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Antibacterial activity of organic extracts of root bark of *Ziziphus jujube* Gaertn (L) var. *Hysudrica* Edgew

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Abstract

Extracts of root bark of *Ziziphus jujube* Gaertn (L) var. *hysudrica* Edgew were prepared by using organic solvents having different polarity i-e methanol, acetone, ethyl acetate, dichloromethane, chloroform and n-hexane. Extracts were then subjected to various characterization studies including UV-Visible and IR profiling. UV-Visible spectrum has shown multiple peaks for all the extracts in UV region. Dichloromethane and chloroform extracts have shown similar pattern as compared to the other extracts. IR spectrum has shown strong –OH (3336.0cm^{-1}) band for methanol and acetone extracts where asethylacetate, dichloromethane, chloroform and n-hexane extracts have shown distinct C-N (1250-1020cm^{-1}) stretch band for amines. All the extracts except n-hexane as it was less in yield were screened for antibacterial potential against two gram negative strains (*Eescherchia coli* and *Pseudomonas aeruginosa*) and two gram positive strains (*Bacillus subtilis* and *Bacillus pumilus*) by using disc diffusion assay. Maximum activity was shown by ethylacetate extract against *Eescherchia coli* and *Bacillus pumilus* having zone of inhibition 13.66mm and 12mm respectively. Dichloromethane extract has shown high activity against *Pseudomonas aeruginosa* having zone of inhibition 10.66mm chloroform extract was high in term of antibacterial activity against *Bacillus subtilis* having zone of inhibition 12mm. data was statistically analyzed by using one-way ANOVA p value less than 0.05 was considered significant. Multiple comparisons were performed by using LSD test.

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Introduction

Zizyphus Jujuba (L.) Gaertn. Var. *Hysudrica* Edgew is a hybrid of two Zizyphus species namely *Zizyphus mauritiana* and *Zizyphus spina-christi* (Azam-Ali *et al.,* 2006). It grows as medium sized tree possessing leaves which appear glabrous on both surfaces. The plant is rarely seen in wild-form and is usually cultivated to obtain its fruit which is edible. The fruit usually attains maximum of 1-inch length in wild-form in contrast to its cultivated form where it may reach up to the length of 3-inch long and is almost half as wide as its length (Chaudhry, 1969).

This variety is distributed in punjab region of Pakistan. *Z. Mauritiana* occurs in form of small shrubs to medium sized tree. The natural habitat of plant is warm subtropics and tropics of South Asia. where it exist in its wild form. The cultivated form of plant spreads through Indo-China and Southren China East ward whereas through Malesia it spreads to South East ward. In contrast Z. spina-christi belongs to drier tropical areas of Middle east, Ethiopia, North-East Africa and Eastren Africa.

In Iran, Saudi Arabia and farther west Turkey it exist in its wild from. In India, Pakistan, Egypt, Syria, the Mahgreb, Saharan Oases and Zanzibar it exists as minor cultivated plant. (Azam-Ali *et al.,* 2006)

The species of *Ziziphus* are enriched in phytochemicals including various Vitaminslike vitamin-C (Bakhshi and singh, 1974; Singh *et al.,*1973), vitamin-B_1 (Thiamine), vitamin-B_2 (Riboflavin) (Troyan and Kruglyakov, 1972; Kuliev and Guseinova,1974), Alkaloids, (Pareek, 2001; Tschesche *et al.,* 1976; Tschesche *et al.,* 1979; Han *et al.,* 1990; Jossang *et al.,* 1996), Carbohydrates (Bakhshi and singh, 1974; Singh *et al.,* 1973) and heteropolysacchrides like Pectin-A (Tomoda *et al.,* 1985), Glycosides like Flavonoid Glycosides/ Spinosins (Woo *et al.,* 1979), various acids like Triterpenoic Acids (Lee *et al.,* 2003), Betulinic Acid (Pisha *et al.,* 1995; Kim *et al.,* 1998; Eizhamer and Xu,2004), Oleanolic acid (Hsu *et al.,* 1997), Saponins like Glycoside saponin (Ogihara *et al.,* 1976), Phospholipids (Goncharova *et al.,* 1990), Inorganic minerals like Calcium and phosphorous (Bakhshi and Singh, 1974; Singh *et al.,* 1973), metal ions like iron (Bakhshi and Singh, 1974; Singh *et al.,* 1973), proteins and carotenes (Bakhshi and Singh, 1974; Singh *et al.,* 1973).

