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Biochemistry

ANTI-MICROBIAL PROPERTIES OF AREGEMONE MEXICANA SEEDS AND ITS PHYTOCHEMICAL EVALUATION.

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ABSTRACT Nature created plant in the world of every cilment and there is cure every disease and man has to find it. The bioactive compound of Argemone mexicane seeds has been evaluated using GC-MS. The chemical composition of the extract of Argemone mexicane seed were investigated using Gas Chromatography –Mass spectrometry .The analysis was carried out with the methanol extracted of the dried plant seeds. This study result concluded that the A. Mexicana have a more phytoconstituents. Medicinal plants have been used in traditional treatments for numerous human diseases for thousands of years and they continue to be an important therapeutic aid for alleviating the ailments of human kind. The results of the study provides basis for subsequent bioactivity guided fractionation the extract of seed aregmone Mexicana and isolation of pure 48 antibacterial compounds. The GC-MS analysis was also carried out in the study to analyse the compounds present in Argemone Mexicana seed extracts and the results are reported respectively.

KEYWORDS : GC-MS, A. Mexicana, Methyl red test, Mexican poppy, alkaloids

Introduction

Medicinal plants have been used in traditional treatments for numerous human diseases for thousands of years and they continue to be an important therapeutic aid for alleviating the ailments of human kind[1]. The therapeutic benefits are generally traced to specific plant compounds; but are specifically due to the active constituents of the plants[2]. Phytochemical screening of various plants has been reported by many workers[3, 4]. These studies have revealed the presence of numerous chemicals including alkaloids, flavonoids, steroids, phenols, glycosides and saponins. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites[5]. A number of studies have focused on the biological activities of phenolic compounds which are antioxidants and free radical scavengers[6].

Plant discerption

Kingdom	:	*Planate*
(Unranked)	:	*Anglosperms*
(Unranked)	:	*Eudicots*
Order	:	Ranunculales
Family	:	Papaveraceae
Genus	:	Argemone
Species	:	*A.Mexicana*
Tamil	:	Ponnumuttai
English	:	Maxican poppy

Fig 1: A.Mexicana

Materials and Method

Collection of the plant sample

The plant sample was collected from residential place of Kolunthampat village, Thiruvannamalai District, Tamilnadu. During spring January. Authentication was carried out at the Department of Botanical Survey of India, (1667 BSI/SRC/5/23/2013-2014/Tech/Dat29.01.2014) Southern Regional Center and Tamil Nadu Agricultural University.

Campus in Coimbatore, where voucher specimens were deposited. The seeds were initially washed with distilled water and dried on a paper towel in laboratory for 24 hours.

Fig 2: Collection of clinical samples

Thirty clinical samples were collected from diabetic patients (from mouth -swab sample and urine sample) from Sai medical and research foundation, Tharagamaruthru, Nagapattinam(DT),Vedaraniyam, Tamilnadu, India.

Preparation of Plant Extract

Seed 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 50◦C. The dried plant materials were then powdered using mixer and grinder. 30g of plant powder were extracted with 200ml of Methanol, for 72hrs by Soxhlet extractor. Then the extracts with solvents were evaporated using rotary evaporator. Extracts were the transferred into pre-weighed sample containers and were stored later was used for phytochemical screening, Antibacterial activity[7,8].

Qualitative screening of phytochemical

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, 2010.The aqueous and methanol extracts were subjected to a preliminary phytochemical screening to identify the various phytoconstituents present in them i.e. Alkaloids, Terpenoids, Glycosides, Steroids, Triterpenoids, Flavonoids, Carbohydrates, Saponins and Tannins. The estimation of carbohydrates by Brindha 1981, alkaloids by Kokate,2001, and Brindha,1981, Test for terpenoids by Kokate, 2001, Test for flavonoids by Kokate ,2001, Lead acetate Test by Brindha ,1981, NaOH Test by Khandewal,2008, Phosphomolybdic acidTest by Brindha,1981, Test for tannins by Mukherjee ,2002, Test for saponins by Kokate ,2001, Legals Test by Brindha ,1981 , Ninhydrin test by Brindha ,1981.

