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FT-IR Spectroscopic Analysis of *Holoptelea integrifolia* (Roxb.) Planch Seed Extracts and their Antibacterial Activity

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ABSTRACT

This study was designed to find out the bioactive functional groups present in seed extracts of *Holoptelea integrifolia* for their antibacterial activity. The characterization of functional groups was achieved by Fourier Transform Infrared Spectroscopy (FT-IR) analysis. Antibacterial activity of methanolic, chloroform and aqueous seed extracts was evaluated against four pathogenic bacterial strains viz. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Salmonella typhimurium* by disc diffusion method. The FT-IR analysis revealed the presence of phenols, alcohols, aldehydes, amines, phosphines, nitriles, aliphatic compounds, azides, esters, β- lactones, amino acids and carboxylic acids etc. Major peaks were observed at 1650-1870 and 3400-3700 cm^{-1} which correspond to C=O and -OH functional groups, respectively. Among the three seed extracts tested, the methanolic extract showed best antibacterial activity against four pathogenic test pathogens. *Staphylococcus aureus* was the most sensitive test organism to seed extract of *H. integrifolia*. The seed extracts have promising antibacterial potential and could be effective natural medicine for the treatment of diseases associated with these pathogenic bacteria. However, further investigation should be done on them to be incorporated in medical applications.

Key words: Bioactivity, disc diffusion, *Holoptelea integrifolia,* phytochemistry, active compounds

INTRODUCTION

Medicinal plants are an important source for the verification of pharmacological effects (Ushimaru *et al*., 2007). It is well known that more than 400,000 species of tropical flowering plants have medicinal properties (Ordonez *et al*., 2006). This enormous number has made traditional medicine cheaper than modern medicine. They can be natural composite sources that act as new anti-infectious agents. Globally, plant extracts are employed for their antibacterial, antifungal and antiviral activities. Development of bacterial resistance to the available antibiotics and increasing popularity of traditional medicine has led researchers to investigate the antibacterial compounds in plants (Hammer *et al*., 1999).

Plant sources provided a good source of anti-infective agents, which are cost-effective and have fewer side effects (Samsam and Moatar, 1991). Natural drugs and bioactive compounds from plants overcome the disadvantage of synthetic drugs (Kumar *et al*., 2013). Plant based drugs have various secondary metabolites like alkaloids, phenols, flavonoids, steroids, tannins, peptides and glycosides etc. Therefore, the analysis of these phytoconstituents would aid in determining pharmacological potentials of the plants. A variety of sophisticated techniques are available, however, chromatography and spectroscopic techniques are the most useful and popular tools used for this

purpose. Fourier Transform Infrared Spectrometry (FTIR) is a physico-chemical analytical technique employed to determine the structure of unknown constituents. It is also employed to measure the intensity of the absorption spectra associated with the molecular composition of the chemical group (Griffiths and de Haseth, 1986; Bobby *et al*., 2012). Moreover, FTIR spectroscopy is an established time saving tool to characterize and identifies functional groups (Grube *et al*., 2008).

Holoptelea integrifolia, commonly known as 'Indian Elm', is a versatile medicinal plant, belongs to Ulmaceae family. It is a large deciduous tree, distributed in temperate and tropical areas of northern hemispheres. The plant is well known for its medicinal properties. It is widely used for the treatment of several human disorders like rheumatism, intestinal tumors, ringworm eczema, cutaneous diseases, common fever, malaria, ringworm and scabies, weakness, polyurea and chronic wound (Sabnis and Bedi, 1983; Sharma *et al*., 1992; Singh and Ali, 1994; Bajpai *et al*., 1995; Mahishi *et al*., 2005; Pawar and Patil, 2007; Khare, 2007; Benjamin and Christopher, 2009; Rajakumar and Shivanna, 2009). Though, several reports are available on the biological activities of *H. integrifolia* but pharmacological knowledge of seeds of *H. integrifolia* is scanty. Therefore, in the present study, seed extracts of *H. integrifolia* were considered for its FTIR analysis and antibacterial activity.

MATERIALS AND METHODS

Plant material: Mature seeds of *H. integrifolia* were collected from various areas of Agra (Uttar Pradesh), India. The seeds were surface sterilized with double distilled water in the laboratory. The seeds were shade dried and powdered with a mixer grinder.