Infectious diseases remain one of the major disasters responsible for morbidity and mortality among human beings and animals. The main factors which drive attention towards natural antimicrobials includes undesirable effects of synthetic antimicrobials, emergence of multi drug resistance and limited antimicrobial spectrum (Ngoci *et al.,* 2014). Since prehistoric time different parts of medicinal plants were used traditionally to cure specific ailments. Pharmacological potential of medicinal plants is associated with presence of different bioactive compounds like alkaloids, phenolics, flavonoids, glycosides, tannins, essential oil, steroids, terpenoids and others. Folklore medicine provide base to develop current drugs available today depending upon information about curative agents in them.

Between 1983-1994 78% of new drugs were obtained from natural source and were used unmodified or in partly synthetic form according to United States Food and Drug Administration (FDA) (Sharmin *et al.,* 2014; Ngoci *et al.,* 2014). Bioactive metabolites vary in amount from plant to plant and in different parts of the same plant. Extraction of these metabolites depends upon two main factors i-e nature of extracting solvent and method adopted for extraction.

In this study the extraction of bioactive metabolites was carried out by using six different solvents having different polarities i.e methanol, acetone, ethyl acetate, dichloromethane, chloroform and n-hexane. These extracts were analysed by UV-Vis spectrophotometer and FT-IR. The antimicrobial activity of all extracts was monitored against two gram negative bacteria i.e. *Escherchia coli* and *Psedomonas* and two gram positive bacteria i.e. *Bacillus subti*lis and *Bacillus pumilus* by using disc diffusion assay.

Materials and method

Collection of Plant Material

Ziziphus jujube Gaertn (L) variety *hysudrica* Edgew was collected from Lahore, Pakistan. The bark of the

root was separated from the inner root by physical means. Which was then shade dried for 10 days and ground, sieved and got properly stored in desiccator.

Chemicals

All the material and reagents used to conduct this study were taken from PCSIR Labs complex and University of the Punjab Lahore. All the chemicals were of AR grade and used as such without further purification.

Preparation of Extracts

Hundred grams of each finely ground root bark and root of *Zizyphus Jujuba* (L.) Gaertn. Var. *Hysudrica* Edgew was poured into six different flasks and extracted against various solvent having different polarities like: methanol, acetone, ethyl acetate, dichloromethane,*n*-hexane and chloroform. Flasks were allowed to continuously stir for 72 hours the material was then filtered and the resulting filtrates were air dried.

Characterization studies

Characterization of root bark extracts in different solvents was done by using UV-Visible and IR spectrum studies.

U.V-Visible Spectrum

The solution of all dried extracts in respective solvents were made and got scanned at 200-800nm to determine λmax (Asif *et al.,* 2014).

IR Spectrum

The dried extracts in different solvents were converted into disc and got scanned at mid IR region (650-4000cm^{-1}) to find the functional groups involved in various vibrations (Asif *et al.,* 2014).

Estimation of Antibacterial Potential

Test Bacteria

Antibacterial activity was performed by using two Gram positive strains i-e *Bacillus subtilis* and *Bacillus pumilus* and two Gram negative strains i-e *Escherichia coli* and *Pseudomonas aeruginosa*. These strains were obtained from microbiology laboratory of University College of pharmacy, university of the Punjab, Lahore. After getting above mentioned strains standard confirmatory tests were performed with them.

Bacterial Culture and Preparation of Media

Nutrient agar media was used to perform in-vitro antibacterial activity. Sterilized water suspensions of six isolated and pure strains of bacteria (*Bacillus subtilis, Bacillus pumilus, Escherichia coli* and *Pseudomonas aeruginosa*.) were prepared and got mixed with nutrient agar separately for evaluation of antibacterial activity. Media temperature was mentained at 45°C which was then poured into petri dishes and allowed to solidify.

Antibacterial activity using Disc Diffusion Method

Disc diffusion method was used for studying antibacterial activity as described by Sharma *et al.,* 2013. Fresh culture suspension of gram positive and gram negative bacteria was spread uniformly on media containing petri dishes. Filter paper disc having 6mm diameter soaked with extract (1000µg/ml) was placed on the surface of media agar plates. Plates were then incubated al 37°C for 24 hours under optimum conditions.