GC-MS Analysis of seed extracts in *Argemone Mexicana*

Presence of individual compounds in the given sample was analyzed using GC-MS/MS of Thermo Fisher make, ITQ900 model. One micro liter of the sample was run in a DB-1 fused silica capillary column with helium (1ml/min) as carrier gas, 250°C injector temperature, 280°C ion-source temperature and isothermal temperature 110°C (2 min),

with an increase of 10°C/mi-n to 200°C then 5°C/min to 280°C and 9 min to 280°C. The mass spectrum interpretation was performed using the library of National Institute Standard and Technology (NIST) and the compounds were identified.

Isolation of microorganism from sample Cultivation of pure cultures

The microorganism was noted as a mass and so they were separated and pure culture isolation was done. The ten different cultures of organize well streaked on to a freshly prepared nutrient agar slants.

Preparation of media

Preparation of nutrient broth cultures

* Test culture *Nutrient broth

Procedure

10 ml of nutrient broth was taken in each sterile test tube. The five cultures was taken and inoculated separately in to each tube marked as A, B.C.D, and E. The tubes were then incubated for about 24 hours for the growth of microorganisms.

Serial dilution

1. The nutrient agar plates were prepared.
2. 0.1ml of serially diluted samples was spreaded evenly over media.
3. The plates were incubated at 37º c for 24 hours

Identification of Microorganism

The culture where biochemically identified by staining and biochemical procedures.

Gram's staining

The Gram staining method is named after the Danish bacteriologist Hans Christian Gram (1853 – 1938) who originally devised it in 1882(but published in 1884), to discriminate between pneumococci and *Klebsiella pneumoniae* bacteria in lung tissue. It is a differential staining method of differentiating bacterial species into two large groups (Gram-positive and Gram- negative) based on the chemical and physical properties of their cell walls. This reaction divides the eubacteria into two fundamental groups according to their stain ability and is one of the basic foundations on which bacterial identification is built. Gram staining is not used to classify archaea, since these microorganisms give very variable responses.

Biochemical test

Imvic test, Indole test, Methyl red test, Vp test citrate test, Catalase test, Oxidase test, Triple sugar iron agar test, Sugar fermentation test

Determination of antibacterial activity by agar well diffusion method

Agar well diffusion method described by Perez, 1990 was employed to determine antibacterial activity. Well of 10mm diameter was prepared with sterilized cork-borer. Standard antibiotics Ampicillin (A10), Amikacin (AK30), Gentamicin (G120) and Kanamycin(K30), Nalidixic acid (30),Nitrofurantion (300),Penicillin (10),Streptomycin (10),Cephotaxime (30)Streptomycin (30),Tetracyline (30) were served as positive controls and methanol as negative control. The plates inoculated with different bacterial species were incubated at 37°C in incubator for 24hours and the zone of inhibition was measured (Diameter in mm). All of the experiments were performed in triplicate. The results are reported as the average of 3 experiments.

Determination of antimicrobial activity by disc diffusion method

Agar disc diffusion method described by Perez, 1990 was employed to determine antibacterial activity[9]. Well of 10mm diameter was prepared with sterilized cork-borer. Standard antibiotics Ampicillin (A10), Amikacin (AK30), Gentamicin (G120) and Kanamycin (K30),Nalidixic acid (30),Nitrofurantion (300),Penicillin (10),Streptomycin (10),Cephotaxime (30)Streptomycin (30),Tetracycline (30) were served as positive controls and methanol as negative control. The plates inoculated with different bacterial species were incubated at 37°C in incubator for 24hours and the zone of inhibition was measured (Diameter in mm). All of the experiments were performed in triplicate. The results are reported as the average of 3 experiments.

Results And Discussion

The presence of secondary metabolites in seed extracts of *Argemone mexicana* are respectively, alkaloids, flavonoids, glycosides, saponins, carbohydrate, protein, terpenoids, phenols, aminoacid, steroids and tannin in methanol extracts.

The water extract showed the presence of saponins, carbohydrate, tannin, saponin, aminoacid phenol and protein. Saponin, aminoacid, protein, phenol and tannin are present in both methanol and water extracts

The extraction of biologically active compounds from the plant material depends on the type of solvent used in the extraction procedure. The most commonly used solvents for investigations of antimicrobial activity in plants are methanol, [10, 11, 12 and 13].The primary estimation of plant extract shows in Table 1.

Isolation Of Microorganisms

After isolation of microorganisms the colonies of bacteria were identified by biochemical characteristics[14].The results of biochemical characteristics of isolated microorganisms are given in Table 2. The results of the gram staining shows that the sample 1,2,4,7,9 and 8 are gram (-) cocci bacteria , sample 3 are gram negative cocci and rod shaped bacteria5,6 gram (-) rod.and sample 10 both gram (-) and positive rod respectively. This result showed in Table 3.

