Bulletin of Advanced Scientific Research 02 [03] (2016) e-ISSN: 2454-3691
www.asdpub.com/index.php/basr

Original Article

Analysis of components in antimirobially effective plant extract of *Pongamia pinnata* by Infra-Red spectroscopy

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Abstract

Plants have been used since olden times, to cure human beings of ailments caused by pathogenic micro-organisms. *Pongamia pinnata* seed extract was found antimicrobially effective against many of the tested pathogenic isolates. It was separated into two components by Thin Layer Chromatography. The aqueous seed extract and the two isolated components were analysed for their inherent ingredients by Infra-Red Spectroscopy. The active chemical groups were interpreted with the help of standard references. They could be responsible for the anti-microbial activity.

Keywords: Pathogenic micro-organisms, antimicrobially effective, Thin Layer Chromatography, inherent ingredients, Infra-Red spectroscopy, anti-microbial activity

1. Introduction

Varieties of herbs have been tried out for the treatment of several severe bacterial diseases. Literature available reveals the positive effects of the medicinal plants against a number of Gram-positive and Gram-negative bacteria [1]. They are promising candidates for development as chemotherapeutic agents and protect against infections and malignancy [2]. Medicinal plants might prove to be prospective potential sources of antimicrobial agents, probably even against some antibiotic resistant strains [3].

The present study has been done considering the advantages of medicinal plants. Seed extract of *Pongamia pinnata* was selected for this investigation. Each part of *P. pinnata* like seeds, flowers, fruits and leaves finds varied uses in the life of mankind. This plant is found to be the anti-microbially efficient. Antimicrobial activity of the aqueous seed extract of *P. pinnata* was tested against commonly occurring pathogenic isolates and was found to be effective. The seed extract was also tested against the pathogens isolated from swab samples of burns wounds. The antimicrobially effective aqueous seed extract of *P. pinnata* was subjected to TLC, its components separated and checked for antimicrobial activity against burns swab isolates. The aqueous *P. pinnata* seed extract and its components were analysed for identification of their active chemical groups by IR spectroscopy.

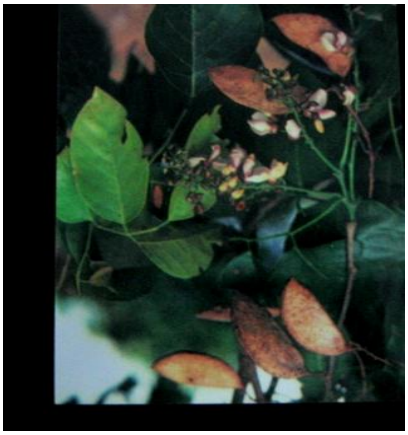
Infrared spectroscopy involves the interaction of infrared radiation with matter. It covers techniques, mostly based on absorption spectroscopy. It can be used to identify and study chemicals. For a given solid, liquid or gaseous sample, the technique uses an infrared spectrometer to produce an infrared spectrum, a graph of infrared light absorbance on the vertical axis vs. frequency or wavelength on the horizontal axis.

The infrared spectrum of a sample is recorded by passing a beam of infrared light through the sample. When the frequency of the IR is the same as the vibrational frequency of a bond or collection of bonds, absorption occurs. Examination of the transmitted light reveals the extent of energy absorbed at each frequency (or wavelength), scanning the wavelength range using a monochromator. This technique commonly analyses samples with covalent bonds. Samples with few IR active bonds show simple spectra and high levels of purity. More complex molecular structures lead to more absorption bands and more complex spectra.

2. Material and Methods

2.1 Preparation of seed extract

Known weight of *P. pinnata* seeds was washed with sterile distilled water, crushed in a grinder, to a very fine paste using a known amount of sterile distilled water (Fresh seeds 90 gm, sterile distilled water 70 ml, dry weight of the aqueous powder 27.90 gm, weight of powder extract 0.3100 i.e. 0.31 X 10^2 gm%). The material was filtered through a muslin cloth and shadow dried. The fine powder obtained was used as the sample for investigation [4].