Results and discussion

The overlay of UV-Visible scan of different extracts of bark of roots was given in Fig. 1. These results showed that the spectra of DCM and chloroform extracts were entirely distinct from the other extracts. All other extracts showed somewhat similar pattern, however, peaks intensities were different which may be due to the difference in concentration of compounds. This is due to the presence of similar compound or compounds having similar UV-Visible absorbance pattern. It is interesting that all the extracts absorbed in UV region indicating the presence of compounds having unsaturated structure and chromophores. For all the extracts multiple peaks were obtained instead of single peak.

Presence of conjugated pi-bonding system having π-π* transition was indicated by the absorbance bands between wavelength range of 200-280nm. As larger the conjugated pi-system became there will be corresponding narrowing of energy gap for π-π*

transition and longer the wavelength of light absorbed have occurred. Second transition which was between non-bonding (lone pair) electrons to aπ* anti-bonding M.O was observed by the presence of absorbance bands in the wavelength range of 300-370nm and was referred to as n- π* transition. The highest bonding p-orbital are lower in terms of energy as compared to non-bonding (n) M.Os. So, the energy gap for π-π* were higher than n-π* transitions that's why n-π* peak was attained at longer wavelength. In general π-π* transitions are stronger than n-π* transitions which are weaker in terms of less light is absorbed to carry out n-π* transition (Armando *et al.,* 2013). ATR-IR spectra of different extracts of root bark of *Ziziphus jujube* Gaertn (L.) Var. *Hysudrica* Edgew were given in Fig. 2. These results showed that the functional groups of acetone and methanol extracts were alike but different from other extracts. Both the extracts showed strong band of OH (3336.0cm^{-1}), C=O (1654.0cm^{-1}), N-O (1094.0cm^{-1}) and N-H (1604.6cm^{-1}). Whereas, DCM, n-hexane, chloroform and ethyl acetate extracts showed similar profile Indicating functional groups such as C-H Stretch (3000-2800cm^{-1}), C-H bending (1000-700cm^{-1}), C=O (1750-1650cm^{-1}), C-O (1250-1000cm^{-1}), C-F (2000-1000cm^{-1}), C=C (1660-1400cm^{-1}) and C-N (1250-1020cm^{-1}). Moreover, α β unsaturated band (1684.8cm^{-1}) was only shown in DCM extract.

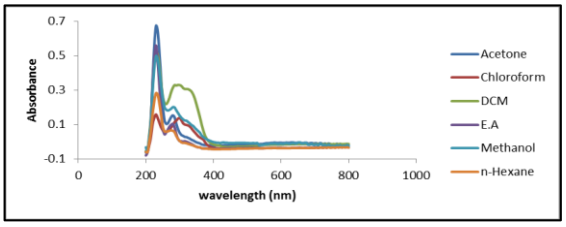

Fig. 1. UV-VISIBLE scans of different extracts of root bark of *Ziziphus jujube* Gaertn (L) var. *Hysudrica* Edgew.

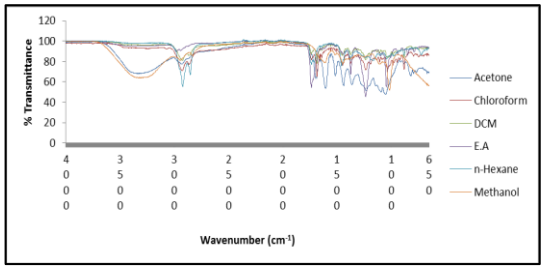

Fig. 2. IR spectrum of different extracts of root bark of *Ziziphus jujube* Gaertn (L) var. *Hysudrica* Edgew.

Results of antibacterial activity of root bark of *Ziziphus jujube* Gaertn (L) var. *hysudrica* Edgew against *Eescherchia coli* were given in Fig. 3. Ethylacetate, dichloromethane and chloroform extracts differ significantly from each other in term of antibacterial activity. Highest activity was shown by ethylacetate extract having zone of inhibition 13.66mm. whereas for other extracts i-e acetone, methanol, dichloromethane and chloroform the zone of inhibition was 8.66mm, 8.33mm, 5.66mm and 5mm respectively. Results of antibacterial activity of root bark of *Ziziphus jujube* Gaertn (L) var. *Hysudrica* Edgew against *Pseudomonas aeruginosa* were given in fig. 4. Extracts do not differ from each other in term of antibacterial activity ($p < 0.05$). Highest activity was shown by dichloromethane extract having zone of inhibition 10.66mm. whereas for other extracts i-e ethylacetate, methanol, chloroform and acetone the zone of inhibition was 9mm, 9mm, 8mm and 5mm respectively.

