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Original Article

Evaluation of Antibacterial Activity Methonolic Extract Prosopis Juliflora Bark

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ABSTRACT

The present study aims to evaluate the antibacterial activity of the methanolic extract derived from the bark of Prosopis juliflora, a plant traditionally recognized for its medicinal properties. The bark extract was prepared using methanol as the solvent through cold maceration. The antibacterial efficacy was assessed against selected Gram-positive and Gram-negative bacterial strains using the agar well diffusion method. The results revealed a significant zone of inhibition, particularly against Staphylococcus aureus and Escherichia coli, indicating potent antibacterial potential. These findings support the traditional use of Prosopis juliflora bark and suggest its potential role as a source of natural antibacterial agents.

Keywords: Prosopis juliflora, antibacterial activity, methanolic extract, bark, medicinal plant, zone of inhibition, phytochemicals

INTRODUCTION

Natural products and their role in drug discovery:

Natural products are defined as the secondary metabolites and byproducts derived from natural sources such as microorganisms, plants and animals (Baker et al., 2000). Some of the natural products derived from animals include chitosan and lysozyme, from plants flavonoids, alkaloids and essential oils and from microbes penicillin, streptomycin and bleomycin (Tiwari et al., 2009). These products have been used and explored for various purposes from thousands of years. In particular, plants are being used as a source of medicine since ancient times. Ancient man discovered the medicinal plants and their curative actions by intimate monitoring the effect of specific plant on sick animal after eating a particular plant or its parts. Even nowadays plant-based medicines and formulations are used by large number of people around the globe (Bensky and Gamble, 1993). Thus, natural products are the traditional path finder compounds contributing an untold diversity of chemical structures (Strobel and Daisy, 2003). Plants have been used for centuries as remedies for human diseases and suggest a new source of biologically active molecules as antimicrobial agent. Medicinal plants represent the richest source of drugs which are used in traditional medicinal systems, nutraceuticals, modern and folk medicines, food supplements and pharmaceuticals (Hammer et al., 1999). It has been estimated that 14-28% of higher plant species are used medicinally and 74% of pharmacologically active plant derived components were discovered after following up on ethno medicinal applications of the plants.

Prosopis juliflora:

an invasive traditional medicinal plant Medicinal plants have been used as therapeutic tools to treat a number of microbial infections caused by viruses, bacteria, fungi and protozoans (Holetz et al., 2002). Furthermore, owing to the growing inefficiency of many synthetic products, unaffordable costs and collateral effects, created a great interest in medicinal plants for the discovery of new antimicrobial compounds. Thus in the present study the plant selected is Prosopis juliflora (Sw.) D.C. The plant has been used in traditional and folk medicine by its native tribes. It is an important species of genera Prosopis, commonly known as “algarroba” or “mesquite”, “Vilayati Kher” (Azhar, 1998) (Fig. 1.1). The plant is native of Mexico, South America, and Caribbean. It has invaded many parts of world including Asia, Australia and Africa by contending

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with native species and is considered as an important weed (Gibbon, 2005). 1.2.1. Classification Class- Dicotyledonae Order-Leguminosae Family- Mimosoideae Genus - Prosopis Species- P. juliflora (Sw.) DC.

Fig. 1.1: *Prosopis juliflora* (Sw.) DC. -Habit

Taxonomy:

The word Prosopis was derived from Greek terminology and it refers to ‘a kind of prickly fruit’ (Burkart, 1976), 'bardane' (Allen and Allen, 1981) and ‘the plant being available in abundance’ (Perry, 1998). The word julus refers to “whiplike” and flora to “flower” (Perry, 1998). The long whip like inflorescence bearing full of flower is the characteristic of the species, hence the name juliflora. Stem is thorny in nature with characteristic catkin inflorescence and belongs to Fabaceae due to the presence of legume fruit.

Geographical distribution:

P. juliflora is distributed throughout the world. It is a native of Central America, South America, Caribbean islands, Panama, Mexico and Northern Peru (Burkart, 1976). Later introduced to other regions of America, North-East Brazil, Bolivia, El Salvador, Nicaragua, Uruguay, West Indies, Colombia, Bahamas and Venezuela. In the last decades, the plant has occupied in all arid regions of world from Africa to South west Asia, North and South America (Ecoport, 2010; Pasiecznik et al., 2001) (Fig. 1.2). Dominant woody plant, spread around 45 million hectares of grazing lands worldwide from sea level to 2,900 m, in hot and dry regions with wide range of temperature between 14 - 48 °C receiving an average annual precipitation of 50–1200 mm (Ecocrop, 2010; Ecoport, 2010). The tree can grow in a variety of soil including saline, alkaline, sandy, and rocky soils with deeply penetrating roots (Orwa et al., 2009). In India, P. juliflora was declared the “Royal Plant” of the State of Jodhpur. The plant is naturalized in India and has spread all over the country, occupying large areas of wasteland (Muthana and Arora, 1983; Pasiecznik et al., 2001) (Fig. 1.3).

MORPHOLOGY:

P. juliflora is an evergreen tree, grows to an average height of 5-10 m. It has a large crown with an open canopy. Stem is green-brown, sinuous and twisted. Bark is rough and dull red with a deep tap root system. Branches and nodes are encircled by axial thorns on both sides. Compound leaves with 13-25 pairs of leaflets. Leaflets oblong (3 x 1.7 mm), dark green, pendulous and bipinnate with 2 pairs of rachis. Light greenish yellow inflorescence with lateral flowers. Calyx with hooded teeth 1.5mm wide and corolla bears 5 petals with 3 mm wide pubescent edges. Fruits are non-dehiscent pod, straight with one segment or divided into several segments. Seeds are hard, dark brown with mucilaginous endosperm surrounding the embryo; flat, rounded cotyledons, epigenous when germinating (Orwa et al., 2009; Pasiecznik et al., 2001).

Economic importance:

P. juliflora is recognized in arid and semi-arid zones of the world for its economic and ecological importance. It is a precious multipurpose tree which is commercial and easily available natural resource for everyone that can be used as fuel or as raw material. All the parts of plant are rich in proteins, carbohydrates, lipids and minerals (FAO, 1981). Pods of P. juliflora were used as foods by early man of new world in the prehistoric era. Pod flour is still admired by people of Amerindian tribes. Pods, Cotyledons and embryos of the plant are rich in protein and sugar. Sweeteners prepared out of the plant are good for diabetic people. Ripen pods contain 12-14% crude protein. Pods are used for the preparation of sweets, bread, syrup and coffee. Toasted seeds are used as tastemaker for coffee. In Argentina, Chile and Peru the pods are used in preparation of alcoholic drinks such as cocktails (Orwa et al., 2009). The pods are also used as feed for domestic animals. For dairy cows, 40-60% of the flour is mixed in their food. In South Africa, Sheep feed contain 90% pod flour. Pigs and poultry are given the fibrous parts of the pod (Orwa et al., 2009). Flowers of P. juliflora are the major source for Bee forage due to the copious nectar flow and good quality honey production. P. juliflora based apiculture is common in Western Australia, Jamaica, Bolivia, Pakistan and Sri Lanka (Pasiecznik et al., 2001). Stem is extensively used for firewood and charcoal production in USA and Northern Mexico. The fiber of the plant is used for the production of paper, paperboard and hardboard in several places. Woods are also used for fence posts, furniture and crafts. In Colombia, bark of the plant is used for roofing. Heartwood is used for extraction of polymeric resins. Exudates of stem and older branches produce a reddish-amber gum similar to arabica gum of Acacia senegal. The seeds produce galactomannan used as carob gum which has a number of applications as thickeners, stabilizers or gelifiers in many recipes (Orwa et al., 2009).