P. pinnata

2.2. Pathogenic isolates used for antimicrobial activity

Common infection causing organisms isolated during the course of investigation included four Gram positive bacteria, eight Gram negative bacteria, one yeast and one fungal culture. They were identified as *Bacillus subtilis, Staphylococcus aureus, Enterococcus fecalis, Micrococcus luteus, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Salmonella paratyphi B, Shigella flexneri, Pseudomonas aeruginosa, Proteus vulgaris, Serratiamar sescens, Candida albicans* and *Aspergillus niger.*

Six isolates from wound swabs of burns patients were identified as *Bacillus cereus, Staphylococcus aureus, Enterococcus fecalis, Pseudomonas aeruginosa, Bacillus subtilis* and *Streptococcus pyogenes.*

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2.3. Separation of the *P. pinnata* seed extract into its components by Thin Layer Chromatography (TLC)

TLC was carried out to verify qualitative presence of constituents in herbal extract under investigation. Stationary phase included pre-coated silica gels, about 5 mm thick. 0.1 ml samples were used. Solvent system (Butanol: Acetic acid: Water: 40: 10: 50) was used for separation. Locating was done by spraying with a developing reagent (0.5% ninhydrin). Sample was separated in the form of coloured spots on the plate [5]. For further study and identification, the developed spot areas were scraped, dissolved in acetone, filtered and dried powder obtained in the filtrate used.

2.4. Sensitivity study of the TLC separated components

Sensitivity study of the components in the effective plant extract separated by TLC was carried out against the isolates from wound infection in burns patients by agar cup diffusion assay method.

2.5. Analysis of antimicrobially active components of the extract by Infra-red (IR) spectroscopy

Analysis of the antimicrobially effective *P. pinnata* seed extract and TLC detected components of the extract was done by IR spectroscopy (Jasco FTIR – 410 – sr. no. AO 11960585) to know its active chemical groups. The groups were identified using standard references, according to their wave numbers and percentage transmission [6].

2.6. Sample preparation for IR spectroscopy:

Liquid samples to be analysed are sandwiched between two plates of a sodium chloride or common salt. The plates are transparent to the infrared light and do not introduce any lines onto the spectra.

3. Observations and results

Aqueous seed extract (0.31 X 10^2gm%) of *P. pinnata* was found effective in inhibiting the growth of Gram positive *B. subtilis*, *S. aureus, Ent. fecalis, M. luteus,* Gram negative *E. coli, Kl. pneumoniae, Sal. typhi, Sh. Flexneri, C. albicans* and *Asp. niger*. In primary screening, response of organisms from swab samples of burns wounds showed that, out of ten, six were sensitive to the aqueous seed extracts of *P. pinnata.*

Preliminary qualitative chemical investigation of the aqueous extract showed the presence of alkaloids, flavonoids, glucose and proteins. Primary screening results of swab samples from burns wounds to aqueous *P. pinnata* seed extract was encouraging. In an additional study, conducted to support the antimicrobial efficacy of the plant extracts against the samples from the burns patients, it was observed that, aqueous extract of *P. pinnata* was found to be effective against six of the burns swab samples.

The extract and its components were analysed for identification of their active groups by IR spectroscopy. The aqueous extract of PP showed the probable presence of hydroxy (O-H $_{Str)}$, aliphatic (C-H $_{Str}$), carbonyl (C=O $_{Str)}$, amide (C=O $_{Str)}$, aromatic nitro / carboxylic acid groups. The first component - PP_1 indicated the presence of aliphatic (C-H $_{Str}$), amide (C=O $_{Str}$), primary and secondary amines (N-H_{def}) and the second component - PP_2 showed N-H_{str}/ O-H_{str}, C-H $_{Str}$, -C = O - O and weak carbonyl, (C=O-NH), carbonyl amide groups (Tables 1,2,3; Graphs 1,2,3).

It was reported that some *N*-substituted amides of long-chain fatty acids showed antimicrobial activity [7]. Sulphanilamide group has all the structural pre-requisites for anti-bacterial action [8]. All members of sulfonamide group differ in the nature of N substitution, which governs solubility, potency and pharmacokinetic property. A free amino group in the para position is required for anti-bacterial activity. Amide (NH_2) group has variable effects on antibacterial activity of a molecule. The aromatic Nitro group, Nitrobenzene group is responsible for antimicrobial activity [9].