Extraction procedure: Soxhlet extraction method following Okeke *et al*. (2001) was used for extraction of *H. integrifolia* seeds.

Fifty grams of seed powder was extracted separately in 400 mL of methanol, chloroform and distilled water. Powder was packed in extraction thimble and placed in an extraction chamber which was suspended above the flask containing the solvent. The flask was heated and the solvent evaporated and moved into the condenser where it was converted into a liquid that trickled into the extraction chamber containing the plant material. The extraction chamber was designed so that when the solvent surrounding the sample exceeded a certain level, it overflowed and trickled back down into the boiling flask. The extraction was done till the dark colour of the seeds turns colourless. At the end of the extraction process, the flask containing the plant extract was removed and solvent was evaporated using a rotary evaporator. The weight of the crude extract was measured and the percentage yield of the plant material was calculated. The soxhleted material was stored at -4°C.

FT-IR analysis: Methanolic, chloroform and aqueous seed extracts of *Holoptelea integrifolia* were scanned for the presence of functional groups by FT-IR spectrophotometer. The molecular functional vibration of chemical groups present in the sample was recorded with Perkin-Elmer FT-IR spectrophotometer, ranging from 4000-400 cm^{-1}.

Bacterial strains: Four pathogenic bacterial strains viz. *Staphylococcus aureus* (MTCC 3160), *Pseudomonas aeruginosa* (MTCC 2581), *Klebsiella pneumoniae* (MTCC 4032) and *Salmonella typhimurium* (MTCC 3224) were used for testing antibacterial activity. The test organisms were

procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The bacteria were cultured on nutrient agar slants and subcultured periodically.

Antibacterial assay: Antibacterial activity of methanolic, chloroform and aqueous seed extracts was evaluated by disc diffusion method (Kohner *et al*., 1994).

For susceptibility testing, different concentrations (200, 100, 50 and 25 mg mL^{-1}) of the plant extracts were prepared by serial dilution. Sterile discs (HIMEDIA) having diameter of 6 mm were impregnated with 25 µL of each serial dilution of extract solution. On other hand, the pathogens were inoculated in nutrient broth and kept overnight at 37°C for exponential growth of cultures. Some colonies from pure culture were mixed in nutrient broth. The bacterial cultures were swabbed on the entire surface of a nutrient agar plate with the culture moistened cotton swab. The sterile discs loaded with plant extracts were placed on inoculated surface of agar plate with the help of sterile forceps. These plates were incubated for 24 h at 37°C. The diameter of the zones of inhibition around each of the disc was taken as a measure of the antibacterial activity. Each experiment was carried out in triplicate and mean diameter of the inhibition zone was measured in millimeter.

Statistical analysis: Results obtained in the present study were analyzed for one way analysis of variance (ANOVA) to find out significant differences between sample means, with significant level being considered at $p<0.05$. All data are expressed as Mean±standard deviation of three values (n=3) obtained from three separate runs.

RESULTS AND DISCUSSION

Yield of extract: Percentage yield of seed extracts of *H. integrifolia* was 3.04% (methanolic extract), 2.85% (chloroform extract) and 2.48% (aqueous extract).

FT-IR spectroscopy analysis of *Holoptelea integrifolia* seeds: The FT-IR spectrum of methanolic, chloroform and aqueous seed extracts of *H. integrifolia* is shown in Fig. 1-3, respectively. The FT-IR analysis revealed the presence of phenols, alcohols, aldehydes, amines, phosphines, nitriles, aliphatic compounds, azides, esters, β-lactones, amino acids and carboxylic acids etc. Major FT-IR peaks were observed at 1650-1870 and 3400-3700 cm^{-1}, which indicates the presence of amides, esters and β-lactones with C=O functional group and phenolic and alcoholic compounds with -OH functional group, respectively (Table 1). Earlier studies on *H. integrifolia* showed the presence of carbohydrates, alkaloids, terpenoids, sterols, saponins, proteins and flavones etc. (Benjamin and Christopher, 2009; Ahmad e*t al*., 2012).

