FORMULATION AND IN VITRO EVALUATION OF DENTAL GEL CONTAINING ETHANGLIC EXTRACT OF MIMOSA PUDICA

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Abstract

Dental caries, one of the globally affecting diseases of the oral cavity and the herbal extracts in clinical practice can benefit the oral hygiene of the patient. The main instigation and progress of dental caries involve acidogenic and aciduric Gram-positive bacteria such as Streptococcus, Lactobacillus and Actinomycetes colonizing the supragingival biofilm which impede with usual nutrition intake, verbal communication, selfworth and daily habitual behavior. Mimosa pudica (from Latin: pudica "shy, bashful or shrinking".) is a creeping annual or perennial flowering plant of the pea/legume family *Fabaceae*, often grown for its curiosity value: the compound leaves fold inward and droop when touched or shaken, defending themselves from harm, and re-open a few minutes later. The in vitro study shows that the ethanolic extract of *M. Pudica* is present flavonoids and tannins contain and the greatest zone of inhibition study indicates anti-bacterial and anti-inflammatory activity.

Keywords: Dental Caries, *Mimosa Pudica*, Herbal Extract, *Fabaceae*, Dental Gel.

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INTRODUCTION

Mimosa pudica is well known for its rapid plant movement. In the evening the leaflets will fold together and the whole leaf droops downward. It then re-opens at sunrise[1]. This type of motion is termed as nyctinastic movement. The foliage closes during darkness and reopens in light. The leaves are drooping because of stimulus, in conditions such as touching, warming or shaking. The stimulus can be transmitted to neighbouring leaves[2]. These types of movements are termed as seismonastic movements. This is due to loss of turgor pressure. The movement is caused by a rapid loss of pressure in strategically situated cells that cause the leaves to droop right before one's eyes[3].

Figure.1: Plant of Mimosa Pudica

Scientific Classification
Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Fabales
Family : Fabaceae
Subfamily: Mimosoideae
Genus: Mimosa
Species: M. pudica

PRINCIPAL PHYTONSTITUENTS OF MIMOSA PLANT

M. pudica contains Mimosine, which is a toxic alkaloid, Adrenalin like substance, Crocetin dimethyl Easter in the extract of the plant. Roots contain tannin, Seeds contain a mucilage which is composed of d-xylose and d-glucuronic acid. The plant extract contains green yellow fatty oil, tubuline[4]. The periodic leaf movement factors are reportedly the derivatives of 4-α-(b-D-glucopyranosyl-6-sulphate) gallic acid[5]. The preliminary phytochemical screening of the M. pudica leaf extract showed the presence of bioactive components such as terpenoids, flavonoids, glycosides, alkaloids, quinines, phenols, tannins, saponins, and coumarins[6].

PHARMACOLOGICAL ACTIVITIES

The study of different journals and book the following pharmacological activate of M. pudica are reported.

Wound healing activity: The M. pudica shoot methanolic extract, *M. pudica* root methanolic extract showed very good wound healing activity. The methanolic extract exhibited good wound healing activity probably due to presence of phenols constituents[7].

Antimicrobial Activity: The antimicrobial activity of methanolic extract of Mimosa was tested against Aspergillus fumigatus, Citrobacter divergens and Klebsiella pneumonia at different concentrations of 50, 100 and 200µg/disc. The antimicrobial activity was attributed to the presence of bioactive constituents like terpenoids, flavonoids, glycosides, alkaloids, quinines, phenols, tannins, saponins and coumarin[8].

Analgesic and anti-inflammatory activity: The ethanolic extract of the leaves of *M. pudica* at the doses of 200 and 400 mg/kg was tested for anti-inflammatory and analgesic activity. The extract produced dose dependent and significant inhibition of carrageenan induced paw oedema. The analgesic activity was found to be more significant on the acetic acid induced writhing model than the tail flick model[9].

Anticonvulsant: The decoction of *M. pudica* leaves were given intraperitoneally at dose of 1000-4000 mg/kg which protected mice against pentylentetrazol and strychnineinduced seizures. M. pudica had no effect against picrotoxininduced seizures. It also antagonized N-methyl-D-aspartateinduced turning behavior[10].

Antidiarrhoeal activity: The ethanolic extract inhibited castor oil induced diarrhoea and PGE2 induced enteropooling in rats and has also reduced gastrointestinal motility after charcoal meal administration. The ethanolic extract at 200 and 400 mg/kg was showed significantly inhibited diarrhoea. The antidiarrhoeal property may be related to the tannin and flavonoids present in the extract[11].

Antifertility activity: *M. pudica* root extract, when administered orally at a dose of 300 mg/kg body weight/day, prolonged the length of the estrous cycle with significant increase in the duration of the diestrous phase and reduced the number of litters in albino mice. The analysis of the principal hormones (Luteinizing hormone,

Follicle-stimulating hormone, prolactin, estradiol and progesterone) involved in the regulation of the estrous cycle showed that the root extract altered gonadotropin release and estradiol secretion[12].

Anti-oxidant activity: The methanol crude extract of the aerial part of *M. pudica* was screened in vitro for antioxidant activity using the 1, 1- diphenyl-2-picrylhydrazyl-hydrate (DPPH) free radical scavenging assay. The methanol crude extract of the aerial part showed moderate antioxidant activity (IC50 296.92µg/ml) compared to ascorbic acid (IC50 131.29µg/ml) suggesting presence of biologically active constituents in the methanolic extract of M. pudica[13].