Table 1. Qualitative analysis of phytochemical compounds

S.NO.	Tested group	Methanol extract	Water extract
1	Tannins	Present	Present
2	Alkaloids	Present	Absent
3	Glycosides	Present	Absent
4	Saponins	Present	Present
5	Steroids	Absent	Present
6	Aminoacid	Present	Present
7	Flavonoids	Present	Absent
8	Protein	Present	Present
9	Phenol	Present	Present
10	Terpenoids	Present	Absent
11	Carbohydrates	Present	Absent

Table 2. Biochemical Characterization Of Microorganisms

	Sample 1	Sample 2	Sample 3	Sample4	Sample 5	Sample 6	Sample 7	Sample 8	Samples 9	Sample 10
Gram staining	Gram (-) cocci	Gram (-) cocci	Gram (-)rods and cocci	Gram (-) cocci	Gram (-) rods	Gram (-) rods	Gram (-) cocci	Gram (+) rods	Gram (-) cocci	Gram (-) & (+) rods
Indole test	Positive Yellow colour	Positive Yellow colour	Positive Yellow colour	Positive Yellow colour	Positive Yellow colour	Positive Yellow colour	Positive Yellow colour	Positive Yellow colour	Positive Yellow colour	Positive Yellow colour
Methyl red test	Red colour positive	Red colour positive	Red colour positive	Red colour positive	Red colour positive	Red colour positive	Red colour positive	Red colour positive	Red colour positive	Red colour positive
VP test	Red colour ring positive	Red colour ring positive	Red colour ring positive	Red colour ring positive	Red colour ring positive	Red colour ring positive	Red colour ring positive	Red colour ring positive	Red colour ring positive	Red colour ring Positive
Citrate utilization test	Positive Blue colour	Positive Blue colour	Positive Blue colour	Positive Blue colour	Positive Blue colour	Positive Blue colour	Positive Blue colour	Positive Blue colour	Positive Blue colour	Positive Blue colour
Catal	Bubb formation	Bubb formation	Bubb formation	Bubb formation	Bubb formation	Bubb formation	Bubb formation	Bubb formation	Bubb formation	Bubb formation
Oxidase test	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
TSI test	Pink colour positive	Pink colour positive	Pink colour positive	Pink colour positive	Pink colour positive	Pink colour positive	Pink colour positive	Pink colourPositive	Pink colour Positive	Pink Colour Positive

Lactose test	White colour positive	White colour positive	White colour positive	White colour positive	White colour positive	White colour positive	White colour positive	Whitecolour positive	White color positive	White colour positive

Table 3. Isolation Of Durg Resistant Bacteria With Different Antibiotics

Patients	A10mcg	T30 mcg	AK30 mcg	K30 mcg	G120 mcg	NA30 mcg	NF300 mcg	Ce30 mcg	P30 mcg	S30 mcg
SAMPLE 1	R	R	R	S	R	S	S	S	S	S
SAMPLE 2	S	R	R	S	S	R	S	S	S	R
SAMPLE 3	R	R	R	R	S	S	R	S	S	S
SAMPLE 4	R	R	R	S	R	S	S	S	S	S
SAMPLE 5	R	R	R	R	S	S	S	S	S	S
SAMPLE 6	S	S	R	R	R	S	R	S	S	R
SAMPLE 7	R	R	R	S	R	S	S	S	S	S
SAMPLE 8	S	R	S	S	R	R	S	R	S	R
SAMPLE 9	R	R	R	R	R	R	R	R	R	R
SAMPLE10	R	R	R	R	R	R	R	R	R	R

R-Resistant, S- Sensitive

A-Ampicillin, T- Tetracycline ,G-Gentamicin, AK-Amikacin ,K-Kanamycin, NA-Nalidixicacid,NF-Nitrofurantoin , Ce - cephotaxime, P –Penicillin G, S – Streptomycin

The indole ,VP,citrate utilization ,methyl red, triple sugar iron,sugar fermentation ,catalase test, showed positive results. It was concluded that the sample culture was identified as *E.coli* respectively. In previous studies many authors have described of hospital wastage sample as a major substrate for it growth. These are found almost everywhere on microorganisms and are found in the skin, nails and body etc.[15]