Antibacterial activity of seed extracts of *Holoptelea integrifolia*: Methanolic, chloroform and aqueous seed extracts of *H. integrifolia* were evaluated for antibacterial activity against different pathogenic bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Salmonella typhimurium).* The activity was analyzed by disc diffusion method at different concentrations (200, 100, 50 and 25 mg mL^{-1}). The extracts showed different inhibitory capabilities towards the tested pathogens. Methanolic extract was found to have considerable activity against all the tested pathogens than the chloroform and aqueous extracts. The maximum activity was observed against *S. aureus* (16.0±2.8 mm) followed by *P. aeruginosa* (15.0±1.0 mm), *S. typhimurium* (10.0±2.0 mm) and *K. pneumoniae* (9.5±0.6 mm) (Table 2). The results were compared with the

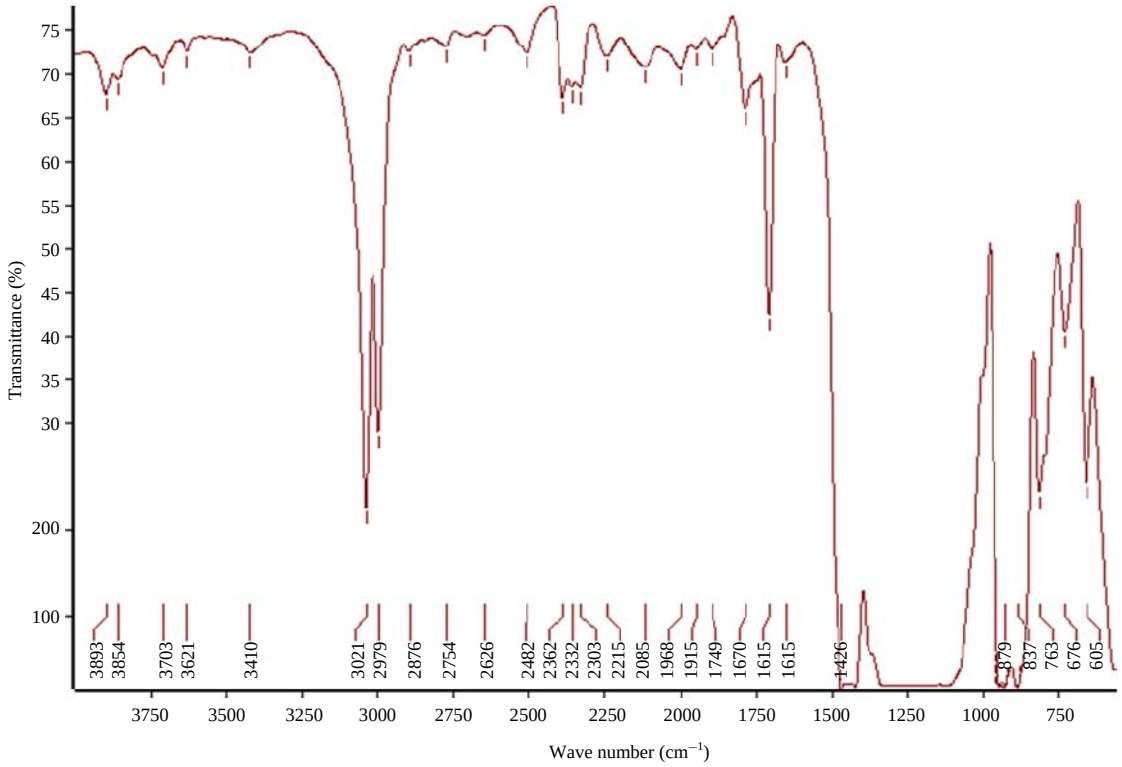


Fig. 1: FTIR Spectrum of methanolic seed extract of *Holoptelea integrifolia*

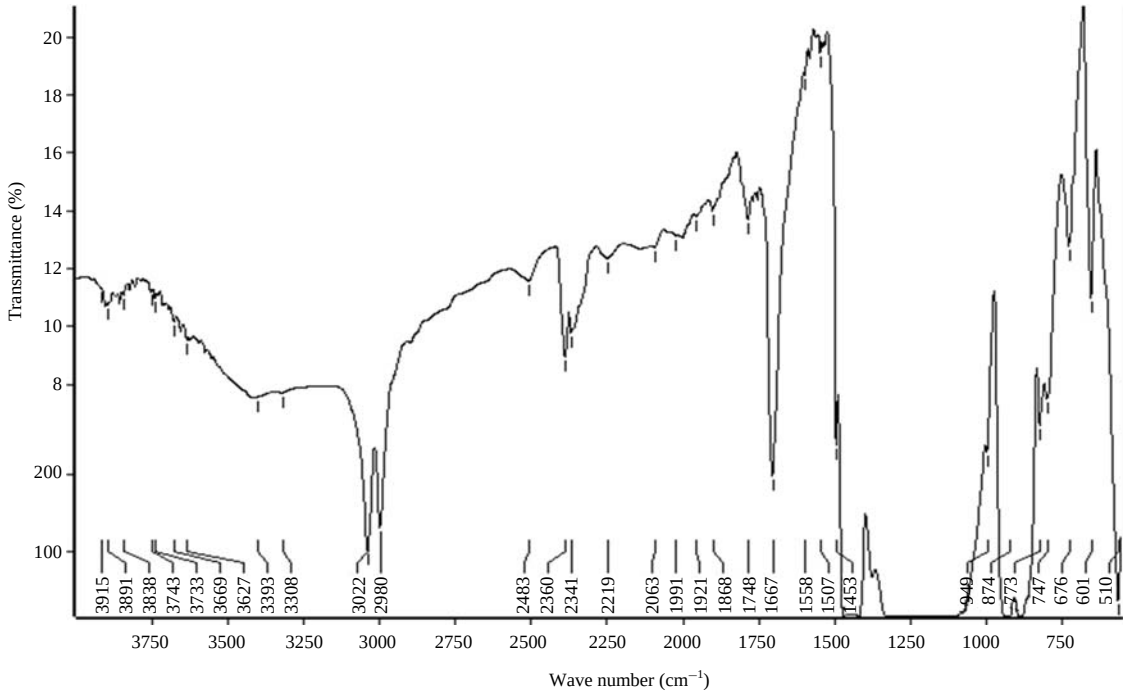


Fig. 2: FTIR Spectrum of chloroform seed extract of *Holoptelea integrifolia*

