Fusarium solani* as compared to other fungal strains. Ethyl acetate fraction also revealed significant anti fungal activity against *Candida albicans* as compared to other strains, however, the same fraction had negligible activity against *Trichophyton longifusus*. These results agree with Dallaeu *et al*. (2008), Zore *et al*. (2011) and Pemmaraju *et al.* (2013) who revealed that terpenoids showed excellent activity against isolates of *Candida* sps., as the subject plants also possessed considerable amount of terpenoids.

Antifungal activity of *L. ustitatissimum* was also investigated during the present study. The methanolic extract exhibited significant activity against *Aspergillus flavus,* which was compatible with standard drug values, however, the same extract was comparatively less effective against other fungal strains including *Trichophyton longifusus*, *Candida albicans* and *Candida glaberata*. It was also observed that methanolic fraction of the same plant was ineffective to control the growth of *Fusarium solani*. Similarly, Methylated spirit fraction showed maximum potential to inhibit the growth of *Fusarium solani* as compared to other fungal strains. Hexane fraction of *L. ustitatissimum* was more effective against *Aspergillus flavus*, followed by *Candida albicans* while the same extract had no activity against *Trichophyton longifusus*, *Candida glaberata* and *Fusarium solani*. These results agree with Dallaeu *et al*. (2008), Zore *et al*. (2011) and Pemmaraju *et al.* (2013) who revealed that terpenoids showed excellent activity against isolates of *Candida* sps., as the subject plants also possessed considerable amount of terpenoids.

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