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Research Article

INVITRO ANTIBACTERIAL ACTIVITY AND PRELIMINARY PHYTOCHEMICAL ANALYSIS OF LEAF EXTRACTS OF *ARGEMONE MEXICANA* LINN – A MEDICINAL PLANT

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ABSTRACT

Argemone mexicana Linn. (Papaveraceae) is used as a medicinal plant in several countries. In this study, methanol, chloroform and petroleum ether leaves extracts of *Argemone mexicana* Linn were evaluated for antibacterial activity against Bacterial (MTCC) strains. Antibacterial activity of this plants extracts were performed using agar disc diffusion method. The antibacterial activity of methanol extracts showed more effective followed by chloroform and petroleum ether extracts against all the bacterial strains. Preliminary phytochemical studies were performed for the presence and absence of phytochemicals in *Argemone mexicana* Linn. The Minimal inhibitory concentrations (MIC) exhibited by *A.mexicana* extract against the bacterial strains ranged between 125 µg/µl to 1000µg/µl.

Keywords: Antibacterial activity, Phytochemical analysis, Leaf extract, *Argemone mexicana* Linn.

INTRODUCTION

Medicinal plants are important to the health of many peoples in developing countries. According to World Health Organization (WHO), approximately 80% of people in developing countries still rely on traditional medicine for their primary health care needs. This usually involves the use of plant extracts (Hsin-sheng Tsay, 1994)[1]. These are the natural and safer source of phytochemicals to fight against new strains of micro organisms. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmological activities, have been extensively investigated as a source of medicinal agents. Thus, it anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Raghavendra MP *et al*., 2006)[2]. The plant *Argemone mexicana* Linn. belongs to family Papaveraceae is commonly known as Mexican poppy or prickly poppy is used as medicinal herbs. The plant contains alkaloids, flavanoids, tannins, sterols and terpenese Quinn-Beatie, M.L. (2002)[3]. The plant is known to possess antimalarial[4], antimicrobial[5], antibacterial[6] and antifungal[7] activities. The roots are useful in guinea-worm infection, skin diseases, leprosy, pruritus, blennorrhagia, inflammations, all type of poisoning, constipation, flatulence, colic, malarial fever chronic skin diseases and vesicular calculus. The leaves are useful in cough, wounds, ulcer, warts, cold sores, cutaneous affections, skin diseases, itches etc. The seeds are useful in vitiated conditions of *kapha*, cough, asthma, pertussis, skin diseases, leprosy, ulcers, wounds, odantalgia, dental caries, constination, rheumatalgia, colic, flatulence and antidote to snake poisoning. The latex is useful in dropsy, jaundice, skin diseases, leprosy, blisters, piles, indolent ulcers, dysentery, tumors, conjunctivitis, inflammations, burning and malarial fever. The oil is useful in indolent ulcers, wounds, leprosy, skin diseases, constipation, flatulence, sensation colic and rheumatalgia[8].

MATERIALS AND METHODS

Bacterial Strains

Bacterial strains *Escherichia coli* MTCC-443, *Pseudomonas aeruginosa* MTCC-7441, *Proteus mirabilis* MTCC-742, *Salmonella typhi* MTCC-441, *Staphylococcus aureus* MTCC-766 and *Klebsiella pneumoniae* MTCC-109 were obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, India. The cells from lyophilized vials were transferred into the liquid nutrient broth medium, and then transferred into nutrient agar slants preserved at 4ºC in the refrigerator.

Plant Materials

The plant materials (leaves) of *Argemone mexicana* Linn were collected from the surroundings of Attur, Salem District, Tamil Nadu, India. The plant species was authenticated by Dr.R.Selvaraj, Professor of Botany, Annamalai University, Tamil Nadu, where voucher specimen was deposited.

Preparation of Plant Extracts

Leaves of the plant samples were thoroughly washed with running tap water 2-3 times and then finally washed with distilled water followed by shade-dried for seven days and then dried in an oven below 50ºC. The dried plant materials were then powdered using mixer and grinder. 30g of plant powder were extracted with 100ml of Methanol, Chloroform and Petrolium ether for 72hrs by Soxhlet extractor. Then the extracts with different solvents were evaporated using rotary evaporator. Extracts were transferred into pre-weighed sample containers and were stored later was used for phytochemical screening, Antibacterial activity and Minimal inhibitory concentration (MIC) (A. Manjamalai and R.Sardar 2010)[8] (Periyasamy Ashokumar 2010)[9].

Preliminary Phytochemical Analysis

The preliminary phytochemical analysis were carried out for the presence and absence of tannins, alkaloids, steroids, phenols, terpenoids, carbohydrates etc. according to the methods described by Periyasamy Ashok kumar *et al*., (2010)[9].