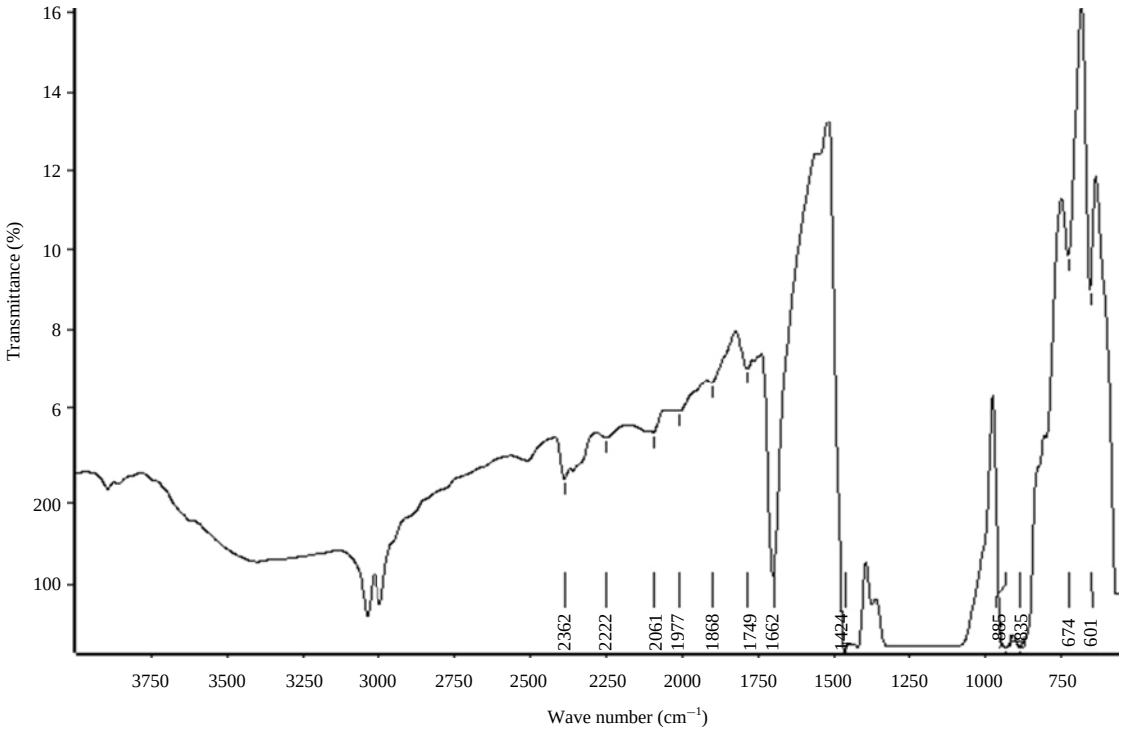


Fig. 3: FTIR Spectrum of Aqueous seed extract of *Holoptelea integrifolia*

Table 1: FTIR profile of seed extracts of *Holoptelea integrifolia*

Extract and peak value	Functional groups	Class
Methanolic seed		
3621	^{-}OH	Phenols, alcohols
3410	^{-}OH	Phenols, alcohols
3021	=CH	Unsaturated hydrocarbons
2979	$^{-}CH_3$ and $^{-}CH_2$	Aliphatic compounds
2876	$^{-}CH_3$ and $^{-}CH_2$	Aliphatic compounds
2754	HC = O: CH	Aldehydes
2626	$^{-}NH_3^{+}$	Amines
2482	$^{-}NH_3^{+}$	Amines
2362	^{-}PH	Phosphines
2332	^{-}PH	Phosphines
2303	^{-}PH	Phosphines
2215	C≡N	Nitriles
2085	$N = N^{+} = N^{+}$	Azides
1968	C = C = C	Allenes
1861	C = O	β-lactones
1749	C = O	Esters
1670	C = O	Amides
1615	$^{-}NH_2$	Amino acids
1426	^{-}OH	Carboxylic acids
879	$CH_2 = C(R)(R')$	Vinylidenes
837	$CH = C(R)(R')$	Tribust alkenes
763	C^{-}S	Sulfonyl chlorides

Table 1: Continue

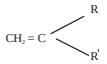
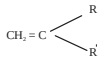
Extract and peak value	Functional groups	Class
676	$^{-}$Ar $^{-}$OH	Phenols
605	$^{-}$Ar $^{-}$OH	Phenols
