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PHYTOCHEMICAL SCREENING OF ARGEMONE MAXICANA AGAINST UTI CAUSING BACTERIA

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ABSTRACT

Powdered leaves of Argemone maxicana (L.) were extracted with ether, methanolic and ethyl acetate. The extract and fractions were tested for antibacterial activity against clinical isolates of Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis and Pseudomonas species using disc diffusion and microbroth dilution technique. The extract and fractions were further subjected to phytochemical tests for the presence of secondary metabolites using standard procedures. Results of sensitivity test showed that methanol extract of the plant was active against *B.subtilis, and Escherichia coli. E. coli* and *B. subtilis* were most sensitive test pathogen to crude extract (500mg/ml) with zone of inhibition 25.32±0.12 and 24.67±0.34 mm respectively. The results of phytochemical screening indicated the presence of alkaloids, flavonoids, protein and carbohydrate in either methanol extract, fraction(s) or both. This indicates that the Argemone maxicana (L.) has the potential for the production of drugs against organisms causing urinary tract infections.

Keywords: Sensitivity, Clinical isolates, Urinary tract, *Argemone maxicana*, Fractions.

1. INTRODUCTION

Medicinal plants are cheap and renewable sources of pharmacologically-active substances and are known to produce certain chemicals that are naturally toxic to bacteria (Basile *et al*., 1999)[1]. *Argemone maxicana* belongs to family Papaveraceae. The plant is commonly found along the road sides and waste places. It grows all over India, mainly in hot climate, at an altitude of 1000-1500 meters found. Svarnaksiri is bitter in taste pungent in the post digestive effect and has cold potency. It alleviates kapha and vata doshas. It possesses light and dry attributes. It is mild laxative, wound cleanser and antiseptic in properties. It is useful in diseases like fever, edema, Dysurea, urinary calculi, burning sensation, blood and skin disorders. It has been used locally in the treatment of urinary tract infections general debility and genito-urinary disorders.

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2. MATERIAL AND METHODS

Plant materials

The whole plant of *Argemone mexicana* Linn was collected from the local surroundings at Vidisha city of M.P, during the month of November to December 2011. The plant was identified by Dr.S.K.Jain Department of Botany S,S,L,Jain CollegeVidisha M.P.. Plant were later air-dried, powdered and stored in an air-tight container for further use.

Preparation of extracts

Sample were shattered and screened with 40 meshes. It was soxhlet extracted three times with petroleum ether for 4hr at 60oC. After drying and levigation, the residues were inverse flow extracted at 75 ^{0}C with ethyl acetate, then were filtrated and the residue was extracted with methanol at 85^0 C for 48hr under reflux condition. The extracts obtained were evaporated in rotary evaporator to get a

powdery mass. The yield of different extracts was calculated. The powder extracts obtained were then subjected to photochemical analysis to detect the chemical constituents present in each extracts.

Preparation and application of disks for experiment

(I) Disc diffusion assay

(i) Disc preparation Sensitivity discs were punched from Whatman No. 1 filter paper, sterilized in Bijou bottles by autoclaving at 121^{o}C for 15mins. Sensitivity discs were prepared by weighing the appropriate amount of the extract or fraction and serial doubling dilution in Dimethylsulfoxide (DMSO) followed by placing the improvised paper discs in the solution such that each disc absorbed 0.01ml to make the disc potency of 500µg, 1000µg, 2000µg and 4000µg (Akinyemi *et al*., 2005; Vallekobia *et al.,* 2001)[2,3].

(ii) Inoculum standardization a loopful of the test isolate was picked using a sterile wire loop and emulsified in 3 – 4mls of sterile physiological saline. The turbidity of the suspension was matched with that of 0.5 McFarland Standard (Cheesebrough, 2000)[4].

(iii) Sensitivity testing Using sterilswab stick, standardized inoculate of each isolate was swabbed onto the surface of Mueller Hinton Agar in separate Petri dishes. Discs of the extracts and standard antibiotic (Augmentin 30µg) were placed onto the surface of the inoculated media. The plates were inverted and allowed to stand for 30mins for the extract to diffuse into the agar after which the plates were incubated aerobically at 350C for 18 hours. This was followed by measurement of zone of inhibition formed by the test organisms around each of the extract and standard antibiotic discs (NCCLS, 1999)[5].

(II) Agar well diffusion assay

The antibacterial diffusion assay was carried out using Agar well diffusion method as described by Perez *et al.* (1990)[6]

One Streptomycin antibiotic drug standard disc (Himedia) of concentration 25 mcg was placed in the centre of each plate as positive control. The assessment of antibacterial activity of the plant alkaloid extract was based on the measurement of diameter of inhibition zone (IZ) in mm formed around the well. Each well was loaded alternately with 100 µl one with 5 mg/ml and the other with 2.5 mg/ml concentration. The assay was carried out in triplicates and the result thus obtained is taken as the mean of the three readings for each concentration and not statistical tools were used to measure the standard deviation.