Secondary metabolites:

Large number of reports is available on the phytochemical study demonstrating the secondary metabolites of P. juliflora (Ahmad et al., 1989; Rastogi and Mehrotra, 1993; Khan et al., 2003; Singh et al., 2011). The phytochemical analysis of different parts has showed the presence of alkaloids, steroids, flavonoids, terpenoids, saponins, phenols, tannins, resins and gum. Leaves are the richest source of secondary metabolites containing large number of alkaloids, flavonoids and phenols (Singh, 2012). Pod and flower extracts have recorded the presence of secondary metabolites of biological importance (Singh et al., 2011). The presence of phenols, flavonoids, terpenes and steroids in low concentrations is reported in stem and roots extracts while saponins are reported only in roots (Singh, 2012). The best solvents for the extraction and isolation of secondary metabolites of P. juliflora includes Methanol (Ahmad, 1991; Raghavendra, 2007; HariPrasad et al., 2011; Sirmah et al., 2011), Ethanol (Silva et al., 2007; Napar et al., 2012; Singh et al., 2011; Singh, 2012), Hexane, Benzene and Acetone (Alsaadi and Maliki, 2014; Kipyegon et al, 2015).

Biological and Pharmacological properties:

of P. juliflora A number of folk remedies are documented by various ethnobotanist and sociologist. Traditional use as folk medicine has inspired several research groups in India and around the world to systematically investigate its beneficial claims and test for novel pharmaceutical properties of the plant. Some of the biological and pharmacological properties of P. juliflora are as follows:

a) Burn wound healing effect- P. juliflora has been reported to possess wound healing property. Studies have showed that the decoction prepared from leaf and seed extract are used as a disinfectant in wound healing. It is also used in several dermatological treatment-like sores, wounds, burns, chapped fingers and lips. Decoction of beans is useful in Sunburn treatment (Mathias et al., 1998; Logeeswari and Shubashini, 2012).

b) Immunomodulatory effect- Immune-modulating activity of leaves of P. juliflora has been reported by Ahmad et al., (1992); Dhyani et al., (2006).

c) Antioxidant effect- The antioxidant potential of different parts of P. juliflora is demonstrated by various workers. The flavonoid and phenolic acid present in the plant is responsible for antioxidant activity (Almaraz- Abarca et al., 2007). The heartwood showed the presence of Mesquitol and Catechin, which demonstrated superior antioxidant

activity than alpha-tocopherol (Sirmah et al., 2011). Phenolic extracts of leaves were studied for antioxidant activity using DPPH scavenging assay and reported proportionate dose dependent antioxidant activity from methanol extracts of leaves (Sathiya and Muthuchelian, 2010; Napar et al, 2012).

d) Anticancer activity- The pharmacological studies of P. juliflora has revealed that alkaloids and flavonoids fraction has the potential to check different types of cancers. Studies of Merzabani et al., (1979) and Ahmad and Sultana (1989) recorded significant activity against lung carcinoma and other types of carcinoma. The flavonoid patulitrin isolated from the flowers and fruits showed significant activity against lung carcinoma in vivo (Wassel et al., 1972). P. juliflora pod extract showed significant anticancer activity against human epithelial tumor cells (HeLa), human hepatic tumor (HepG2), and two fibroblast cell lines F26 and F57 (Batatinha, 1997). Total alkaloid extract from leaves of P. juliflora showed dose and time dependent cytotoxicity against human T-cell leukemia (Molt-4) cells in MTT assay determined by Sathiya and Muthuchelian, (2011).

e) Antibacterial activity- Numerous literature reports the antibacterial properties of different extracts of leaves, pod, flower and bark of P. juliflora. Methanol, ethanol and aqueous extracts showed significant antibacterial activities against Aggregatibacter actinomycetemcomitans, Bacillus subtilis, Bacillus cereus, Bacillus coagulans, Escherichia coli, Enterobacter aerogenes, Enterococcus faecalis, Klebsiella pneumonia, Micrococcus luteus, Prevotella intermedia, Porphyromonas gingivalis, Pseudomonas aeruginosa, Pseudomonas vulgaris, Staphylococcus aureus, Staphylococcus epidermis Streptococccus sp. Salmonella typhimurium, Salmonella typhi, Shigella dysentriae, S. agalactiae, Vibrio cholera (Kanthaswamy et al., 1989; Kay, 1996; Sathiya and Muthuchelian, 2008; Raghavendra et al., 2009; Seetha Lakshmi et al., 2010; HariPrasad et al., 2011; Napar et al., 2012; Sukritha and Growther, 2012; Sharma et al., 2012; Jesudoss et al., 2014; Srivastava et al., 2014; Tajbakhsh et al., 2015).extracts of leaves also showed inhibitory potential against plant pathogenic bacteria Xanthomonas axonopodis pv. malvacearum, Xanthomonas axonopodis pv. phaseoli, X. campestris pv. vesicatoria, Xanthomonas campestris and Agrobacterium rhizogenes, (Raghavendra et al., 2009; Sheikh et al., 2012).

f) Antifungal activity- The alkaloid fraction inhibited the growth of several dermatophytes at a concentration of 2.5 and 5 mg/ml. Alkaloidal fraction of P. juliflora and juliflorine exhibited antifungal activity against Trichophyton. Aqeel et al., (1989) studied the antifungal activity of alkaloid juliflorine isolated from P. juliflora against Candida tropicalis. In a recent study, the leaves extract showed significant growth inhibition of Candida albicans (Rechab et al., 2011). The leaves extract showed potential growth inhibition against a panel of clinical and environmental isolates. The studies also demonstrated the potential of the plant as a bio-fungicide. The antifungal activity of P. juliflora was studied against fungi such as, Alternaria alternata, Aspergillus niger, Fusarium oxysporum, Aspergillus fumigatus, A. candidus, A. columnaris, A. flavipes, A. flavus, A. fumigatus, A. niger, A. ochraceus, and A. tamarii, species of Colletotrichum and Fusarium, Mucor rouxii, Penicillium citrenigrum, (Ahmad et al., 1989; Kanthasamy et al., 1989; Kaushik et al., 2002; Satish et al., 2007; Raghavendra et al., 2009; Sheikh et al., 2012). Raghavendra, (2007) isolated a novel alkaloid Julifloravizole from leaves of P. juliflora and has demonstrated the broad-spectrum antifungal activity against species of Fusarium, Drechslera and Alternaria.