Chloroform seed		
3669	$^{-}$OH	Alcohols and phenols
3627	$^{-}$OH	Alcohols and phenols
3393	$^{-}NH_2$	Amines
3308	$^{-}$OH	Oximes
3022	= CH	Unsaturated hydrocarbons
2980	CH_3 and $^{-}CH_2$	Aliphatic compounds
2483	$^{-}NH_3^{+}$	Amino hydrohalides
2360	$^{-}NH_3^{+}$	Amino hydrohalides
2341	$^{-}$PH	Phosphines
2219	C ≡ N	Nitriles
1868	C = O	β-lactones
1748	C = O	Esters
1667	C = O and $^{-}NH_2$	Primary amides
1558	$^{-}NO_2$	Aliphatic nitro compounds
1507	$^{-}$NH	Secondary amides
1453	$^{-}CH_2$	Aliphatic compounds
949	$CH = CH_2$	Vinyl compounds
874		Vinylidenes
773	C^{-}S	Sulfonyl chlorides
747	C^{-}S	Sulfonyl chlorides
676	C^{-}OH	Alcohols
601	NO_2	Aliphatic nitro compounds
510	C^{-}C = O	Ketones
Aqueous seed		
2362	$^{-}NH_3^{+}$	Amino hydrohalides
2222	C≡N	Nitriles
1977	C = C = C	Allenes
1868	C = O	β-lactones
1749	C = O	Esters
1662	C = O	Primary amides
1424	$^{-}$OH	Carboxylic acids
885		Vinylidenes
835	Si$^{-}CH_3$	Silanes
674	C^{-} C^{-} CHO	Aldehydes
601	C = O	Amides

Table 2: Zones of inhibition of *Holoptelea integrifolia* seed extracts against different bacteria

