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Phytochemical Studies and Anticariogenic Activityof Argemone Mexicana Seeds.

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ABSTRACT:
Oral diseases are a major health problem worldwide. The increasing failure of chemotherapeutics and antibiotics resistance exhibited by pathogenic micro-organisms has led to the screening of several medicinal plants for their anticariogenic activity.The present study was undertaken to evaluate the anticariogenic activity of Argemone Mexicana seeds. Itis belonging to family Papaveraceae. It grows as weed almost all parts in India. The plant have alkaloids ,terpenoides ,glycosides and flavonoides .Antibacterial activity of Argemone mexicana seeds was evaluated against the bacteria that causes dental carries. The ethanolic extract of crude seeds were tested by Agar well diffusion method, the ethanolic extract shows antibacterial activity against the test cariogenic bacterial stains. The study concludes that Argemone Mexicana have biologically active compounds which are effective in treatment of Dental carries.

Keywords: Anticariogenic activity, Argemone mexicana, Agar well diffusion method

I. INTRODUCTION:

Oral health is integral to general well-being and relates to the quality of life that extends beyond the functions of the craniofacial complex concerns [1]. Dental caries and periodontal diseases are among the most important global oral health problems, although conditions such as oral and pharyngeal cancers and oral tissue lesions are also significant health concerns [2]. According to WHO, dental caries is defined as localized post eruptive pathological process of external origin involving softening of the hard tooth tissue. It is an infectious disease caused by microbe's results in decalcification n and destroying particular infected tooth. It develops by complicated mechanism such as interaction of tooth or saliva with acidogenic bacteria. [3]As the enamel losses its minerals, it starts to breakdown, resulting into formation of cavity [4]. Medicinal plants and their products used as a primary source of many drugs from several thousands of years. These plants play a significant role in prevention, diagnosis and treatment of various diseases in humans. The advantage of using plants asmedicine is, they are not producing any side effects compared to chemically produced drugs or medicines. Lactobacillus acidophilus, Lactobacillus casei, Streptococcus mutans and Staphylococcus aureus were the bacteria used to producecariogenic effect. As a result some bacterial infections are now essentially untreatable with antibiotics. In a 2003, Institute of medicine report, microbial threat to health, antimicrobial resistance was noted as a paramount microbial threat of the twenty first century, some strain of bacteria are now resistant to essentially available antimicrobial drugs and some remain susceptible to only one. The lack of new drug classes is a consequence of difficulties in discovery of new compounds that has persisted for many years [5].Now-a-days, dental caries is very common in industrialized as well as under developed and low-income countries. Inferring a history, plants are rich in antimicrobial drug source. So, it is priority to discover an herbal treatment for dental caries. The present study was taken up to conduct phytochemical and anticariogenic activity studies of seeds of Argemone Mexicana.

II. MATERIAL AND METHODS:

2.1 Collection f plant material:-

Argemone mexicana Seeds were collected between June and July were collected from the surrounding fields of Mangalwedha. The plant was identified and authenticated by botanist.

2.2 Extraction of plant material:-

The Argemone mexicana Seeds were shade dried and coarsely powdered. The coarse powder was first extracted by maceration and then by Soxhlet extraction method. About 50 gm. of Seeds powder was subjected to cold maceration with 200 ml of 80% aqueous ethanol for 24 hours

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[6]. Flask was securely plugged with absorbent cotton and was shaken periodically. The extract was filtered and the marc was air dried.

2.3 Bacterial strains for anticariogenic activity

A group of bacteria that cause toothcaries were selected. Lactobacillus acidophilus (NCIM5306), Lactobacillus casei (NCIM5303), Streptococcus mutans (MTCC890) and Staphylococcus aureus were used in present study to study the antimicrobial activity of the prepared extracts. L. acidophilus and L. casei were procured from National Collection of Industrial Microorganism (NCIM), CSIR-National Chemical Laboratory, Dr. Homi Bhabha Road, Pune-411008, India.S. mutans was procured from Microbial Type Culture and Gene Bank (MTCC), Institute of Microbial Technology, Shanti Path, 39A, Sector 39, Chandigarh, 160036,India. S. aureus procured from BLDE's Shri B. M. Patil Medical College Hospital & research Centre, Solapur road, Vijayapur 586103.

2.4 Preliminaryphytochemical Screening

Crude extract obtained from extraction process were subjected to phytochemicalstudies.

Procedure for Detectionof alkaloids [7]:

Mayer'stest: Filtrate of drugs extract was treated with freshly prepared Mayer'sreagent. Yellow colored precipitate shows presence of alkaloids.

Wagner's test: Filtrate of drugs extract was treated with Wagner's reagent. Formation of reddish brown precipitate confirms presence of alkaloids.

Dragendroff's test: Filtrates of drugs extract were treated with Dragendroff's reagent. Red precipitate indicates presence of alkaloids.

Hager's test: Few drops of Hager's reagent were added in filtrate so drugs extracts. Yellow precipitate indicates presence of alkaloids.