Fig 3: I Mvic Test

Fig 4: Isolation Of Microorganisms

Fig 5: Indole Test

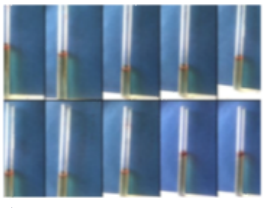


Fig 6: Methy Red Test

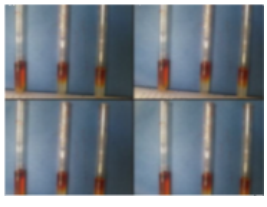


Fig 7: Citrate Test

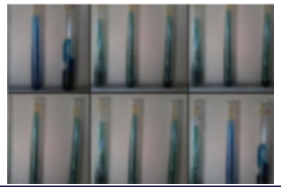


Fig 8: Vp Test

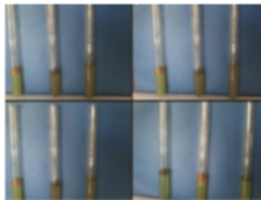


Fig 9: Triple Sugar Iron Test

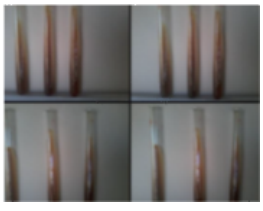


Fig 10: Lactose Fermentation

Fig 11: Oxidase Test

Fig 12: Catalase Test

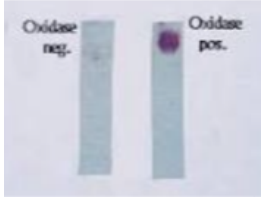
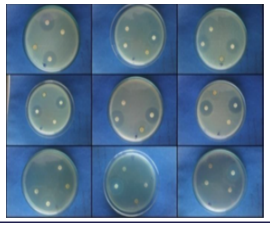


Figure 13: Isolation Of Durg Resistant Bacteria



A-Ampicillin, T- Tetracycline ,G-Gentamicin, AK-Amikacin ,K-Kanamycin, NA-Nalidixicacid ,NF-Nitrofurantoin , Ce - cephotaxime, P –Penicillin G, S – Streptomycin

Figure: 14

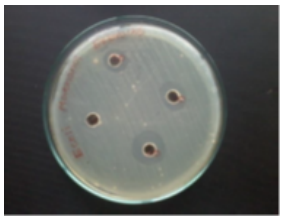


Fig 14.1(SAMPLE 1)

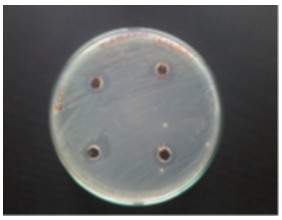
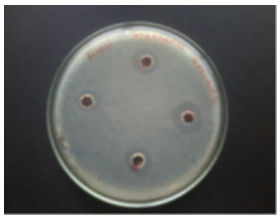


Fig 14.2(sample 2)



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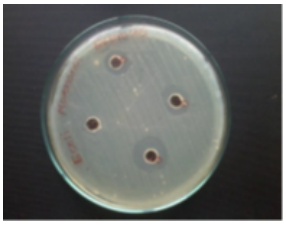

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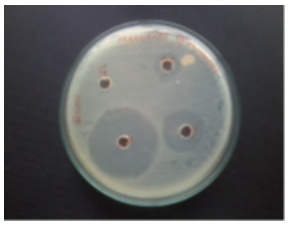

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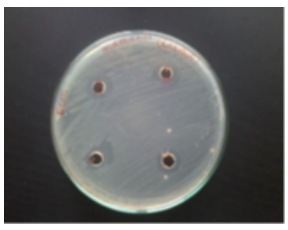

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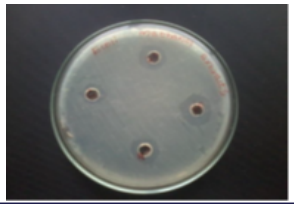

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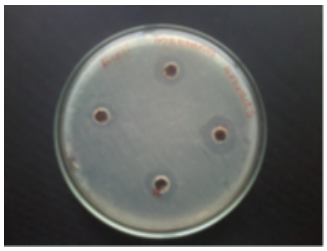

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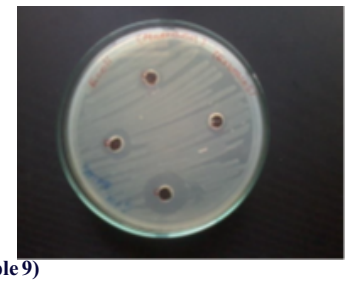