(III) Micro-broth dilution technique

(i) Minimum inhibitory concentration (MIC) Minimum inhibitory concentrations of the extract and fractions were prepared by serial doubling dilution using distilled water to obtain concentrations of 4000µg/ml, 2000µg/ml and 1000µg/ml. Equal volume (2mls) of extract and Mueller – Hinton broth were mixed. Specifically 0.1ml of standardized inocula (3.3 x 106 CFU/ml) was added to each of the test tubes above. The tubes were incubated aerobically at 35 ^{0}C for 24 hours. Tubes containing broth and extracts without inoculate which served as positive control while tubes containing broth and inoculate served as negative control. The tubes were observed after 24 hours of incubation to determine minimum inhibitory concentration. That is the lowest concentration that showed no evidence of growth (Akinyemi *et al.,* 2005; Vallekobia *et al*., 2001)[7,3].

(ii) Minimum Bactericidal Concentration (MBC) Sterile Mueller-Hinton agar plates were separately inoculated with sample from each of the test tubes that showed no evidence of growth. The plates were further incubated at 35 ^{0}C for 24 hours and observed. The highest dilution that yielded no bacterial growth was regarded as MBC (Akinyemi *et al*., 2005; Vallekobia *et al*., 2001)[7, 3].

3. RESULTS AND DISCUSSION

High yield of methanol extract was obtained at the end of extraction, with brown colour and hard texture. The high yield may be due to the stronger extraction capacity of methanol as indicated by Tschehe (1971)[8]. Both ether and ethyle acetate fractions had same colour but different texture of being hard and being gummy, which may be as a result of the difference in polarity of the different solvents.

Table 1: Showing percentage yield of crude extract of *Argemone mexicana* L. in different solvents.

S. No.	Solvent	Weight of powdered material (gm)	Volume of solvent (ml)	Weight of crude extract (gm)	Characters of extract	Percentage yield %
1	Petroleum ether extract	200	500	1.62	dark green	0.81
2	Ethyl acetate extract	200	500	2.31	Green	1.15
3	Methenol extract	200	500	1.82	Brown	0.91

The results of phytochemical screening of p ether, ethyl acetate, and methanol extracts and fractions of argemone maxicana revealed the presence of alkaloids, flavonoids, saponins, terpenoids (Table 2). These metabolites have been reported to possess antimicrobial activity (Cowan, 1999)[9]. In particular the flavonoids were reported to be responsible for antimicrobial activity associated with some ethnomedicinal plants (Singh and Bhat, 2003)[10].

Table 2: Phytochemical Screening of crude extracts of Petroleum ether, Ethyl acetate and methanol from *Argemone maxicana* Linn

S. No.	Tests	Observation for extracts		
		Pet. Ether	Ethyl acetate	Methanol
1	Test for carbohydrates			
	Fehling's Test	_	+.	+
2	Test for Alkaloid	_	_	+
	Wagner's test	_	_	+
3	Test for Flavonoids			
	Shinoda test	_	+	+
	aline reagent test	_	+	_
4	Test for Terpenoids			
	Salkowski test	+	_	_
5	Test for Saponins			
	Foam test	-	+	-
6	Test for proteins	_	+	+

Sensitivity of the test isolates to argemone maxicana extracts and fractions was indicated by observation and measurement of inhibition zones formed around discs prepared from various concentrations of the extracts and fractions (Table 3, 5 next pages). Absence of turbidity in tube cultures indicates the activity of the extract or fraction using micro-broth dilution technique, the least concentration amongst the tubes without evidence of turbidity was considered the minimum inhibitory concentration (MIC). Microbroth dilution technique employed in this research is important in determining whether the extract and fractions are capable of inhibiting the growth or completely killing the test isolates. The results of sensitivity tests using both procedures indicated that methanol extracts of the plant powder were more active than ether and acetate extract on the isolates tested. The activity exhibited by the extracts may be related to the presence of tannins that are well documented for antimicrobial activity (Tschehe, 1971)[8] in addition to alkaloids and flavonoids, which were reported to be responsible for antimicrobial properties of some ethnomedicinal plants (Singh and Bhat, 2003)[10].