Procedure for Detectionof glycosides [8]:

Modified brontrager's test: 1gm. of drugs extract was added in 5 ml of dilute HCL. 5 ml of ferric chloride wasadded and boiled on water bath for 10 min. filtrate was treated with carbon tetrachlorideand equal amount of ammonia solution. Formation of pink to red colour indicates presence of glycosides.

Legal'stest: Drug extract was added in equal amount of water. 0.5 ml of lead acetate solutionwas added. Filtrate was treated with equal amount of chloroform. 2 ml of pyridine and sodium nitropruside was added in above mixture. Appearanceof pink color confirmspresenceof glycosides.

Baljet test: Drug extract was treated with sodium picrate solution. Yellow color shows presence of glycosides.

Liebermann burchard test: Concentrated sulphuric acid was added from the side wall of test tube. Appearance of violet ring and blue color indicates presence of glycosides.

Procedure for Detectionof saponins [9]:

Froth'stest: 3 gm. of extract was mixed with 10 ml of distilled water. Mixture was shaken vigorously. Honey comb froth indicates presence of saponins.

Detection of phytosterols [10]:

Liebermann test:

Filtrates of drugs were treated with sulfuric acid and acetic anhydride solution.
Blue color shows presence of phytosterols.

Salkowski test: Extract of drugs was treated with chloroform and few drops of concentrated sulfuric acid. Red color indicates presence of sterols.

Detectionofphenolsandtannins [11]:

Ferricchloridetest: Drugs extractswere mixedwithwarm water. 2mlof 5% ferric chloridewasadded. Green or blue colour indicates presence of phenolic compounds.

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Gelatintest:

1% of gelatin solution containing 10% sodium chloride was treated with drug extract. Formation of precipitation indicates presence of phenolic compound**.**

Lead acetate solution test:

Drug extract was treated with 0.5ml of 1% lead acetate solution. Formation of precipitation indicates presence of tannins and phenolic compounds.

Sodium hydroxide test:

Few drops of sodium hydroxide solution were added infiltrate. Formation of precipitation indicates presence of phenolic compounds.

Shinoda test:

Few drops of magnesium ribbon and concentrated hydrochloric acid were treated with extract. Appearance of magenta color indicates presence of phenolic compounds**.**

Detectionofproteinsandaminoacid [12]:

Millionstest:

1 ml of Millions reagent was added in 1 ml of filtrate. Mixture was boiled for 1min. after cooling 1% sodium nitrate was added. Development of brick red precipitation indicates presence of proteins.

Biurettest:

1ml of filtrates of drug extracts was treated with 1ml of Biuret reagent. Development of blue color represents presence of proteins.

Ninhydrin test:

2% of Ninhydrin solution was prepared by dissolving 2 gm of Ninhydrin in 10 mlof distilled water. Few drops of Ninhydrin solution were added in filtrate. Deep blue color indicates presence of amino acid.

Detection of carbohydrates [13]:

Molisch'stest:

2 drops of alcoholic α-naphthol were treated with filtrate. 2 ml of concentrated sulphuric acid was added in the tube by side walls. Formation of violet ring indicates presence of carbohydrate.

Benedict'stest:

Filtrates were treated with Benedict's reagent and heated on water bath. Formation of orange red precipitate indicates presence of reducing sugar.

Fehling'stest:

Filtrate was treated with dilute hydrochloric acid. Fehling's A and B solutions were added. Red precipitation indicates presence of reducing sugar.

Barfoed'stest:

1 ml of filtrate was treated 1 ml of Barfoed's reagent. Brick red precipitation showspresenceof carbohydrate.

Detectionoffixedoil [14]:

Staintest:

small quantity of extract was placed between two filter papers. Oil stain on pape rindicates presence of fixed oil.

Soaptest:

Few drops of 0.5N alcoholic potassium hydroxide were added tothe extract.1drop of phenol phthalein was added in mixture. Mixture was heated on water bath for 2hrs. Formation ofsoap indicates presence of fixed oil.

2.5 Preparation of bacterial inoculums:

Broth cultures of the selected bacteria were prepared by inoculating a loop full of bacteria into organism-specific media and incubated at optimal temperature. MRS media was used for L. acidophilus and L. casei. Enriched infusion heart media for S. mutans and Nutrient broth media for S. aureus were employed respectively. The bacterial suspension was compared with 0.5 Mc Farland turbidity standards, which is equivalent to approximately 1×10^8 bacterial cell count per ml. Such prepared bacterial suspensions were used for antimicrobial studies [15].

2.6 Bioassay for anticariogenic activity of plant extracts:

2.6.1 Agar well diffusion method:

The anticariogenic activity of extracts of Argemone mexicana (seed) was studied by agar well diffusion method. The plant extracts were dissolved in dimethyl sulfoxide and were tested at three concentrations viz. 50, 100 and200 mg/ml.The agar plateswere prepared and labeled for specific bacteria and extract. A fresh bacterial culture of 100 µl having 1×10^8CFU/ ml was spread

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on agar plates using sterile cotton swab.6mm diameter well was made with a sterile borer. The prepared wells were filled with 100 µl of respective plant extracts. Plates were placed in the refrigerator for 30 min. for diffusion of extracts. Then plates were incubated at 37˚C for24 hrs. The zone of inhibition was measured. Tetracycline (100 µg/ml) was used as a standard drug for comparison [16].

