ejpmr, 2017,4(1), 322-326

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

FT-IR ANALYSIS AND *IN VITRO* ANTIBACTERIAL ACTIVITY OF *PROSOPIS JULIFLORA*

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Article Received on 10/11/2016 **Article Revised on 01/12/2016** **Article Accepted on 22/12/2016**

ABSTRACT

Prosopis juliflora, an Indian Mallow has been used in the treatment of various diseases in different parts of the world since time immemorial. The present investigation was carried out to find the effect of methanolic extracts of flowers of *Prosopis juliflora* through FT-IR Spectroscopy method and its antibacterial activity. The FT-IR analysis confirmed the presence of phenol, alcohols, alkanes, carboxylic acids, ketones, alkenes, aromatics, ethers, and aliphatic amines compounds, which showed major peaks. The anti bacterial activity of the methanolic extracts of *Prosopis juliflora* (25,50,75,100, 200,300mg/ml) was also tested against two human pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus.* The anti bacterial potent of the extracts was found to be dose dependent. The phytochemical analysis of the plant was carried out. The results revealed the quantitative analysis of following classes of natural constituents: flavonoids, alkaloids, saponins, phenols and tannins in the range of (25%), (15%), (10%), (22%) and (18%), respectively. The crude fiber and ash content were also analyzed and found as (12%) and (7%), respectively whereas, pectic substances were calculated as (12%). The activity of the flower of *Prosopis juliflora* was due to the presence of various secondary metabolites. Hence the plant can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities.

KEYWORDS: *Prosopis juliflora*, FT-IR analysis, *in vitro* antibacterial activity.

INTRODUCTION

Plants are potent biochemists and have been components of phytomedicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc i.e. any part of the plant may contain active components. The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories. Scientific analysis of plant components follows a logical pathway. Plants are collected either randomly or by following leads supplied by local healers in geographical areas where the plants are found (Parekh *et al.*, 2006).

Fourier Transform-Infra Red Spectroscopy (FT-IR): this is one of the most widely used methods to identify the chemical constituents and elucidate the compounds strutures and has been used as requisite method to and identify medicines in pharmacopoeias of many countries. FT-IR a quick andeffective analysis method used for the complicated mixture syatem had played an important role in pharmaceutical analysis in recent years (Mu and Shi, 2002; Druy, 2004; Sohrabi *et al.*, 2005). Thus, the application of IR Spectroscopy in herbal analysis is still very limited compared to its application in other areas (Food and beverage, Pharmaceutical) (Chewoon sim *et al*., 2004).

Medicinal plants are rich sources of antimicrobial agents. Plants are used medicinally in different countries and are the source of potential and powerful drugs (Srivastava *et al*., 1996). According to World health organization (WHO) more than 80% of the world population relies on traditional medicine for their primary health care Needs (Diallo *et al*., 1999). The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the north east, but it is thoughtless as art as old as mankind (Mahesh and Satish, 2008). The potential of higher plants as a source for new drugs is still largely unexplored.

Man has used plants to treat common infectious diseases, and some of the traditional medicines are still included as part of the habitual treatment of various maladies (Heinrich *et al*., 2004; Rios *et al*., 2005). Scientific interest in medicinal plant has burgeoned in recent times due to increased efficiency of new plant derived drugs

and rising concerns about the side effects of modern medicine. It is a well known fact that intensive use of antibiotics often followed the development of resistant strains (Ahmad *et al*., 1987). The continuing emergence of drug resistant organisms and the increasing evolutionary adaptations by pathogenic organisms to commonly used antimicrobials have reduced the efficacy of antimicrobial agents currently in use. This propensity of drug resistance requires the search for new, effective and safe drugs. Therefore, the search for new drugs from plants continues to be a major source of commercially consumed drugs. Even most synthetic drugs have their origin from natural plant products (Sofowara, 1982). The scientific analysis of medical plants has led to the discoveries of many important drugs (Pelletier *et al*., 1817; Ahmad *et al*., 1992). Hence the present investigation is to screen the of *Prosopis juliflora* for *in vitro* antibacterial activity.

Prosopis juliflora is an evergreen tree with a large crown and an open canopy, growing to a height of 5-10m. stem green-brown, sinuous and twisted, with axial thorns situated on both sides of the nodes and branches. Flowers lateral to the axis with a tubular, light greenish-yellow, 1.5 mm wide calyx with hooded teeth; corolla light greenish-yellow, composed of 5 petals with 3 mm. Pharmacological properties under in vitro study shown that antimicrobial, antifungal, anti- inflammatory and hemolytic activities attributed to leaves extract of *Prosopis juliflora*. Additionally; cytotoxic, antitumoral activity against human epithelial tumor cell (HeLa), human hepatic tumor (HepG2) (Raghavendra *et al*., 2009). *Prosopis juliflora* is native to tropical America, but is naturalized in many countries including Egypt and India. (Basavaraja *et al.,* 2006). Extracts of *P. juliflora* seeds and leaves have several in vitro pharmacological effects such as antibacterial (Ahmad, *et al*., 1986; Aqeel *et al*., 1989; Kunthasamy *et al*., 1989; Caceres *et al*., 1995; Al-Shakh-Hamed *et al*., 1999) antifungal (Kursheed *et al*., 1989) and anti-inflammatory properties.These properties have been attributed to piperidine alkaloids (Batatinha, 1997) It is also known for its ethno-medicinal properties, mainly used for boils, rheumatic pain, digestive disturbances (Vyas, 2002).