Table 3: Antimicrobial activity of methanol crude extract of *Argimone Mexicana*

S.No.	Bacteria	Zone of inhibition (mm)				
		Concentration 500mg/ml				Streptomycin
		100%	75%	50%	25%	10µg/ml
1	*B.subtilis*	24.67±0.34	21.29±0.41	16.05±0.17	10.14±0.23	25.32±0.21
2	*P. vulgaris*	12.95±0.12	9.78±0.42	0	0	12.13±0.24
3	*P. aeruginosa*	19.52±0.39	15.23±0.32	0	0	9.9±0.11
4	*S. aureus*	13.72±0.17	11.00±0.23	0	0	21.4±0.14
5	*Enterobacter*	22.91±0.16	17.95±0.25	0	0	23.6±0.22
6	*S.choni*	12.92±0.13	10.5±0.36	0	0	14.6±0.05
7	*E.coli*	25.32±0.12	21.26±0.21	0	0	16.1±0.16
8	*P. mirabilis*	8.96±0.02	6.32±0.24	5.83±0.14	0	11.31±0.12
9	*K. pneumoniae*	10.39±0.01	8.8±0.08	0	0	11.6±0.06

Table 4: Separation of constituents from methanol fraction

S. No.	Solvent System	Ratio	Fractions	Solvent system for TLC	Yield
1	CHCl3:MeOH	(100:0)	1-12	Chloroform: methanol (90:10)	No residue after evaporation
2	$CHCl_3$:MeOH	(95:5)	13-24		---do---
3	$CHCl_3$:MeOH	(90:10)	25-34		Less yield
4	$CHCl_3$:MeOH	(80:20)	35-47		Less yield
5	$CHCl_3$:MeOH	(50:50)	48-70		0.65gm
6	$CHCl_3$:MeOH	(0:100)	71-80		No residue after evaporation

Table 5: Antimicrobial activity of Column purified Fraction of *Argimone Mexicana*

S.No.	Bacteria	Zone of inhibition (mm)				
		Concentration 500mg/ml				Streptomycin
		100%	75%	50%	25%	10µg/ml
1.	*B.subtilis*	27.21±0.02	24.98±0.08	22.43±0.03	20.36±0.16	25.32±0.21
2.	*P. vulgaris*	18.84±0.08	15.12±0.04	11.98±0.23	9.37±0.06	12.13±0.24
3.	*P. aeruginosa*	25.78±0.11	21.51±0.33	18.61±0.52	16.02±0.31	9.9±0.11
4.	*S. aureus*	20.84±0.06	18.33±0.04	15.63±0.24	11.65±0.12	21.4±0.14
5.	*Enterobacter*	26.91±0.13	23.87±0.05	19.54±0.17	18.48±0.08	23.6±0.22
6.	*S.choni*	19.36±0.18	17.52±0.04	13.88±0.06	12.03±0.01	14.6±0.05
7.	*E.coli*	29.32±0.01	26.16±0.22	23.28±0.08	19.65±0.33	16.1±0.16
8.	*P. mirabilis*	13.92±0.41	10.32±0.09	9.01±0.22	6.53±0.06	11.31±0.12
9.	*K.pneumoniae*	16.51±0.07	13.31±0.21	10.81±0.15	8.21±0.31	11.6±0.06

Table 6: minimum bactericidal concentration (MBC) of fraction of A.maxicana.

Sl. No.	Bacteria	Minimum Bactericidal Concentration							MBC (mg/ml)
		Concentration mg/ml							
		100	50	25	12.5	6.25	3.13	1.56	
1	***B.subtilis***	-	-	-	-	-	-	+	**3.13**
2	***P. vulgaris***	-	-	-	+	+	+	+	**25**
3	***P.aeruginosa***	-	-	-	-	-	-	+	**3.13**
4	***S. aureus***	-	-	-	-	+	+	+	**12.25**
5	***Enterobacter***	-	-	-	-	-	+	+	**6.25**
6	***S.choni***	-	-	-	-	+	+	+	**12.5**
7	***E.coli***	-	-	-	-	-	-	+	**3.13**
8	***P.mirabilis***	-	-	-	+	+	+	+	**25**
9	***K.pneumoniae***	-	-	-	-	+	+	+	**12.5**

Table 7: Minimum Inhibitory Concentration (MIC) of fraction of *Argimone mexicana*

S. No.	Bacteria	MIC (mg/ml)
1	*B.subtilis*	3.125
2	*P. vulgaris*	12.5
3	*P. aeruginosa*	1.56
4	*S. aureus*	6.125
5	*Enterobacter*	3.125
6	*S.choni*	6.125
7	*E.coli*	3.125
8	*P. mirabilis*	12.5
9	*K. pneumoniae*	6.125

4. CONCLUSION

From the results of this work, it can be concluded that argemone maxicana has the potential for the production of drug for the treatment of urinary tract infections.

RECOMMENDATIONS

In view of the results obtained in this work, it is recommended that scientists should;

a) Isolate and identify the active compound(s) present in the methanol extract and fractions.

b) Determine the toxicity level of both crude extract and the active compound(s).

c) Screen more plants view of finding alternative treatments to microbial infections.

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