g) Anti-inflammatory activity- Several studies have showed the anti-inflammatory activity of P. juliflora (Ahmad et al., 1989; Kanthasamy et al., 1989). Anti-inflammatory activity of P. juliflora bark was studied using carrageenan and histamine induced paw oedema in acute models and formation granulation tissues by cotton pellets in chronic model in rats. Alkaloids from leaves reduced the formation of granulation tissues by cotton pellets in rats (Sivakumar et al., 2009). Choudhary and Nagori (2013) investigated on the use of P. juliflora in oral treatment to ameliorate the inflammatory responses against carrageenan induced paw edema in rats.

h) Antimalarial activity- Antiplasmodial activity of ethanol extracts of P. juliflora was studied by Ravikumar et al., (2012) against Plasmodium falciparum. Al-Musayeib et al., (2012) studied the antimalarial activity of methanol extract of the P.

juliflora collected from Saudi Arabia. Bansal et al., (2012) showed that the methanol and acetone extracts were effective against larvae of Anopheles stephensi, Aedes aegypti and culex quinquefasciatus exhibiting antimalarial activity.

i) Anthelmintic activity- Odhiambo et al. (2014) studied the anthelmintic activity of P. juliflora against gastroinstestinal nematodes. Due to the presence of tannins, saponins, and alkaloids and other chemical constituents, P. juliflora showed significant anthelmintic activity. Kipyegon et al. (2015) studied the ovicidal activity of encapsulated extract of P. juliflora on Haemonchus contortus eggs using egg hatch inhibition assay.

j) Analgesic activity- Analgesic activity of P. juliflora extract was studied in acetic acid induced writhing in mice. 250 and 500 mg/kg of ethanol extract of P. juliflora was administered to mice orally. The ethanol extract at concentration of 250 and 500mg/kg showed reduction in Writhing by 75.16% and 78.34% respectively (Gopinath et al., 2013a).

k) Antipyretic activity-The ethanolic extract of P. juliflora was evaluated for anti-pyretic activity by Gopinath et al. (2013b). The ethanol extract at the concentration of 250 mg/kg showed reduction in the rectal temperature after 3 h of treatment. A significant dose-dependent reduction in rectal temperature was reported.

l) Antihyperlipidemic activity
Antihypercholesterolemic effect of P. juliflora leaf powder, as a dietary supplement was investigated in hypercholesterolemic male albino rats. The study suggested that P. juliflora leaf possess antihyperlipidemic properties and reverses the hypercholesterolemic conditions

m) Antihyperglycemic activity- 4-(2'-Hydroxyethyl) phenol isolated from P. juliflora pods was tested on fasted alloxan induced diabetic rabbits. The oral administration of terpene compounds decreased the blood glucose level significantly in hyperglycemic rabbits in 4 hours (Alsaadi and Al-Maliki, 2014).

n) Antiprotozoal activity- The plant P. juliflora shows antitrypanosomal activity against both Trypanosoma cruzi and T. brucei (Al-Musayeib et al., 2012). It showed the highest selectivity index with pronounced activity against T. brucei with IC50 value 2.0 µg/mL, IS 24.9). Garbi et al. (2014)

evaluated the leaves extract of P. juliflora for Antigiardial and Amoebicidal activity. P. juliflora leaves petroleum ether and methanol extract (1000 ppm) was tested against Giardia lamblia. Petroleum ether extract and Methanol extract showed mortality of 78.91% and 71.97% respectively after 72 hours of treatment against Entamoba histolytica.

o) Cytotoxic effect- In 1989, Kandasamy et al., studied the effect of alkaloids isolated from P. juliflora on normal erythrocytes and on rat. Tabosa et al. (2006) reported similar neural damage and degeneration of nerves in the mandibular and trigeminal. Alkaloids present in pods of this plant are reported to be the main cause of neural damage. It also induces the cytotoxic effect and reaction in glial cells (Hughes et al., 2006; Silva et al., 2007). Tabosa et al. (2000) studied the cytotoxic effect of consumption of P. juliflora pods in goats. These studies have clearly shown that all parts of P. juliflora have the potential to produce bioactive compounds of biological importance. Thus P. juliflora can be explored for the discovery of novel antimicrobial compounds from different parts of the plant.

Table 1.1. Biological activities of aqueous and solvent extracts of different parts of *P. juliflora*

Plant part	Extraction Procedure	Solvent used	Bioactivity	Screening method/model	Reference
Leaves	Cold maceration	Methanol, Ethanol	Antibacterial	Disc diffusion	Singh *et al.*, 2011, Prasad *et al.*, 2011, Sathiya and Muthuchelian, 2008, Napar *et al.*, 2012, Sheikh *et al.*, 2012
	Cold maceration	Methanol	Glial activation, cytotoxicity and NO production.	Astrocyte primary of newborn Wistar rats by MTT test and LDH activity	Silva *et al.*, 2007
	Cold maceration	Methanol	Acute systemic toxicity	Swiss albino mice of both sexes by LD50	Ahmed *et al.*, 2012
	Cold maceration	Aqueous,	Allelopathy activity	Seed germination and Seedling growth	Meher, 2011, Getachew *et al.*, 2012, Siddiqui *et al.*, 2009
	Cold maceration	Acetone, Methanol	Anti-tumor	Human T-cell leukemia (Molt-4) cells by cell viability and MTT	Sathiya and Muthuchelian, 2011
	Cold maceration	Acetone, Water	Antioxidant Activity	DPPH Radical Scavenging Method	Lakshmibai *et al.*, 2015, Napar *et al.*, 2012
	Cold maceration	Methanol	Antifungal	Poison food technique, MIC	Raghavendra *et al.*, 2009, Rajkumar and Murugeasan, 2013, Bazie *et al.*, 2014
	Cold maceration	Methanol	Ovicidal Activity	Egg hatch assay	Kipyegon *et al.*, 2015
	Cold maceration	Methanol	Antibacterial and Hepatoxicity	Disc diffusion, Induced oxidative stress in the liver of rats	HariPrasad *et al.*, 2011a,b
	Cold maceration	Ethanol, Methanol	Hemolysis	Human Erythrocytes	Kandasamy *et al.*, 1989
	Powder		Antipyretic Activity	Brewer's yeast induced hyperthermia in Rats	Gopinath *et al.*, 2013
	Boiling	Water	Anti-Pustule	Well diffusion, Growth curve and MIC	Rajadurai *et al.*, 2014
	Soxhlet	Methanol, Water	Antifungal	Poison food technique	Raghavendra, 2007
	Soxhlet	Methanol	Anticryptococcal Activity	Disc diffusion activity	Valli *et al.*, 2014
	Soxhlet	Methanol	Antibacterial	Disc diffusion activity	Patel *et al.*, 2015, Sukirtha and