The main goals of this study are to analyse the functional groups of phytoactive compounds present in the methanol extracts flowers of *Prosopis juliflora* by FTIR spectroscopic analysis and study the *in vitro* antibacterial activity of methanolic extract of *Prosopis juliflora* against bacterial species such as *Escherichia coli* and *Staphylococcus aureus* by disc diffusion method and compare the activity using *amoxycilin* as standard drug.

MATERIALS AND METHODS

Plant Collection and Identification

The plant species namely *Prosopis Juliflora* flowers were collected in and around Mannargudi, Thiruvarur (Dt), Tamil Nadu.

Preparation of plant powder

The plants were air dried under shade for 10-15 days. Then the dried materials were grinded to fine powder using an electric grinder and stored in air tight bottles. The powder matter was used for phytochemical analysis, *in vitro* antibacterial activity.

Extraction of Plant Material

Methanol extracts were prepared according to the methodology of Indian pharmacopoeia (Anonymous, 1996). The coarse powder material was subjected to soxhlet extraction using methanol. The extracts were concentrated to dryness in flash evaporator under reduced pressure controlled at a temperature (40°C-50°C) and stored in air tight container stored in refrigerator.

Preliminary Phytochemical Screening

Qualitative phytochemical analysis was carried out for all the extracts as per the standard methods of Kokate *et al.* (1995).

Quantitative phytochemical analysis

Determination of flavonoids, (Okwu and Ukanwa, 2007); alkaloids, (Poornima and Ravishankar, 2009); saponins, (Aliyu, 2008); phenols, (Hussain *et al.*, 2011); tannins, (Price and Butler, 1977); crude fibre, (Patil and Gaikwad, 2011); Ash content (Anon, 1973) and pectic substances, (Ranganna, 1979).

Fourier Transform Infrared Spectrophotometer (FT-IR)

Dried powder of methanol extract of *Prosopis juliflora* flowers was used for FT-IR analysis. FT-IR analysis was performed using Perkin Elmer spectrophotometer system which was used to detect the characteristic peaks and their functional groups. The peaks values of the FT-IR were recorded.

In vitro antibacterial activity

The antibacterial activity was performed by disc diffusion method.

RESULTS AND DISCUSSION

Phytochemical screening

In the present study, the investigation of phytochemical screening of Methanol extract of leaves in *Prosopis juliflora*. The results revealed that the methanolic extract of *Prosopis juliflora* recorded the presence of alkaloid, flavonoid, phenol, tannins, saponins, phytosterol and glycosides followed by other extract (Table1).

Quantitative phytochemical analysis

The air dried leaves of *Prosopis juliflora was* analyzed for its chemical composition and to the best of our knowledge proximate analyses of the leaves of this plant has not been carried out. The results revealed the presence of following important classes of natural constituents such as flavonoids, alkaloids, saponins,

phenols and tannins. The crude fiber and ash content were also analyzed and quantified (Table 2).

Table 1: Preliminary phytochemical screening of *Prosopis juliflora*

S. No.	Phytochemicals	Methanolic Extract of *Prosopis juliflora*
1.	Alkaloid	+
2.	Flavonoid	+
3.	Terpenoid	-
4.	Tannins	-
5.	Phenol	+
6.	Saponin	-
7.	Phytosterrol	+
8.	Glycoside	+