	Zone of inhibition (mm)			
Extract and microorganism	200	100	50	25
Methanolic seed				
S. aureus	16.0±2.8	13.5±1.5	8.3±0.8	7.5±0.7
P. aeruginosa	15.0±1.0	12.6±0.9	8.6±0.5	7.9±0.6
S. typhimurium	10.0±2.0	9.3±0.8	8.5±0.6	7.6±0.5
K. pneumoniae	9.5±0.6	8.2±0.5	7.0±0.4	6.8±0.7
Chloroform seed				
S. aureus	13.5±1.6	11.3±1.3	9.5±0.7	6.5±0.7
P. aeruginosa	11.0±0.8	10.5±0.6	9.0±0.5	7.5±0.3
S. typhimurium	9.5±0.7	9.0±0.6	7.5±0.6	-
K. pneumoniae	9.5±0.8	8.3±0.3	7.0±0.2	-
S. aureus	8.2±0.8	7.4±0.6	7.0±0.5	6.5±0.3
Aqueous seed				
P. aeruginosa	-	-	-	-
S. typhimurium	7.5±0.6	7.0±0.8	6.5±0.2	-
K. pneumoniae	-	-	-	-

No significant difference between means of samples at 5% level of significance (p>0.05), Mean±Standard deviation

standard antibiotic, cefotaxime. In comparison to cefotaxime, the methanolic extract showed maximum of 65.21% activity against *P. aeruginosa* while chloroform and aqueous extracts had maximum activity of 54 and 34.89%, respectively, against *S. aureus* (Fig. 4a-c). The MIC values varied from 1.56-45 mg mL^{-1}. The MIC results are summarized in Table 3.

In the present study, *S. aureus* was found to be the most sensitive test organism to seed extract of *H. integrifolia*. It may be due to its Gram-positive nature. Generally Gram-positive bacteria are more susceptible to commercial antibiotics, crude extracts and isolated compounds from natural sources, which may be related to cell wall structure. According to Tortora *et al*. (2001) the cell wall