					Growther, 2012
	Cold & Soxhlet	Petroleum ether, Chloroform, Methanol	Antibacterial	Well diffusion	Thakur *et al.*, 2014
	Cold maceration	Methanol	Anti-inflammatory & Acute toxicity	Carrageenan-Induced Paw Oedema in Rats- LD-50	Choudhary and Nagori, 2013
Pollen Grain	Cold maceration	Ethanol, Methanol, Water	Antioxidant	Lipid peroxidation on mouse hepatic microsomal	Almaraz-Abarca *et al.*, 2007
Pods	Cold maceration	Methanol	Antibacterial	Disc diffusion	Singh *et al.*, 2011, Tajbakhsh *et al.*, 2015
	Soxhlet	Methanol	Antibacterial	Disc diffusion	Srivastava *et al.*, 2013
	Acid/base fractionation	Methanol	Antibacterial	Disc diffusion	Santos *et al.*, 2013
	Soxhlet extraction	Hexane, Benzene, Acetone, Ethanol, Methanol	Hyperglycemic	Alloxan induced diabetic rabbits by GOD-PAP method	Alsaadi and Maliki, 2015
	Cold extraction	Methanol	Cytotoxicity and cell membrane integrity assays	GL-15 cell line, derived from glioblastoma multiform	Hughes *et al.*, 2005
	Hot extraction	Hexane, Benzene, Acetone, Ethanol, Methanol	Hypoglycemic Effect	Phenols on glucose level in alloxan –induced diabetic rabbits	Alsaadi and Maliki, 2014
	Acid/Base extraction		Cytotoxicity effect	Cerebral hemispheres of newborn Wistar rat pups	Hughes *et al.*, 2006
Bark	Soxhlet extract	Methanol	Anti-inflammatory	Carrageenan-Induced Paw Oedema in Swiss Albino mice, Histamine Induced Inflammation	SivaKumar *et al.*, 2009
	Cold maceration	Methanol	Allelopathy activity	Seed germination	Getachew *et al.*, 2012
	Cold maceration	Hexane, Benzene, Acetone, Ethanol, Methanol	Antioxidant activity	Methyl linoleate oxidation inhibition	Sirmah *et al.*, 2011
Stem(Heart wood)	Cold maceration	Hexane, Acetone, Ethanol, Methanol	Antioxidant activity	Methyl linoleate oxidation inhibition	Sirmah *et al.*, 2011
Roots	Cold maceration	Water, Acetone	Allelopathy activity	Seed germination	Getachew *et al.*, 2012
	Cold maceration	Methanol	Ovicidal Activity	Egg hatch assay	Kipyegon *et al.*, 2015

Human pathogenic bacteria:

The bacterial disease in human are generally caused by Streptococcus sp., Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus cereus and E. coli. These bacteria infect human through infection of food, water, air and physical contact with infected persons. The pathogens causing diarrhoea in humans includes Shigella, Salmonella, E. coli and Enterococcus faecium, pathogens causing respiratory tract infection are Klebsiella pneumoniae, Mycobacterium tuberculosis and Pseudo. aeruginosa, urinary tract pathogens are E. coli and other Enterobacteriaceae members. These pathogens are the prominent cause of death from a single infectious disease worldwide (CDC, 2015). According to Centers for Disease Control and Prevention (CDC) the acute foodborne diseases leads to a loss of $52 billion annually in developed country as a major economic fall back. In the present investigation the human pathogenic bacteria selected for the study are descried below with colony morphology, diseases caused by them and important symptoms. Bacillus cereus The bacteria are encapsulated spore forming bacilli with square ends or in short chains, strictly aerobic or facultative anaerobe. They cause gastroenteritis and produce heat stable enterotoxin causing diarrhea, illness with abdominal pain, vomiting nausea and bacteremia (Prescott, 2005; Mehrdad, 2007). Bacillus subtilis They are encapsulated spore forming (endospore), rod shaped bacteria which are obligate aerobic in nature. They cause food poisoning, ropiness in bakery foods and diseases in immune-compromised patients (Prescott, 2005). Staphylococcus aureus These are cocci shaped bacteria appear in clusters and facultative anaerobe. They are the common cause of infection in hospitalized patients and also cause endocarditis, infects heart valves, tissues, furuncles, carbuncles, bacteremia, food poisoning and toxic shock syndrome (Prescott, 2005).

Escherichia coli:

They are rod shaped, motile, non-spore forming bacteria. They are aerobic or facultative anaerobic. They cause gastro enteritis, urinary tract infections, neo-natal meningitis, peritonitis, mastitis and septicemia (Prescott, 2005). Klebsiella pneumoniae They are rod shaped, non-motile, capsulated and facultative anaerobic bacteria. It causes Pneumonia rhinoscleroma, ozaena and infection in alimentary tract, urinary tract, genital tract and eyes. Nosocomial infections leads to morbidity and mortality (Prescott, 2005). Pseudomonas aeruginosa They are rod shaped and flagellated. They are aerobic in nature. They produce fluorescent green pigment poverdin. They colonize in damaged sites (burns, wounds), respiratory tract, known to cause destructive lesions and cystic fibrosis (Prescott, 2005). Salmonella typhi They are rod shaped, motile, non-spore forming, facultative anaerobe, smooth colonies. They cause food borne illness, typhoid, para typhoid fever, gastro intestinal symptoms like diffuse abdominal pain and constipation, Bone marrow infection, inflammation and necrosis (Prescott, 2005). Enterococcus faecalis They are rod shaped, non-motile, commensal bacteria. They are known to cause life threatening infection in humans. They also cause endocarditis and bacteremia, urinary tract infections (UTI) and meningitis (Prescott, 2005).

Bacterial resistance:

Disproportionate use of antibiotics to treat bacterial diseases has led to the emergence of resistance in bacteria. These resistant bacteria are responsible for causing large number of mortality globally. Antibiotic resistance reduces the probability of effective treatments and increases the risk of impediments or death for the patient (Woodford and Livermore, 2009). According to CDC report, antibiotic resistant microorganisms are nightmare bacteria that stance a catastrophic threat to people throughout the world (CDC, 2013). Development of resistance is a serious and multifaceted problem that was developed by many factors (Al-Masoudi et al., 2013; NIAID, 2014):

1. Inappropriate use of antibiotics in human medicine (e.g., for viral infections)
2. Overuse of antibiotics in agriculture
3. Overprescribing of broad-spectrum drugs
4. Poor health care facilities
5. Global travel and trade
6. Poor sanitation (contaminated water systems and spread resistant bacteria in

sewage) Antimicrobial resistance can be classified into 3 groups: intrinsic, mutational and acquired.