2.6.2 Determination of Minimum Inhibitory Concentration (MIC) of plant extracts:

MIC was done by broth dilution method. The plant extracts were tested at 30, 40, 50, 60, 70 80, 90 and 100 mg/ml. 100 µl of bacterial suspension was added to each tube. The tubes were incubated for 24 hrs at 37˚C. 100 µl of 0.1% growth indicator 2, 3, 5- triphenyl tetrazolium chloride was added to each tube to find out bacterial growth inhibition. Tubes were incubated for 30 min. under dark condition. Bacterial growth was determined when colorless growth indicator converted into red color [17].

III. RESULT AND DISCUSSION:

In present study, the anti-cariogenic assay of plant material extract formulation against oral pathogenic organism was carried out for the purpose of checking of sensitivity of cariogenic bacteria. Plant material were extracted by using 80% aqueous Ethanol and used for further anti cariogenic activity. The presence of common phytochemical constituents such as alkaloids, tannins, saponins, terpenoids, steroids, phenolic compounds, and cardiac glycosides were tested qualitatively as per the methodology and presented in Table 1. Glycosides, were absent in Argemone mexicana.

TableNo.1:Preliminary phytochemical screening of seed extracts of A. mexicana.

Phytochemicals	Results
Alkaloids	+
Anthraquinones	+
Cardiac glycosides	+
Tannins,	+
Steroids	-
Terpenoids	+
PhenolicCompounds	+

Key Absent = (-), Present = (+)

The anticariogenic activity of the plant extracts and their efficacy were quantitatively assessed by measuring the zone of inhibition. The plant extracts were tested for anticariogenic activity by agar well diffusion method. It was tested at three concentrations viz. 50, 100 and 200 mg/ml. Dimethyl sulfoxide was used to dissolve the plant extracts. The result of sensitivity of cariogenic organisms was assessed by recording the presence or absence of Zone of inhibition in diameter. The zone of inhibition was observed at 50, 100 and 200mg/ml concentration of ethanolic plant extracts and results are summarized as under Table no.2.

Table no. 2 Anti cariogenic activity of ethanolic extract at different concentration

Sr. no	Concentration (mg/ml)	Zone of inhibition (mm)			
		L. acidophilus	L. casei	S. mutans	S. aureus
1	50	14	10	11	12
2	100	19	16	17	18
3	200	24	20	19	22

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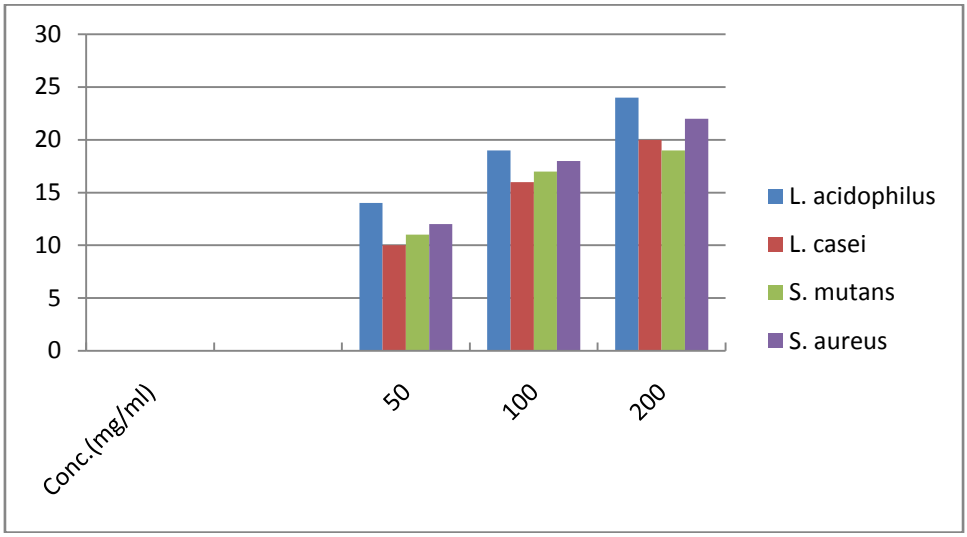
Fig no. 1.**Anti cariogenic activity of ethanolic extract at different concentration**



The Minimum Inhibitory Concentration (MIC) values of seed extract of Argemone Mexicana showing highest activity against selected organisms was assessed and summarized in Table no 3. Examining the MIC values of samples extracts showing highest anticariogenic activity againstall four bacteria, L. acidophilus, L. casei, S.mutans and S. aureuswiththeir MIC values ranging from40 to 90 mg/ml.

Table No.3–MIC values of ethanolic extract in mg/ml.

Sr. no	Minimum Inhibitory Concentration (MIC)in mg/ml			
	L. acidophilus	L. casei	S. mutans	S. aureus
1	40	50	50	40

IV. CONCLUSION:

The result of this study highlights the significance of anticarriogenic activity against the selected bacterial stains from ethanolic seed extract of A. mexicana. It will find a place in formulation of herbal medicine to control Dental carries.

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