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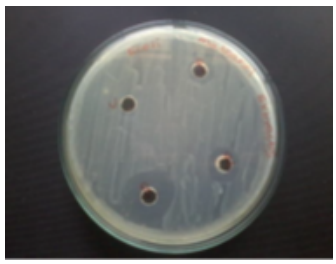

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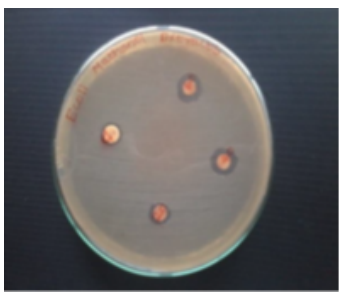
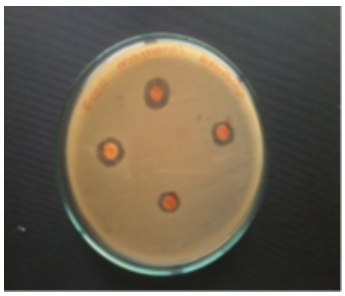


Figure: 15



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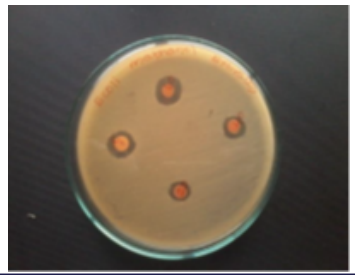

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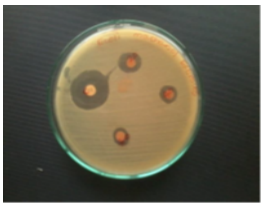

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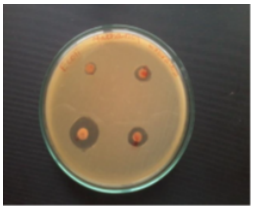

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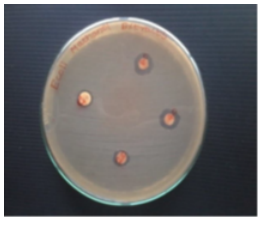

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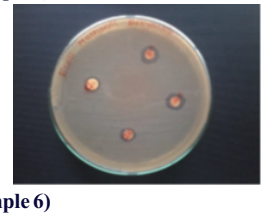

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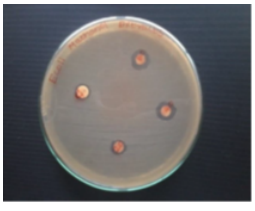

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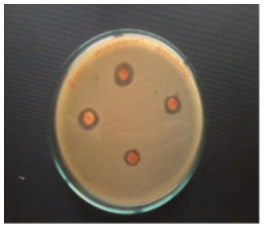

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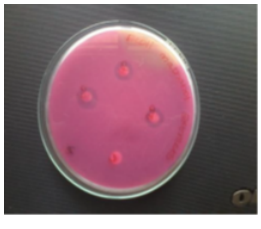

ANTIBIOTIC SUSCEPTIBILITY TEST

Ten isolated strains and the standard strain of E.coli were subjected to antibiotic sensitivity test by the disc diffusion method (Table 5, Table 6) following the standard antibiotic susceptibility chart of National Committee for Clinical Laboratory Standards (NCCLS) guidelines.[16]

Different concentrations of seed extracts in the methanol were tested to find out the inhibition zone for the bacterial strains. The lowest concentrations (1.0µg/ml) were not effective against any of the test organisms, however inhibition zones were observed at increasing concentrations (20.0-60.0 µg/ml). The minimum inhibition activity of E.coli was observed at a concentration of (20.0 µg/ml) -and the maximum inhibition activity of E.coli was observed at a concentration of (60.0 µg/ml) Mexicana seed extract in water had no antibacterial property. The water soluble flavonoids (mostly anthocyanins) have no antimicrobial significance and water soluble phenolics are only important as antioxidant compounds.[17] The antimicrobial activity can be enhanced if the active components are purified and adequate dosage determined for proper administration. The study area was highly affected by dental carries patients for many diseases. A number of microorganisms such as lactobacillius, strepyococci, E.coli, lactobacilli, and kelebsiella is one of the predominant microorganisms, which cause the fecal oral transmission.[18]

This study was focused on the antibiotic resistance pattern of E.coli spp.in dental carries and urinary tract infection in diabetic patients.E.colispp, is an enteric organisms that has been isolated from different patients. In this study 30 samples were collected and it was analyzed for their total bacterial population on nutrient agar and macconkey agar medium. Out of the bacterial population, ten isolates were identified as E.coli based on the conventional method.