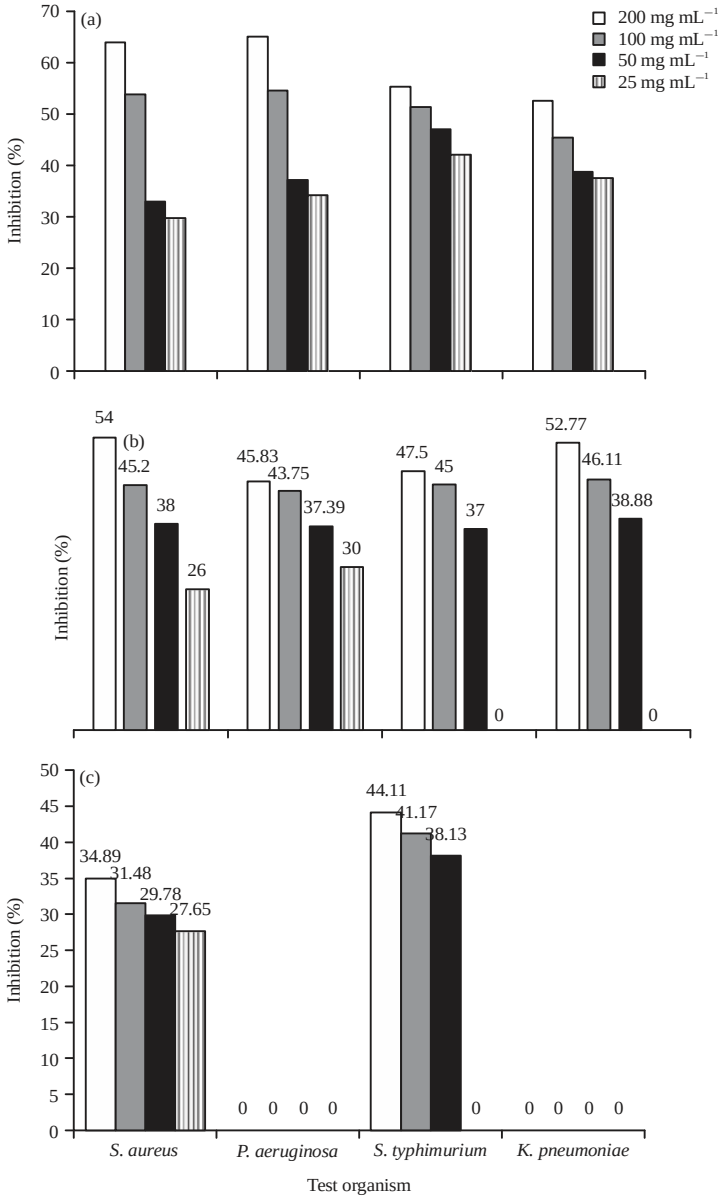


Fig. 4(a-c): Antibacterial activity of (a) Methanolic seed (b) Chloroform seed and (c) Aqueous seed extract of *Holoptelea integrifolia*, S: *Staphylococcus*, P: *Pseudomonas*, S: *Salmonella* and K: *Klebsiella*

Table 3: Minimum inhibitory concentration values of different seed extracts of *Holoptelea integrifolia*

	Minimum inhibitory concentration (mg mL^{-1})			
Extracts	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Salmonella typhimurium*	*Klebsiella pneumoniae*
Methanolic seed	1.56	3.125	6.25	6.25
Chloroform seed	2.34	4.68	35.00	35.00
Aqueous seed	12.5	-	45.00	-

of Gram-negative bacteria acts as a barrier to a number of substances, including antibiotics. It was also found that the same plant extract was effective against both Gram-positive as well as gram- negative bacteria. According to Kostova and Dinchev (2005) the effectiveness of plant extract against both types of bacteria may be due to the presence of a wide spectrum of bactericidal substances, or the action of toxins produced by the plant.

Methanolic seed extract was the dominant one for the antibacterial activity in the present study. Tomar and Tomar (2015) also reported better antibacterial activity of alcoholic leaf extracts of *Holoptelea integrifolia* than acetone and aqueous extracts. The better antibacterial activity in the methanolic extract may be due to the presence of more phytochemicals, extracted in methanol from *H. integrifolia* seeds.

All the test extracts worked in a dose dependent manner, as the concentration of the extract was decreased the activity also decreased. This is due to susceptibility of the pathogen towards concentration of the extracts, after which the extract damages that microbe which is not tolerable for it (Ordonez *et al*., 2006).

Plants have a wide range of compounds which are responsible for bioactivity. Presence of phenols, alcohols, aldehydes, amines, phosphines, nitriles, aliphatic compounds, azides, esters, β-lactones, amino acids and carboxylic acids etc., in the seeds of *H. integrifolia*, as indicated by FT-IR analysis, might be responsible for the present antibacterial activity. These medicinally bioactive components exert antimicrobial action through distinct mechanisms.

Results regarding the antibacterial activity of the seed extracts are in concordance with other findings that the *H. integrifolia* had strong antibacterial activity against a wide panel of tested pathogenic bacteria (Vinod *et al*., 2010; Durga and Paarakh, 2011; Ahmad *et al*., 2012; Hallikeri *et al*., 2013). In addition, Reddy *et al*. (2008) have also reported similar range of antibacterial activity (9.6-14.9 and 11.3-20.4 mm) in methanolic leaf and stem bark extracts of *H. integrifolia*, respectively. The results of our study are promising in the aspect of a new drug discovery from plant sources. *Pseudomonas aeruginosa* has emerged as one of the most troublesome gram-negative pathogens, with the alarmingly high antibiotic resistance rates (Savas *et al*., 2005). Even with the most effective antibiotics against this pathogen, the resistance rates were detected at 15-20.4% amongst 152 *P. aeruginosa* strains. This pathogen was found to be sensitive to the crude extracts of *H. integrifolia* seeds.

CONCLUSION

The plant investigated in the present study is a rich source of antibacterial compounds, with the extracts exhibiting strong antibacterial activity. With regard to the functional group of compounds, the extracts showed C=O and -OH as the major groups, which indicate the presence of amides, esters, β- lactones, phenolic and alcoholic compounds. The results may serve as a scientific basis to develop safe and effective drugs for the treatment of some infectious diseases. However, additional studies, especially with regard to *in vivo* toxicity, should be conducted.

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