Analysis of antimicrobially active components of the extract and its components by Infra-red (IR) spectroscopy Analysis of the antimicrobially effective *P. pinnata* seed extract (PP) and TLC detected components of the extract was done by IR spectroscopy (Jasco FTIR – 410 – sr. no. AO 11960585) to know its active chemical groups. The groups were identified using standard references, according to their wave numbers and % transmission [6].

The antimicrobially effective seed extract of *Pongamia pinnata* (PP) and its component fractions, PP_1 and PP_2 (detected by TLC) were analysed by IR spectroscopy. The active chemical groups were interpreted with the help of standard references [6].

PP, PP_1 and PP_2 were tested for antimicrobial activity against burns swab samples, isolates from the sample and were found effective. The aqueous extract PP showed the probable presence of hydroxy (O-H $_{Str)}$, aliphatic (C-H $_{Str}$), carbonyl (C=O $_{Str)}$, amide (C=O $_{Str)}$, aromatic nitro / carboxylic acid groups. The first component - PP_1 indicated the presence of aliphatic (C-H $_{Str}$), amide (C=O $_{Str}$), primary and secondary amines (N-H_{def}) and the second component - PP_2 showed N-H_{str}/ O-H_{str}, C-H $_{Str}$, -C = O - O and weak carbonyl, (C=O-NH), carbonyl amide groups (Tables 1,2,3; Graphs 1,2,3).

Table 1: Data of spectral analysis of PP on IR spectra

Peak Number	Wave Number (cm. $^{-1}$)	Peak Height (% Transmission)	Probably Present Groups
9.	3395.55	41.6340	O-H $_{Str}$(Hydroxy)
10.	2924.04	34.1341	C-H $_{Str}$(aliphatic)
11.	2853.17	47.8047	C-H $_{Str}$(aliphatic)
12.	1746.23	45.1590	C=O $_{Str}$(carbonyl)
13.	1652.70	31.5888	C=O $_{Str}$(amide)
14.	1558.68	28.8028	Aromatic Nitro / carboxylic acid

Graph 1: IR spectral analysis of PP

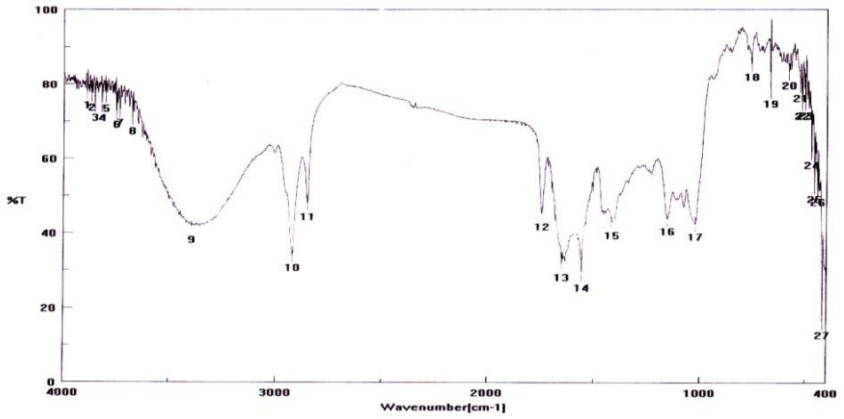

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Table 2: Data of spectral analysis of PP_1 on IR spectra:

Peak Number	Wave Number(cm $^{-1}$)	Peak Height (% Transmission)	Probably Present Groups
33.	2925.97	79.4245	C-H $_{Str}$(aliphatic)
37.	1693.68	87.6764	C=O $_{Str}$(amide)
38.	1662.82	85.6236	C=O $_{Str}$(amide)
39.	1596.29	81.3126	N-H_{def} Primary and secondary amines