1. Intrinsic resistance refers to an inherent resistance to an antibiotic which is a naturally arising feature of the microorganism. (Example oral bacteria, Streptococci lack the nitroreductase necessary to convert metronidazole to its active metabolites and therefore are not affected by the drug).

2. Mutational resistance arises due to a spontaneous chromosomal mutation that leads to a genetically-altered bacterial population. (Example: Mutations resulting from the alteration of a single nucleotide base can end in resistance for aminoglycosides and rifampin).

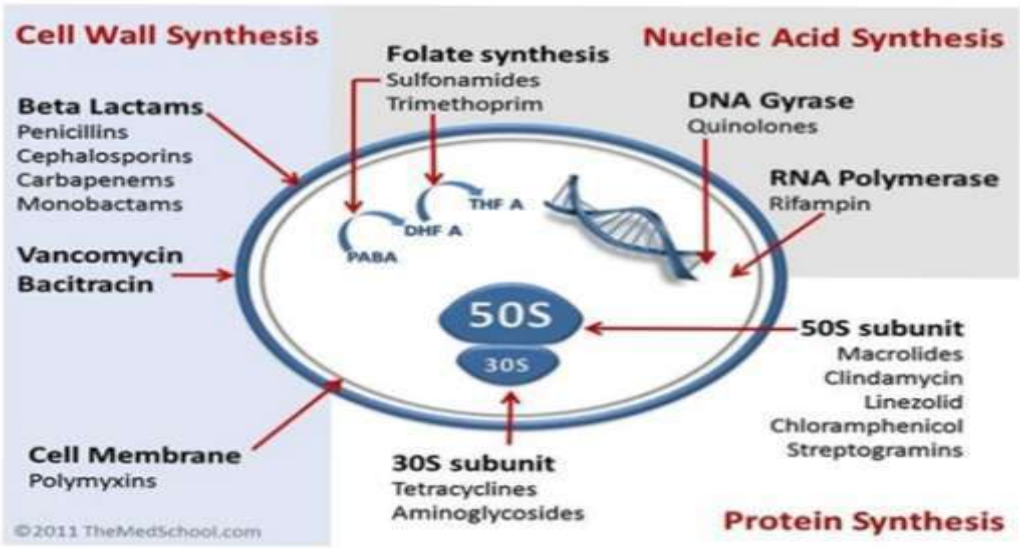
3. Acquired resistance refers to the horizontal acquisition of genetic element that encrypts antibiotic resistance through transduction, transformation or conjugation. Three main strategies used by bacteria to acquire resistance against different antibiotics includes:

a. avoiding the drug from reaching its target (Nikaido, 2009),

b. fluctuating the target (Spratt, 1994) and

c. inactivating the antibiotic (Robicsek et al., 2006).

Thus, a new generation of antimicrobial drugs is of immediate need because the effectiveness of antibiotics is progressively compromised by the rise of drug resistance and multiple drug resistant bacteria, which become new clinically common and represent a serious threat to public health (Aly and Bafiel, 2008; Neville et al., 2013). The mechanism of action of the drugs must be studied for the development of target-based drug to understand the mechanism of resistance in bacteria.



MATERIALS AND METHODS

Collection of plant material:

Different parts of P. juliflora viz: leaf, young stem, bark, inflorescence and pod were collected from surroundings of kukarahalli lake area of Mysore ((12.30°N 76.65°E, altitude above sea level: 770 m /2,526 ft) Karnataka, India (Fig. 2.1). The plant materials were washed thoroughly in running tap water, shade dried and powdered. The powdered material were hermetically sealed and stored at 4oC until use.

Fig. 2.1: Different parts of Prosopis juliflora
a) Leaves b) Inflorescence c) Bark d) Young stem e) Pods

Test pathogens:

Plant pathogenic Bacteria:

The plant pathogenic bacteria were obtained from National Centre of Industrial Microorganisms (NCIM), NCL, Pune and Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India.

Xanthomonas oryzae was isolated from diseased paddy seeds and R. solanacearum was isolated from infected potato plant (Table 2.2).

Table 2.2: Selected test Plant pathogenic bacteria

S No.	Bacteria	Abbreviated as	Source
1	*Xanthomonas oryzae*	*X. oryzae*	Diseased plant
2	*Xanthomonas campestris* pv. *vesicatoria*	*X. c. vesicatoria*	MTCC 2286
3	*Xanthomonas campestris* pv. *campestris*	*X. c. campestris*	NCIM 5028
4	*Pseudomonas syringae*	*Pseudo. syringae*	NCIM 1502
5	*Ralstonia solanacearum*	*R. solanacearum*	Diseased plant

Human pathogenic bacteria:

Human pathogenic bacteria were obtained from the Microbial Type Culture Collection (MTCC) IMTECH, Chandigarh, India (Table 2.3).

Table 2.3: Selected test Human pathogenic bacteria

S No.	Bacteria	Abbreviated as	Source
1	*Bacillus subtilis*	*B. subtilis*	MTCC 1272
2	*Bacillus cereus*	*B. cereus*	MTCC 121
3	*Escherichia coli*	*E.coli*	MTCC 7410
4	*Enterococcus faecalis*	*E. faecalis*	MTCC 2729
5	*Staphylococcus aureus*	*Staph. aureus*	MTCC 7433
6	*Pseudomonas aeruginosa*	*Pseudo. aeruginosa*	MTCC 424
7	*Salmonella typhi*	*Sal. typhi*	MTCC 733
8	*Mycobacterium smegmatis*	*Myco. smegmatis*	MTCC 6
9	*Klebsiella pneumoniae*	*Klb. pneumoniae*	MTCC 7407

All the test bacteria were maintained on Nutrient agar (NA) medium and periodic sub culturing was carried out.

Preparation of plant extract:

Aqueous extraction:

Fresh plant material was collected and washed thoroughly in running tap water. Fifty gram of fresh material was grounded in 100ml of distilled water, filtered through a double layered muslin cloth and then centrifuged at 5000g for 15 min. The supernatant was sterilized and stored at 40C until further use (Saadabi and Ayoub, 2009).

Solvent extraction:

The powdered material were extracted by soaking four 50g of powder in 100 ml of Petroleum ether followed by Chloroform, Ethyl acetate, and Methanol. Extract were filtered through Whatmann No. 1 filter paper and concentrated by evaporating to dryness using rotary flash evaporator (Dalli et al., 2007). The yield was then dissolved in methanol to a final concentration 100mg/ml. 25gms of powdered material was subjected to soxhlet extraction successively with Petroleum ether, chloroform, ethyl acetate, and methanol (Ncube et al., 2008). The extract was concentrated to dryness using rotary flash evaporator. The yield was then dissolved in methanol to a final concentration of 100mg/ml.

Antibacterial activity assay:

Antibacterial potential of aqueous and solvent extracts of different parts of the plant was tested following the procedures of well diffusion and disc diffusion method with standard antibiotics as a positive control and organic solvents as negative controls.