All the E.coli spp. not only produce large scale pandemics and epidemics but characterized by multi drug resistance for antibiotics Ampicillin, Tetracycline, Gentamicin, Kanamycin, Penicillin, Nalidixic acid , Cefpodoxine, Nitrofurantion respectively. The mainstay of treatment is an appropriate antibiotic but development is drug resistance posses a series therapeutic challenge to cure the urinary tract infection disease and dental carries. The pattern of resistance of the staring studied was resembles that reported by Ama bile-Cuevas200319.

In our study, the MAR index value was observed and the isolates might have originated from high sources like human fecal contamination.[19]

SUMMER AND CONCLUSION

The present study was done to analyse the phytochemical compounds qualitatively and to evaluate the antimicrobial activity of Aregemone Mexicana seeds. The study was carried out on multidrug resistant bacteria among diabetic patients. The clinical sample was collected from KSR Dental Science and Research Insititute,Tiruchengode.10 isolates were isolated from 30 patients both male and female.40-70 age groups of patients depicted the high rate of infection. Bacteriological examinations clearly indicated that the overall prevalence of bacterial infection is 80 % for women. From that, the prevalence rate of isolated pathogenic organisms illustrated describes that high rate of prevalence was exhibited by E.coli.

The results of the phytochemical analysis of the seed extract of argemone mexicana revealed the presence of secondary metabolites arerespectively, alkaloids, flavonoids, glycosides, saponins,carbohydrate,protein,terpenoids,phenols,aminoacid,steroids and tannin.2.4 mg of tannin and 4.8 mg of phenol was estimated in the seed sample.

The present study the estimation of secondary metabolites of argemone Mexicana demonstrated the presence of tannin and phenol. Hence the present investigation suggests the extract shows good antioxidant activity, reducing power of free radical scavenging activity.

The antibiotics sensitivity test has been done for all the pathogens. From the 30 mouth swab and urine sample, 10 E.coli pathogens was isolated which is a multidrug drug resistant strain,, because it is a hospitalized organisms and it was highly exposed to antibiotics. For the antibiogram pattern different antibiotics were used.

Different concentrations of seed extracts in the methanol were tested to find out the inhibition zone for the bacterial strains. The lowest concentrations (1.0µg/ml) were not effective against any of the test organisms, however inhibition zones were observed at increasing concentrations (20.0-60.0 µg/ml). The minimum inhibition activity of E.coli was observed at a concentration of (20.0 µg/ml) and the maximum inhibition activity of E.coli was observed at a concentration of (60.0 µg/ml).

The results of the study provides basis for subsequent bioactivity guided fractionation of the extract of the seed of aregmone Mexicana and isolation of pure 48 antibacterial compounds. The GC-MS analysis was also carried out in the study to analyse the compounds present in Argemone Mexicana seed extracts and the results are reported respectively.