Graph 2: IR spectral analysis of PP $_1$

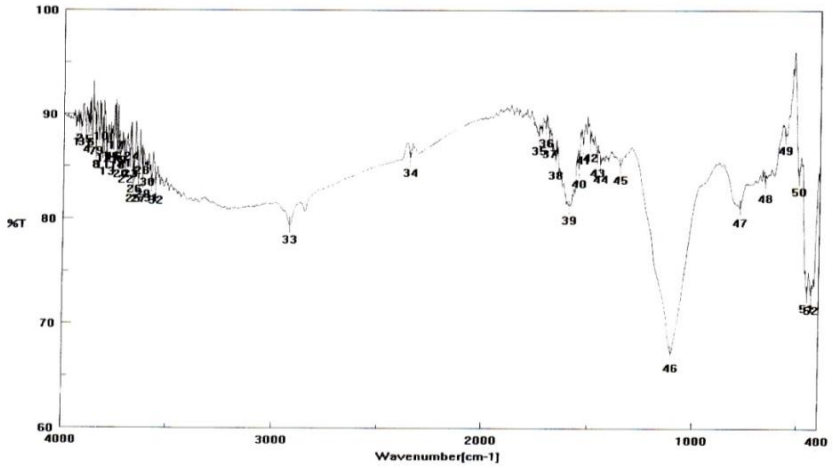
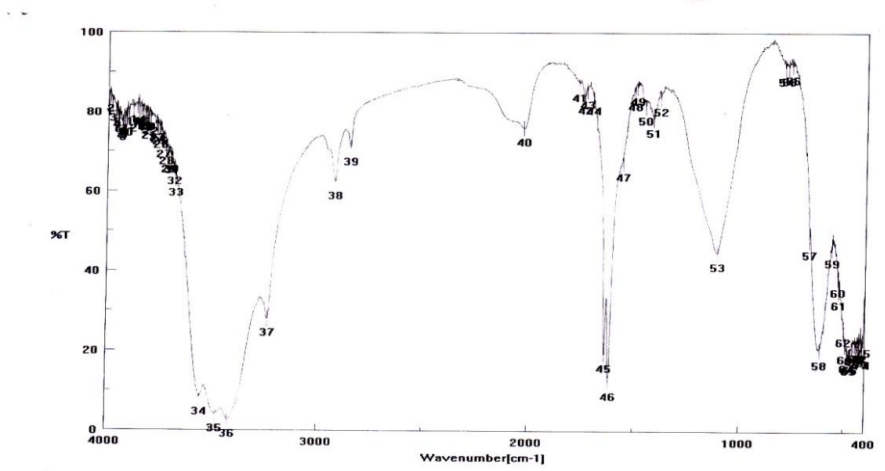


Table 3: Data of spectral analysis of PP_2 on IR spectra

Peak Number	Wave Number (cm $^{-1}$)	Peak Height (% Transmission)	Probably Present Groups
34.	3550.31	8.1564	N-H_{str}/ O-H_{str}
35.	3477.03	3.9753	N-H_{str}/ O-H_{str}
36.	3416.76	2.6374	N-H_{str}/ O-H_{str}
37.	3235.00	27.9643	N-H_{str}/ O-H_{str}
38.	2924.04	62.3912	C-H $_{Str}$
39.	2852.68	70.9834	C-H $_{Str}$
40.	2029.23	75.9759	-C = O -O (weak, carbonyl)
45.	1637.75	19.2059	C=O-NH (carbonyl amide)
46.	1617.50	12.1869	C=O-NH (carbonyl amide)

Graph 3: IR spectral analysis of PP $_2$



4. Conclusion

P. pinnata is anti-microbially active and its components when analysed using IR spectroscopy showed the presence of groups which may be responsible for its anti-microbial nature. The study is compatible with certain references in this context.

The seed extract of *P. pinnata* Linn with methanol and ethanol solvent at 100µg/ml concentration showed significant antibacterial activity on selected pathogens in clinical isolates [10].

A study of oil content and fatty acid composition of seed powder and seed oil of *P. pinnata* by near infrared spectroscopy by Near

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infrared reflectance spectroscopy (NIRS) coupled with a geometric study was used to estimate the oil content and fatty acid composition of *P. pinnata* seed powder and seed oil. Calibration equations showed the presence of individual fatty acids (palmitic acid C16:0, stearic acid C18:0, oleic acid C18:1, linoleic acid C18:2, and behenic acid C22:0) in seed oil [11]. *P. pinnata* seeds have also been qualitatively tested for their use as biodiesel. Attempt to compile and document information on different aspect of *P. pinnata* and its potential use as a source of biodiesel was made [12].

Further quantitative analysis may throw more light on the build-up!

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