Preparation of inoculum:

The inoculum was prepared according to the procedures of CLSI (2002). The test bacteria were cultured on Mueller Hinton agar (Himedia Laboratories Pvt Ltd., India) and incubated for 18 to 24 hours at 37oC. The colonies were suspended in 5ml of Mueller Hinton broth. The density of the bacterial culture required for the test was adjusted to 0.5 McFarland Standard, (1.0 x 108 CFU/ml) (Schwalbe et al., 2007).

Well diffusion method:

Antibacterial activity of aqueous extract was determined by well diffusion method on Muller Hinton agar medium (Perez, 1990). 15 ml of molten sterilized Mueller-Hinton agar (MHA) media was poured into sterile Petri dishes and allow to solidify. Wells of 5mm diameter was made in the center of the Petri dishes using sterile cork borer. 100 µl of test inoculum was seeded on to the medium and allowed to dry. 50 µl of the test extracts were added into the wells and plates were incubated at 37oC for 24 h and the zone of inhibition, if any was determined. All experiments were conducted in triplicates with appropriate positive and negative controls (Kurhekar, 2006).

Disc diffusion method:

Disc diffusion method was performed according to CLSI M44-A document. Sterile discs of 6 mm diameter were impregnated with 50µl of solvent extracts and kept under a laminar hood for 20 min to dryness. The test bacteria was swabbed evenly with a cotton swab onto the surface of solidified Mueller-Hinton agar (MHA) plates and the discs containing different solvent extracts were placed on the surface of the medium. The disc impregnated with a respective solvent of the same volume served as negative control and standard antibiotics served as positive control. The plates were incubated at 28oC for 24h (for plant pathogenic bacteria) and 37±2oC for 24 h (for human pathogenic bacteria). Each test was done in three replicates and the results were tabulated.

RESULTS:

Extractive yield of solvent extracts from different parts of P. juliflora:The quantitative yield and nature of different solvent extracts obtained from various parts of the P. juliflora are presented in Table 2.4. Methanol extract of leaves, Bark and stem showed higher extractive yield than pod and inflorescence. In Pod, petroleum ether extract yield was higher and in inflorescence, chloroform extract had higher yield.

Table 2.4: Percentage yield of extracts of different parts of P. juliflora

Plant parts	Quantity Powder taken (g)	Solvent	Physical appearance of Extract	Extract Yield (g)	Percentage yield (%)
Leaf	30	Petroleum ether	Oily, greenish	1.154	3.84
	30	Chloroform	Solid, dark green	1.101	3.67
	30	Ethyl acetate	Solid, pale brown	0.832	2.77
	30	Methanol	Solid, dark brown	1.713	5.71
Bark	30	Petroleum ether	Oily, pale brown	0.021	0.07
	30	Chloroform	Solid, dark brown	0.102	0.34
	30	Ethyl acetate	Solid, reddish brown	0.040	0.134
	30	Methanol	Solid, brownish red	1.113	3.71
Pod	30	Petroleum ether	Oily, colorless	0.934	3.11
	30	Chloroform	Solid, dark green	0.511	1.70
	30	Ethyl acetate	Solid, pale green	0.693	2.31
	30	Methanol	Solid, greenish brown	1.072	3.57
Young Stem	30	Petroleum ether	Oily, colorless	0.851	2.31
	30	Chloroform	Solid, pale yellow	0.937	3.12
	30	Ethyl acetate	Solid, brownish yellow	1.115	3.17
	30	Methanol	Solid, pale brown	2.367	7.89
Inflorescence	30	Petroleum ether	Oily, pale yellow	0.934	3.11
	30	Chloroform	Solid, greenish yellow	1.113	3.71
	30	Ethyl acetate	Solid, yellow	1.072	3.57
	30	Methanol	Solid, yellow	1.065	3.55

Comparative evaluation of antibacterial activity of different parts of P. juliflora against plant pathogenic bacteria:

The antibacterial activity of aqueous and solvent extracts of different parts of P. juliflora is as follows. Leaves: Petroleum ether, chloroform, methanol and aqueous extract of leaves recorded significant antibacterial activity against X. c. vesicatoria, X. o. oryzae, X. c. campestris and Pseudo. syringae, but did not showed activity against R. solanacearum (Table 2.5). Methanol and aqueous extract showed highest with zone of inhibition 30.0± 0.57 mm and 32.5± 0.57 against X. c. vesicatoria respectively. Pods: The antibacterial activity of aqueous and solvent extracts of pod against plant pathogenic bacteria is presented in Table 2.6. Petroleum ether, methanol and aqueous extract showed significant activity against all the test pathogens except R. solanacearum. Highest inhibitory activity was observed in methanol extract against X. c. campestris (22.33 ± 0.04 mm) and aqueous extract against X. c. vesicatoria and X. o. oryzae with zone of inhibition of 25.6±0.33 and 30.0±0.57. Inflorescence: Methanol and aqueous extract showed significant antibacterial activity against X. c. vesicatoria and X. oryzae while no activity was observed in ethyl acetate and chloroform extracts. Highest inhibitory activity was observed in methanol extract against X. c. vesicatoria (30.03 ± 0.57 mm) (Table 2.7). Young stem and Bark: Solvent extract of bark and stem did not showed antibacterial activity against plant pathogenic bacteria. The study clearly demonstrate that among different solvents, methanol and aqueous extract showed significant antibacterial activity against plant pathogenic bacteria. Among different parts of the plant leaves pod and inflorescence extract of P. juliflora showed significant antibacterial activity compared with extracts of other parts of the plant (Fig. 2.4)

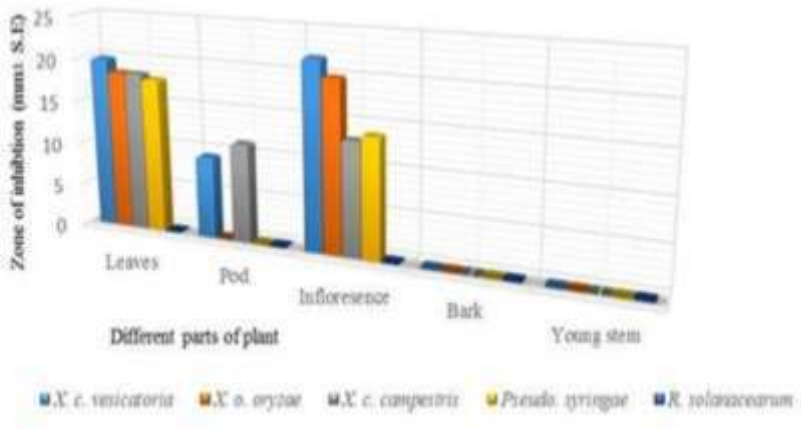


Fig. 2.4: Comparative antibacterial activity of different parts of P. juliflora against plant pathogenic bacteria Table 2.5: Antibacterial activity (zone of inhibition) of aqueous and solvent extracts leaves against plant pathogenic bacteria