Results of antibacterial activity of root bark of *Ziziphus jujube* Gaertn (L) var. *hysudrica* Edgew against *Bacillus pumilus* were given in Fig. 5. Extracts do not differ from each other in term of antibacterial activity ($p < 0.05$). Highest activity was shown by ethylacetate extract having zone of inhibition 12mm. whereas for other extracts i-e acetone, chloroform, methanol and dichloromethane the zone of inhibition was 10.33mm, 9.33mm, 6mm and 5mm respectively.

Results of antibacterial activity of root bark of *Ziziphus jujube* Gaertn (L) var. *Hysudrica* Edgew against *Bacillus subtilis* were given in Fig. 6. The extracts do not differ from each other in term of antibacterial activity ($p< 0.05$). Highest activity was shown by chloroform extract having zone of inhibition 12mm. whereas for other extracts i-e ethylacetate, acetone, dichloromethane and methanol the zone of inhibition was 11.66mm, 11.33mm, 10.66mm and 10mm respectively.

In present study it was clearly seen that antibacterial activity was shown by all extracts in different organic solvents. Mostly in plants antimicrobial activity was attributed to alkaloids and flavonoids both of which

are secondary metabolites (Compean and Ynaluz, 2014). The solvents having high polarity may extract phenolics and flavonoids better that's why activity shown by these extracts i-e methanol and acetone and was attributed to phenolics and flavonoids present in respective extract. Whereas solvents having low polarity may extract alkaloids better that's why activity shown by these extracts i-e ethylacetate dichloromethane and chloroform was attributed to alkaloids present in respective extracts.

These statements were also strongly supported by IR spectrum of various extracts given in Fig. 2. It was evident from IR spectrum that very distinct peak for –OH (3336.0cm^{-1}) group was present in both methanol and acetone extracts.

Whereas ethylacetate, dichloromethane and chloroform a distinct peak for –C-N (1250-1020cm^{-1}) group was observed which was an amine stretch peak confirms the presence of alkaloids in these extracts.

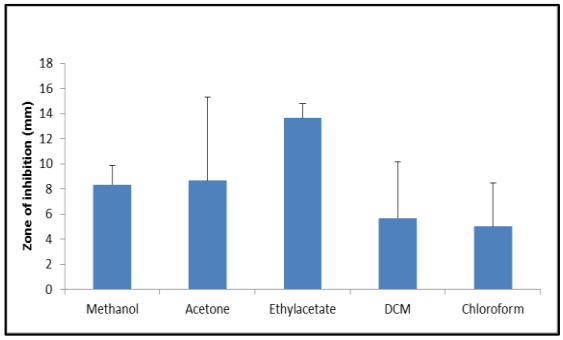

Fig. 3. Antibacterial activity of different extracts of root bark of *Ziziphus jujube* Gaertn (L) var. *Hysudrica* Edgew against *Escherchia coli.*

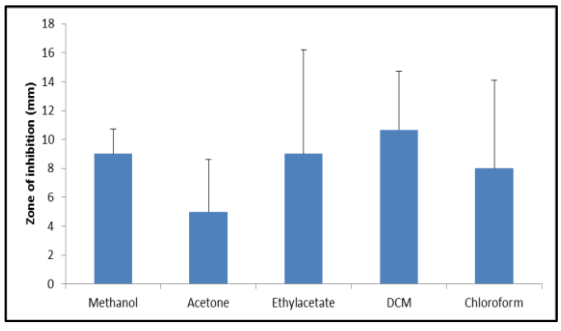

Fig. 4. Antibacterial activity of different extracts of root bark of *Ziziphus jujube* Gaertn (L) var. *Hysudrica* Edgew against *Pseudomonas aeruginosa*.