REFERENCES

1) Abee-Lund, (2002). Antibiotic resistance in food-related bacteria - a result of interfering with the global web of bacterial genetics. Intl. J. Food Microbiol., 78: 43-56.
2) Ahn JW (1994). Cytotoxic limonoids from Melia azedarach var. japonica. Phytochemical. 36: 1493-1496.
3) Akueshi CO, Kadiri CO, Akueshi EU, Agina SE, Ngurukwem B(2002). Antimicrobial 4.potentials of Hyptis sauvedens Poit (Lamiaccae), Nigeria. J Bot 15: 37-41.
4) Alexander M. (1981) why microbial predators and parasites do not eliminate their prey and hosts Ann Rev Microbiol; 35: 113-133.
5) Almagboul AZ, Bashir AK, Farouk A , Salih A K M, (1985), Fitoterapia, 56,331.
6) Ama´bile-Cuevas CF (2003) Gathering of re-sistance genes in Gramnegative bacteria: an overview. In: Ama´bile-Cuevas CF (ed) Multidrug resistant bacteria. Horizon Scientific Press, Wymondham, pp 9–31.
7) In the Wealth of India A Dictionary of Indian Raw Materials and Industrial Products. Council of Scientific and In-dustrial Research, New Delhi, Vol. I, 2004: 86-87.
8) Artizzu N, Bonsignore L, Cottiglia F , Loy G ,(1995), Fitoterapia, 66,174. Izzo A A, Carlo Di , Biscardi G , Fusco D, Mascolo R , Borreli N ,Capasso F, Fasulo F, Autore M P, 1995. Phy-tother Res, 9,281.
9) Bennett RN, Wallsgrove RM (1994). Secondary metabolites in plant defence mechanisms. New Phytol 127: 617-633. Bhattacharjee I, Chatterjee S.K., Chatterjee S., Chandra G: (2006) Antibacterial potentiality of Argemone mexicana solvent extracts against some pathogenic bacteria. Mem Inst Oswaldo Cruz.; 101(6): 645-8Brown SA (1987)Minimum inhibitory concentrations and postantimicrobial effects as factors in dosage of antimicrobial drugs. J Am Vet Med Assoc 19: 871-872.
10) Brinda P, Sasikala P, Purushothaman KK. Phar-macognostic studies on Merugan Kizhangu. Bullet in Medical 18 Eth-anobotanical Research, 3, 1981, 84-96.
11) Capasso, A., S. Piacente, C. Pizza, N. Tommasi, C. Jativa and L. Sorrentino, (1997) Isoquinoline alkaloids from Arge-mone mexicana reduce morphine withdrawal in guinea pig isolated ileum. Plant Med., 63: 326-328.
12) Chang Y.C. Chang F.R., Khalil A.T., Hsieh P.W., Wu Y.C:Cytotoxic benzophenanthridine and benzylisoquinoline alka-loids from Argemone mexicana. Z Naturforsch 2003; 58(7-8): 521-6.
13) Chopra R.N., Chopra I.C., Varma B.S. (1979) Supplement to Glossary of Indian Medicinal Plants with active princi-ples, Publications and Information Directorate, New Delhi,(part I), 85-86.
14) Chopra RN, Nayer A, Chopra IC (1986) Glossary of Indian Medicinal plants, (including the supplement). Council of Scientific and Industrial Research, (CSIR), New Delhi.
15) Chopra RN, Nayer SL and Chopra IC (1988) Glossary of Indian medicinal plants council, scientific and industrial research: 30-45,
16) Cohen ML (1992). Epidemiology of drug re-sistance: implications for a post-antimicrobial era. Science 257: 1050-5.
17) Cova (1995) Accumulation of chemicals has af-fected by biological environments Ethanopharmocology: Page no 76-98
18) Cowan, M.M., (1999). Plant products as antimi-crobial agents. Clinical Microbiol. Rev., 12: 564-582.
19) Cox S (1985) simple soures such as plants pro-duced antimicrobial agents Rev (45): 78-131
20) Dubey, N.K., R. Kumar and P. Tripathi,(2004). Global promotion of herbal medicine: Indian's opportunity. Current Sci., 86: 37-41
21) Senthamilselvan et al, protective role of vitis vinif-era seed on isoproterenol induced myocardial infarction in rate, Indian journal of medical Science, Sep 2009, 343
22) Senthamilselvan et al, Analysis of bioactive com-pounds in ethanol extracts argemone mexicane plant seeds using gc-ms techniques, Indian Journal of Applied science, May 2015
23) Senthamilselvan et al 2015, Anti-hyperlipidemic activity of the bark extract ofterminalia arjuna in caffeine induced mice, Indian Journal of Applied science, May 2015
24) Senthamilselvan et al, Analysis of cladophora glomerata in high performance Liquid chromatography-mass spec-trometry (HPLC – GCMS) International journals of Science and research, May 2015
25) Senthamilselvan et al, Analysis of phytochemical component and nutrients component in ethanol extracted oldenlandia corymbosa, World Journal of Pharmaceutical research 2015
26) Senthamilselvan et al, Regenerating activity of cit-rus aurantifolia on paracetamol induced heaptic damage, Asian Journal of Bioscience May 2009
27) Senthamilselvan et al Impact of hemidesmus indi-cus on mosquito coil exposed rat Journal of Medicinal Plant studies, May2009
28) Senthamilselvan et al , Analysis of bioactive com-pounds in methanol extract of cissus vitiginea leaf using GC-MS, Ra-sayan Journal of Chemistry 2009.