Test Bacteria	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
Negative control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
X. c. vesicatoria	0.0±0.0	0.0±0.0	0.0±0.0	22.0±0.57	18.5±0.57
X. oryzae	0.0±0.0	12.3±0.33	0.0±0.0	17.3±0.57	20.3±0.88
X. c. campestris	0.0±0.0	0.0±0.0	0.0±0.0	12.5±0.66	25.3±0.33
Pseudo. syringae	0.0±0.0	8.5±0.0	0.0±0.0	18.3±0.33	0.0±0.0
R. solanacearum	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

(*Values represents the Mean ± SEM of three replicates)

Table 2.6: Antibacterial activity (zone of inhibition) of aqueous and solvent extracts of pods against plant pathogenic bacteria

Test bacteria	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
Negative control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
X. c. vesicatoria	0.0±0.0	0.0±0.0	0.0±0.0	11.3±0.33	8.6±0.33
X. oryzae	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
X. c. campestris	0.0±0.0	0.0±0.0	0.0±0.0	12.5±0.66	11.5±0.57
Pseudo. syringae	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
R. solanacearum	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

(*Values represents the Mean ± SEM of three replicates)

Table 2.7: Antibacterial activity (zone of inhibition) of aqueous and solvent extracts of inflorescence against plant pathogenic bacteria

Test bacteria	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
Negative control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
X. c. vesicatoria	15.3±0.33	0.0±0.0	0.0±0.0	30.3±0.57	14.3±0.33
X. oryzae	18.6±0.33	0.0±0.0	0.0±0.0	28.3±0.33	12.0±0.57
X. c. campestris	7.0±0.0	8.7±0.0	0.0±0.0	11.3±0.66	15.3±0.33
Pseudo. syringae	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	14.0±0.57
R. solanacearum	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

(*Values represents the Mean ± SEM of three replicates)

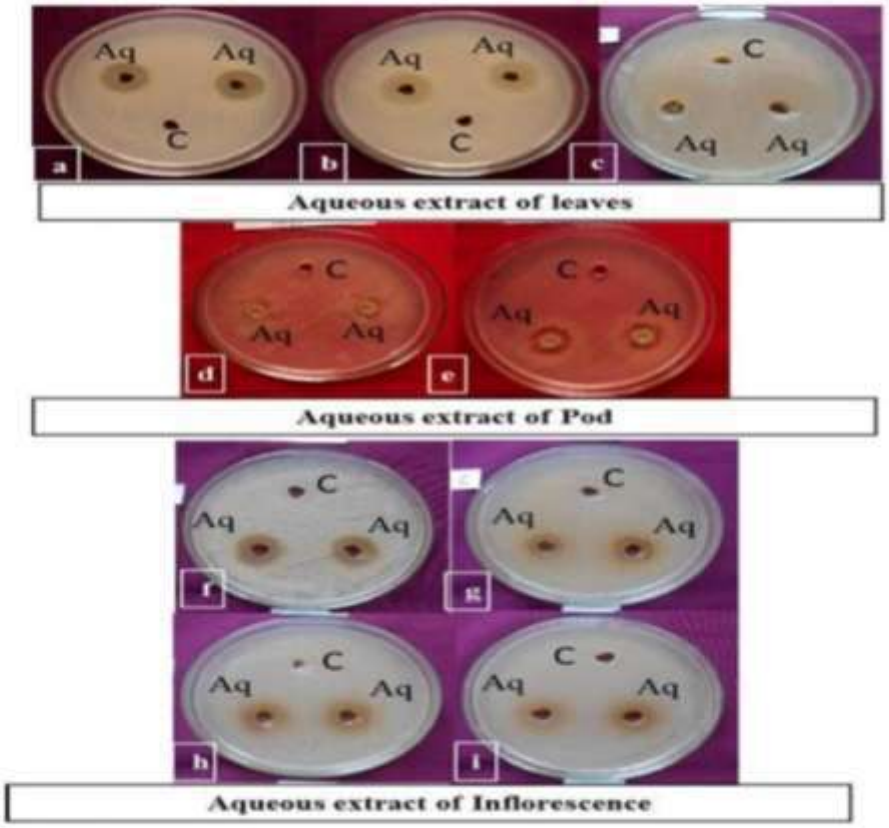


Fig. 2.5: Antibacterial activity of aqueous extract of different parts of P. juliflora against plant pathogenic bacteria Aqueous extract of leaves against a) X. c. vesicatoria b) X. oryzae c) X. c. campestris Aqueous extract of pod against d) X. c. vesicatoria e) X. c. campestris Aqueous extract of inflorescence against f) X. c. vesicatoria g) X. oryzae h) X. c. campestris i) Pseudo. syringae C-Control; Aq-Aqueous extract 6