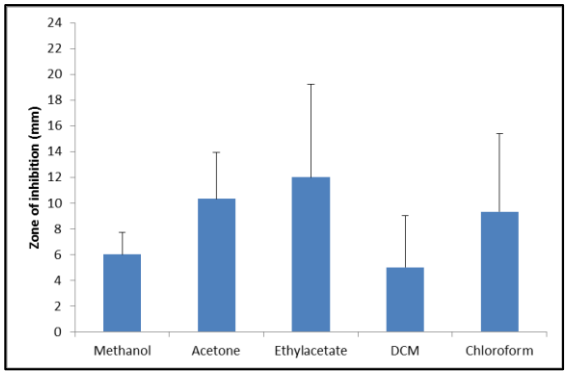

Fig. 5. Antibacterial activity of different extracts of root bark of *Ziziphus jujube* Gaertn (L) var. *Hysudrica* Edgew against *Bacillus pumilus.*

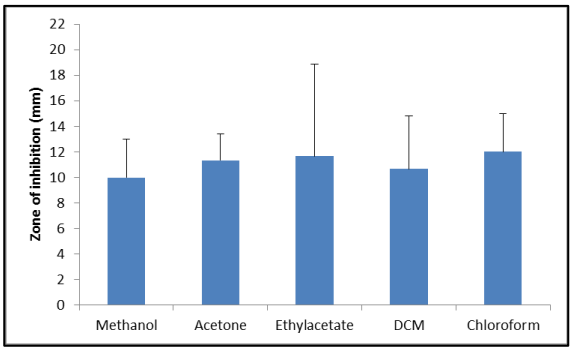

Fig. 6. Antibacterial activity of different extracts of root bark of *Ziziphus jujube* Gaertn (L) var. *Hysudrica* Edgew against *Bacillus subtilis.*

Genus *Ziziphus* comprised up of 40 different species distributed in subtropical and warm temperate zones. *Ziziphus* has been used since past many years as a traditional medicine for the treatment of various diseases like anemia, diabetes, fever, liver complaints, urinary infections, skin infections, liver complains, digestive disorders, bronchitis, diarrhea and others (Mishra and Bhatia.,2014; Goyal *et al.,* 2012). Plants belonging to genus *Ziziphus* have demonstrated high potential to develop natural antibiotics as indicated in literature (Emad *et al.*,2017; Mirza *et al.,* 2016; Mishra and Bhatia., 2014; Hossain *et al.,* 2013; Dhunmati *et al.,* 2013; Dangogoo *et al.,* 2012; Ahmad *et al.,* 2011; Abalaka *et al.,* 2010).

It was also evaluated that bioactive components responsible for antibacterial activity were phenolics and alkaloids present in plant extracts (Rizwan *et al.,* 2017; Bukar *et al.*,2015; Compean and Ynaluz, 2014; Mehbuba *et al.,* 2010).

The possible mechanisms of action through which extracts have shown antibacterial effect includes interference with the formation of extracellular polysaccharides resulting in disruption of cell morphology (Hasnah *et al.,* 2019; Yenugu *et al.,* 2006), disruption of membrane integrity resulting in electrolyte leakage from cell (Walsh *et al.,* 2003) inhibition of bacterial enzyme activity and interference with microbial DNA functioning resulting in cell death (Omojate *et al.,* 2014).

Conclusion

Extraction of root bark of *Ziziphus jujube* Gaertn (L) var. *Hysudrica* Edgew was done by using six different organic solvents of varying polarities (methanol, acetone, ethyl acetate, dichloromethane, chloroform and n-hexane). Characterization was done by using UV-Visible and IR profiles of extracts in order to understand how the nature of unsaturation and different functional groups will be affecting the activity of extracts. Antibacterial potential of all the extracts except *n*-hexane (as its yield was very low) was estimated by using disc diffusion method against two gram negative strains (*Eescherchia coli* and *Pseudomonas aeruginosa*) and two gram positive strains (*Bacillus subtilis* and *Bacillus pumilus*) bacterial strains. Maximum activity was shown by ethylacetate extract against *Eescherchia coli* and *Bacillus pumilus* having zone of inhibition 13.66mm and 12mm respectively. Dichloromethane extract has shown high activity against *Pseudomonas aeruginosa* having zone of inhibition 10.66mm. chloroform extract was high in term of antibacterial activity against *Bacillus subtilis* having zone of inhibition 12mm. Antibacterial activity was shown by all the extracts to different extentand was attributed to C-N band (1250-1020cm^{-1}) in IR spectrum for alkaloids in ethylacetate, dichloromethane and chloroform extracts and OH band (3336.0cm^{-1})in IR spectrum for phenolics/lavonoids in methanol and acetone extracts.

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