Screening for Antibacterial Activity

Disc Diffusion method was used to test the antibacterial activity of the extracts against seven bacteria. The essential leaf extracts were used for studying their antibacterial activity. A loopful of bacterial strains were inoculated into 5ml of nutrient broth and incubated for 24hrs at 37ºC to get active strain by using disc diffusion method. Muller Hinton Agar plates were prepared by pouring 20ml of molten media into sterile Petri plates. After solidification of media, inoculum of MTCC strains was swabbed uniformly and the inoculum was allowed to dry for 5 minutes.

The extracts were dissolved in Dimethyl Sulfoxide (DMSO). The different concentrations of extracts (100µg, 200µg, 300µg, 400µg and 500µg/disc) were loaded on 5mm sterile disc using micropipette. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5mins and the plates were kept for incubation at 37ºC for 24hrs. At the end of incubation, zones formed around the disc were measured with transparent ruler in millimetre. Based on the diameter of the zone of inhibition, antibacterial susceptibility was ranked (Periyasamy Ashok kumar 2010)[9].

Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) was determined by Micro dilution method using serially diluted plant extracts. The extracts

were diluted into different concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.81 mg/ml respectively with DMSO. Then each tubes was filled with 1ml of sterile nutrient broth and inoculated with 0.1ml of broth culture of the test organism (inoculums contains $1\text{-}2\times10^{-7}$ CFU/ml). The tubes were incubated aerobically at 37ºC for 18-24hrs. The control tubes were maintained for each test tube. Inhibition of growth observed in those test tubes (No turbidity) which has lowest or minimum concentration of extract. This lowest or minimum concentration was considered as Minimum Inhibitory Concentration (MIC) (Ammara Hassan *et al*., 2009)[10].

RESULTS AND DISCUSSION

The antibacterial activity of the plant extracts from *Argemone mexicana* Linn was studied against Bacterial MTCC strains *Escherichia coli* (MTCC-443), *Pseudomonas aeruginosa* (MTCC-741), *Proteus mirabilis* (MTCC-742), *Salmonella typhi* (MTCC-441), *Staphylococcus aureus* (MTCC-766) and *Klebsiella pneumoniae* (MTCC-109) using agar disc diffusion method. The result revealed that inhibitory effects of test samples was dose dependent as the concentration increased the zone of inhibition was also increased. This is also evidenced by B. Uma Reddy[11] who supported the presence of antibacterial activity of *A.mexicana* against gram positive and gram negative bacteria by dose dependent manner.

The methanol extracts of this plant (leaves) showed maximum activity against *Salmonella typhi* followed by *Klebsiella pneumoniae, Bacillus subtilis*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*. The chloroform extracts of this plant (leaves) showed maximum activity against *Salmonella typhi* followed by *Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Petroleum ether extracts of this plant (leaves) showed maximum activity against *Salmonella typhi* followed by *Bacillus subtilis*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae.* The antibacterial activity of methanol extracts showed more effective followed by chloroform and petroleum ether extracts against all the bacterial strains. The results were recorded and tabulated (Table-1). A similar study was obtained by Indranil Bhattacharjee[6] reported that methanolic extract of *A.mexicana* leaves and seeds showed greater antibacterial activity against some species of gram positive and gram negative pathogenic bacteria than the water extracts.

Table 1: Antibacterial Activity of Different Solvent Extracts of *Argemone mexicana Linn.* against Bacterial (MTCC) Strains

Name of the Solvents	Concentration of the Disc	Name of the Bacterial (MTCC) Strains Zone of Inhibition (in mm)						
		E.coli	***S. typhi***	***S.aureus***	***B.subtilis***	***K.pneumoniae***	***P.aeruginosa***	***P.mirabilis***
Methanol	100 µg	-	-	-	-	-	-	-
	200 µg	-	10mm	-	-	-	10mm	-
	300 µg	10mg	11mm	13mm	12mm	12mm	11mm	11mm
	400 µg	14mm	15mm	13mm	13mm	15mm	13mm	14mm
	500 µg	15mm	18mm	15mm	16mm	17mm	15mm	16mm
Chloroform	100 µg	-	-	-	-	-	-	-
	200 µg	-	-	-	10mm	10mm	11mm	-
	300 µg	11mm	11mm	11mm	12mm	12mm	12mm	10mm
	400 µg	13mm	15mm	12mm	13mm	15mm	13mm	14mm
	500 µg	15mm	16mm	14mm	16mm	17mm	15mm	15mm
Petroleum Ether	100 µg	-	-	-	-	-	-	-
	200 µg	-	-	-	-	-	-	-
	300 µg	-	-	-	-	-	-	-
	400 µg	-	11mm	10mm	10mm	-	10mm	10mm
	500 µg	13mm	13mm	12mm	12mm	11mm	12mm	13mm

Table 2: Preliminary Phytochemical Screening of *Argemone mexicana* Linn.