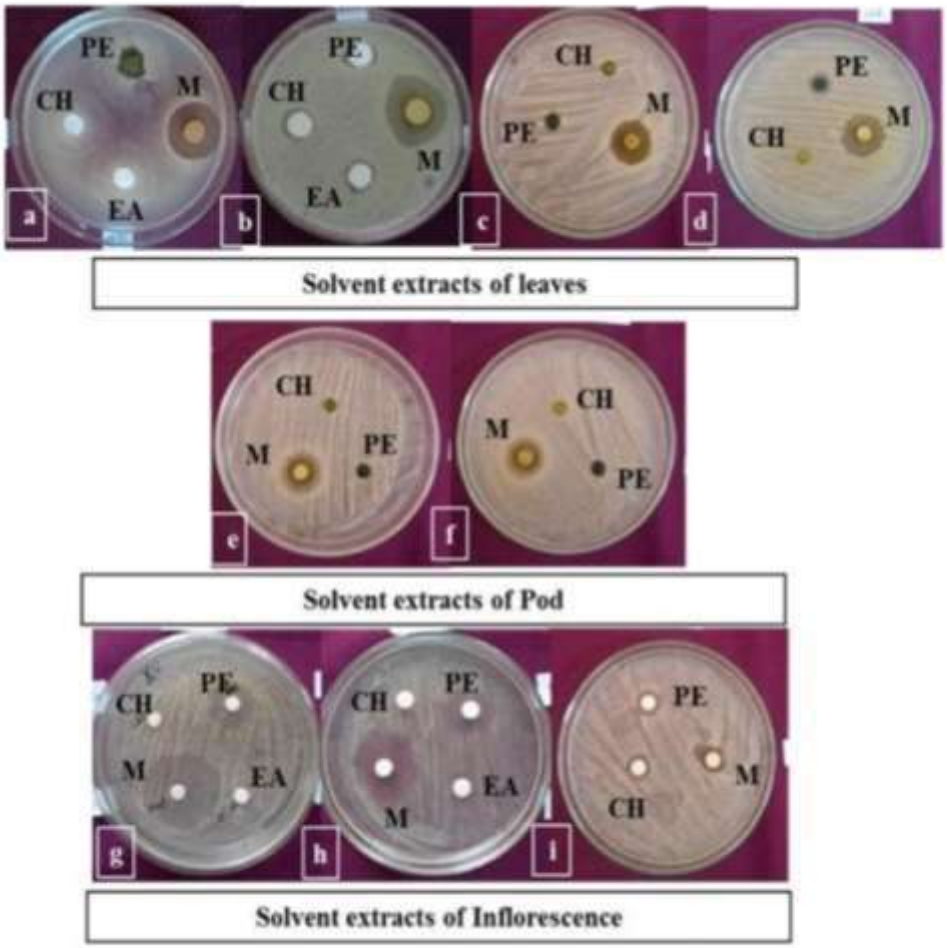


Fig. 2.6: Antibacterial activity of solvent extracts of different parts of P. juliflora against plant pathogenic bacteria Solvent extracts of leaves against a) X. c. vesicatoria b) X. oryzae c) X. c. campestris d) Pseudo. syringae Solvent extracts of pod against e) X. c. vesicatoria f) X. c. campestris Solvent extracts of leaves against g) X. c. vesicatoria h) X. oryzae i) X. c. campestris PE- Petroleum ether extract; CH-Chloroform extract; EA-Ethyl acetate extract; M Methanol extract

2.4.3. Comparative evaluation of antibacterial activity of different parts of P. juliflora against human pathogenic bacteria: The antibacterial activity of aqueous and solvent extracts of different parts of P. juliflora is as follows. Leaves: Methanol and aqueous extract showed significant activity against Staph. aureus, B. cereus, E. coli and Klb. pneumoniae (Table 2.8). Aqueous extract showed significant against B. subtilis with zone of inhibition of 25.43±0.67 mm. Methanol extract showed activity against Sal. typhi and E. faecalis and no activity was observed against P. aeruginosa and Myco. smegmatis. Petroleum ether, Chloroform and Ethyl acetate extracts did not showed any antibacterial activity (Fig. 2.8). Pod: Methanol extract of pod showed moderate antibacterial activity against B. cereus, E. coli, and Klb. pneumoniae. Aqueous extract showed significant activity against B. subtilis. No activity was recorded against Staph. aureus, Sal. typhi, E. faecalis, P. aeruginosa and Myco. smegmatis. Petroleum ether, chloroform and ethyl acetate extracts did not showed any antibacterial activity against test pathogens (Table 2.9). Inflorescence: Methanol extract of inflorescence showed moderate activity against E. coli, Klb. pneumoniae, Staph. aureus, Sal. typhi and B. cereus. Aqueous extract of inflorescence showed mild activity against E. coli and no activity was recorded against P. aeruginosa, B. subtilis and Myco. smegmatis (Table 2.10) (Fig. 2.9). Young stem and bark: Extracts did not showed any antibacterial activity against human pathogenic bacteria. The respective organic solvents which served as a negative control did not showed any activity. In different parts of P. juliflora, leaves showed significant antibacterial activity against different human pathogenic bacteria compared to other parts of the plant (Fig. 2.7). Methanol and aqueous extract were found to be effective.

Isolation and characterization of the active alkaloid fraction from P. juliflora leaves:

Thin layer chromatography and Bioautography:

The chromatogram of total alkaloid fraction illustrated, 4 bands with Rf values of 0.52, 0.6, 0.81, 0.84 respectively (Fig. 2.17). The bioautography of the different alkaloid fraction obtained in TLC revealed the antimicrobial active fraction Rf value by

clear zone of inhibition. Band II with Rf value 0.6 revealed the significant antimicrobial activity against X. c. vesicatoria, X. oryzae, X. c. campestris, Pseudo. Syringae, E. coli, B. cereus, B. subtilis, Staph. aureus, F. semitectum Microsporum canis and Microsporum gypseum on bioautogram (Fig. 2.18).

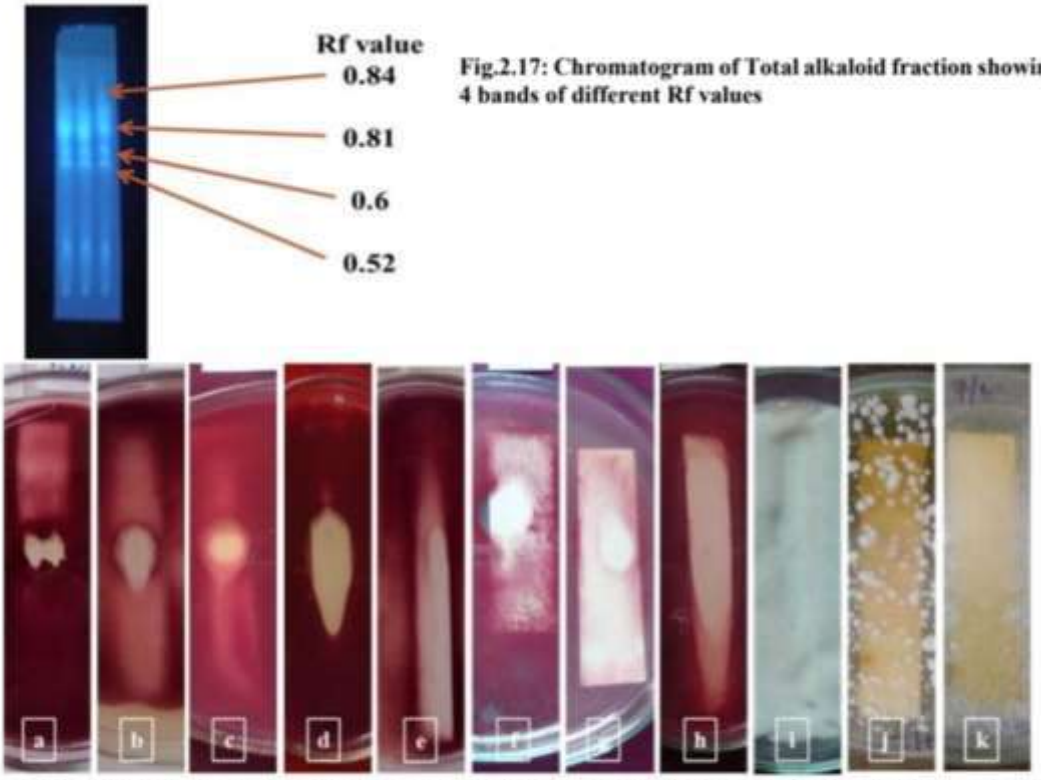


Fig.2.17: Chromatogram of Total alkaloid fraction showing 4 bands of different Rf values

Fig 2.18: (a-k): Bioautography showing growth inhibition effect of band II of Total Alkaloid Fraction against test bacteria and fungi (a) X. c. vesicatoria (b) X. oryzae (c) X. c. campestris (d) Pseudo. syringae (e) B. cereus (f) E. coli (g) B. subtilis (h) Staph. aureus (i) F. semitectum (j) M. canis (k) M. gypseum

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