+ = presence
- = absence

Table 2: Quantitatvie phytochemical analyses of *Prosopis juliflora*

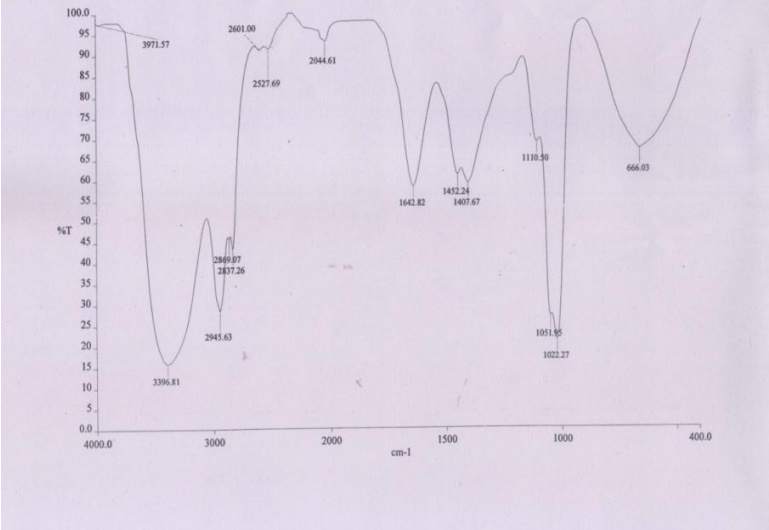
S.No.	Phytochemicals	Concentration in %
1.	Flavonoids	25g
2.	Alkaloids	15g
3.	Saponins	10g
4.	Phenols	22g
5.	Tannins	18g
6.	Crude fiber	12g
7.	Ash content	7g
8.	Pectic substances	13g

FT-IR ANALYSIS

The FT-IR spectrum was used to identify the functional groups of the active components present in extract based on the peaks values in the region of IR radiation. When the extract was passed into the FT-IR, the functional groups of the components were separated based on its peaks ratio. The results of FT-IR analysis confirmed the presence of alcohol, alkanes, aromatic carboxylic acid, and halogen compound, alkyl halide (Table 3 and Figure-1). FT-IR analysis concludes with a graph of 15 major peaks indicating the vital functional groups. Secojuliprosopinal (Choudhary *et al*., 2005). Juliflorine, Juliprosine (Ahmad *et al*., 1989), Isojuliprosine, Juliprosinene (Hiroshi *et al*., 2004) are the major alkaloids found in leaf of *Prosopis juliflor* (Muhammad Ibrahim *et al*., 2013). Structures of the alkaloids include functional groups such as primary amines, alcohols, alkenes, aromatics. FTIR analysis of AEP reveals the presence of above mentioned functional groups in the wavenumber range 3423.65, 2924.09, 1625.99, 1419.61, 1118.71 of phenols, alkanes, primary amines, aromatics, alkyl halides indicating that AEP might have contain Juliflorine, Juliprosine, Juliprosinene. Thus this acetone extract was rich in alkaloids (Dhananjaya Seturama Prabha *et al*., 2014).

Table 3: FTIR spectral peak values and functional groups obtained for the flower extract in methanol of *Prosopis juliflora*

Extracts prepared in	Peak values	Functional groups
Methanol	3971.57	Phenols
	3396.81	Phenols and Alcohols
	2945.63	Alkanes
	2869.07	Alkanes
	2837.26	Alkanes
	2601.00	Carboxylic acid
	2527.69	Aromatics
	2044.61	Alkanes
	1642.82	Ketones
	1452.24	Aromatics
	1407.67	Aromatics
	1110.50	Ethers
	1051.95	Aliphatic amines
	1022.27	Aliphatic amines
	666.03	Alkanes



Figrue 1: FTIR spectrum of Methanolic extract of *Prosopis juliflora*

Antibacterial activity

The antibacterial activity of methanolic extracts of *Prosopis juliflora* were studied in different concentrations (25mg/ml, 50mg/ml, 75mg/ml, 100mg/ml, 200mg/ml 300mg/ml) against two pathogenic bacterial strains (*Escherchia coli*, *Staphylicoccus aureus*). Antibacterial potential of methanolic extracts was assessed in terms of zone of inhibition of bacterial growth. The results of the antibacterial activity are presented in (Table 4).

Table 4: Antibacterial activity of methanolic extracts of *Prosopis juliflora* against bacterial strains

S.No.	Test Microorganisms	Zone of inhibition in mm					
		25 mg	50 mg	75 mg	100 mg	200 mg	300 mg
1.	*Escherichia coli* (mm)	14± 0.28	14± 0.28	16± 0.42	17± 0.49	19± 0.56	22± 0.84
2.	*Staphylococcus aureus* (mm)	13± 0.25	14± 0.28	15± 0.35	15± 0.35	17± 0.49	20± 0.70

The inhibitory effect of *Prosopis juliflora* plant methanolic extracts showed at 25, 50, 75, 100, 200, 300mg/ml were (14, 14, 16, 17, 19, 22mm) for *E. coli* and (13, 14, 15, 17, 20mm) for *Staphylococcus aureus* for bacterial strains. The results showed that *Prosopis juliflora* extracts were found to be more effective against all the bacterial species tested and the methanolic extract showed greater zone of inhibition.

From the results obtained in the present study, it can be concluded that the flower extracts in methanol of *Prosopis juliflora* their phytoconstituents may act as source of antibiotics. Further research will be needed to findout the structural analysis of flavonoid compound by use of different analytical methods such as NMR and Mass spectrophotometer.

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