Constituents/ Tests	Methanol	Chloroform	Petroleum Ether
Alkaloids			
Mayers test	+	+	-
Dragendorffs test	+	-	-
Hangers test	-	-	-
Wagers test	-	+	+
Proteins and amino acids			
Millons test	-	-	-
Ninhydrin test	-	+	-
Biuret test	+	-	-
Anthraquinone glycosides			
Borntragersn test	+	-	-
Flavonoids			
Shinodas test	+	+	-
Ferric chloride test	+	-	-
Tannins and phenolic compounds			
Ferric chloride test	+	-	+
Lead acetate test	+	+	-
Gelatin contains Nacl test	+	-	+
Carbohydrates			
Molischs test	-	-	-
Barfoeds test	+	-	-
Fehling test	-	+	+
Saponins			
Frothing test	+	-	+
Phytosterol			
Libermann-Burchards test	-	+	-

Table 3: MICs of Various Solvents Extracts of *Argemone mexicana* Linn. against Bacterial (MTCC) Strains

Experimental Flora	Name of the Organisms	Name of the Solvents	Extract Concentration (µg/ml)							
			7.8	15.6	31.2	62.5	125	250	500	1000
***Argemone mexicana* Linn.**	*Escherichia coli*	Methanol	-	-	-	-	β	+	+	+
		Chloroform	-	-	-	-	β	+	+	+
		Petroleum ether	-	-	-	-	-	β	+	+
	Salmonella typhi	Methanol	-	-	-	-	β	+	+	+
		Chloroform	-	-	-	-	β	+	+	+
		Petroleum ether	-	-	-	-	-	β	+	+
	Staphylococcus aureus	Methanol	-	-	-	-	β	+	+	+
		Chloroform	-	-	-	-	β	+	+	+
		Petroleum ether	-	-	-	-	β	+	+	+
	Bacillus subtilis	Methanol	-	-	-	-	β	+	+	+
		Chloroform	-	-	-	-	β	+	+	+
		Petroleum ether	-	-	-	-	-	β	+	+
	Klebsiella pneumoniae	Methanol	-	-	-	-	β	+	+	+
		Chloroform	-	-	-	-	β	+	+	+
		Petroleum ether	-	-	-	-	β	+	+	+
	Pseudomonas aeruginosa	Methanol	-	-	-	-	β	+	+	+
		Chloroform	-	-	-	-	β	+	+	+
		Petroleum ether	-	-	-	-	-	β	+	+
	Proteus mirabilis	Methanol	-	-	-	-	β	+	+	+
		Chloroform	-	-	-	-	β	+	+	+
		Petrolium ether	-	-	-	-	β	+	+	+

- = Resistance (Bacterial growth (or) Turbidity), + = Concentration showing no turbidity (inhibition on bacterial growth),

β = MIC value

All the test phytochemicals as alkaloids, flavanoids, tannins and Phenolic compounds, Phytosterols, saponins, Anthraquinone and glycosides were detected in different solvent extracts, but carbohydrates, Proteins and amino acids were absent in all three solvent extracts (Table-2). Many compounds belonging to these secondary metabolite groups have been reported to their antimicrobial activity[12]. Ibrahim et al, [13] who reported that alcoholic extracts of *A.mexicana* leaves contains reducing sugars, flavanoids, tannins, sterols/terpenes and alkaloids.

The results of the Minimum Inhibitory concentrations (MICs) of leaf extracts of *Argemone mexicana* Linn. determined against *Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis*, *Proteus mirabilis*, *Pseudomonas aeruginosa Salmonella typhi* and *Staphylococcus aureus* were presented in Table-3. MICs of methanol and chloroform extracts of *Argemone mexicana* Linn against *Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis*, *Proteus mirabilis*, *Pseudomonas aeruginosa Salmonella typhi* and *Staphylococcus aureus* were 125µg/µl. MICs value of petroleum ether extract of *Argemone mexicana* Linn against *Staphylococcus aureus* and *Klebsiella pneumonia* were 125µg/µl and 250 µg/µl against *Escherichia coli, Bacillus subtilis*, *Proteus mirabilis*, *Pseudomonas aeruginosa Salmonella typhi* (Table-3).

CONCLUSION

This present study suggests that the methanolic extract of the *Argemone mexicana* leaves possesses bioactive compounds with antibacterial activity against the bacterial strains. It is also suggest that *A.mexicana* used for the treatment of disease caused by some bacteria tested in this study. These *A.mexicana* plant extract can be used to formulate the new antibacterial drugs against the diseases.

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