Antimalarial activity: The ethanolic extract of *M. pudica* leaves was investigated for antimalarial activity against Plasmodium berghei infections in mice. The extract of P. niruri and M. pudica leaf demonstrated significant antiplasmodial activity in all the three models of the antimalarial evaluations. Phytochemical screening revealed the presence of some vital antiplasmodial constituents such as terpenoids, flavonoids and alkaloids. The leaf extract of P. niruri and M. pudica possesses antimalarial activity[14].

Anti-hepatotoxic activity: The ethanol extract of *M. pudica* leaves was evaluated for its hepatoprotective against carbon tetrachloride (CCl4)-induced liver damage, in Wistar albino rats. The ethanol extract of M. pudica (Mimosaceae) leaves (200 mg/kg body weight, p.o.) was administered to the experimental rats for 14 days. The substantially elevated levels of serum SGOT, SGPT, ALP and total bilirubin, due to CCl4 treatment, were restored towards near normal by M. pudica (Mimosaceae), in a dose[15].

Antihelminthes activity: The present study was undertaken to evaluate anthelmintic activity of different extracts of seeds of *M. pudica*. The different successive extracts namely petroleum ether, ethanol and water using Pheretima posthuma as a test worm to the different concentrations (100, 200, 500 mg/kg) were tested for bioassay which involved determination of paralysis and time of death of the worms[16].

Antihyperglycemic activity: Chloroform extract showed significant hyperlipidemic effect by lowering the serum levels of biochemical parameters such as significant reduction in the level of serum cholesterol, triglyceride, LDL, VLDL and increase in HDL level which was similar to the standard drug Atorvastatin. Chloroform extract exhibited significant atherogenic index and percentage protection against hyperlipidemia[17].

Antiulcer activity: Antiulcer potential of ethanolic extract of *M. pudica* leaves was evaluated by pylorus ligation, aspirin and ethanol induced ulcer models. The ethanolic extract of the leaves of M. pudica was given by oral route at a dose of 100 mg/kg b.w. The extract, dose dependently reduce, the total acidity, ulcer index, and an increase in pH of gastric juice in pylorus ligated ulcer model[18,19,20].

Antivenom activity: The Aqueous extract of dried roots of *M. pudica* displayed a significant inhibitory effect on the lethality, phospholipase activity, edema forming activity, fibrinolytic activity and hemorrhagic activity. About 0.14 mg and 0.16 mg of M. pudica extracts were able to completely neutralize the lethal activity of 2LD50 of Naja and Bangarus caerulus venoms respectively[21].

MATERIAL AND METHODS

Collection of plant material

The fresh steam and leaves of plant *Mimosa pudica* were collected in the month of March 2020 from the areas of Gondia Dist.

Preparation of extract

Mimosa pudica Folium leaves are separated from the stems, sorted dry and wet, shed dried until a moisture content below 10%, and a blender to obtain a coarse powder. 20 grams of the powdered thorns of 250 ml of ethanol were subjected to Successive extractions using Soxhlet extractor. After condensing the extract using rotary evaporator, the thorn extract was labelled and stored at 5°C[22].

IDENTIFICATION OF PHYTOCHEMICAL

The identification of phytochemicals were:

Flavonoids: Samples coupled with a little water in a test tube, add a bit of metal magnesium and 5 drops of 2 N HCL, heated for 5-10 minutes, hot filtered and allowed to cool, the filtrate plus amyl alcohol, strong shaking. A positive reaction to the formation of a layer of red in amyl alcohol.

Alkaloids: Extracts basified with ammonia, chloroform added. Chloroform liquid is filtered, the filtrate is placed in a test tube and then added 2 N Hall, shaken, until separation occurs. The filtrate plus reagent Dragendorff show sediment or turbidity colorless to brown, and other filtrate

added reagent Mayer showed white precipitate or turbidity.

Saponin: 1g of extract added with warm water, shaken vertically for 10 seconds, allowed to stand for 10 seconds. 1-10 cm tall foam forming stable for not less than 10 minutes, indicating the presence of saponins. In addition 1 drop of HCl 2 N, the foam does not disappear.

Tannin: 200 mg of extract diluted in 20 ml of hot water and then shaken until homogeneous. After cooling added FeCl3 3% showed a positive result if the solution formed a blue-black or brownish green.

ANTIMICROBIAL ASSAY

Well-diffusion method

Antimicrobial activity was determined by agar well diffusion method. About 25 ml agar medium along with the inocoulm was poured into a sterile Petri plate. The plates were allowed to solidify. After five minute setting of the type strains, a sterile cork borer was used to make 5 mm well on the agar in a uniform manner. The plant extracts were dissolved in sterile saline and loaded into wells with definite concentrations. The solvent saline loaded well served as negative control. Ciprofloxacin (25µg/ml) well served as positive control for bacteria. The plates were incubated for 24 hours at 37°C in an incubator. The antibacterial activity was determined by measuring the diameter of the zone of inhibition around the well using antibiotic zone scale[23].

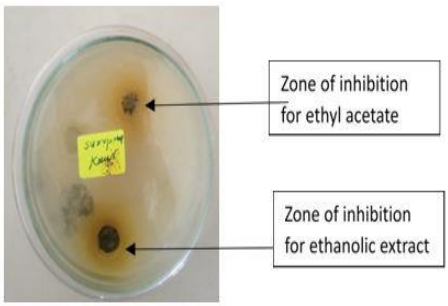

Figure.2: Antimicrobial assay (Well-diffusion method)

IN VITRO ANTI-INFLAMMATORY ACTIVITY ASSAY

Inhibition of protein denaturation assay

Protein denaturation results loose of biological properties of protein molecules. Protein denaturation has been correlated with the formation of inflammatory disorders. In this assay either egg albumin or bovine serum albumin (BSA) are used as protein.

Denaturation of protein is induced by keeping the reaction mixture at 70°C in a water bath for 10 minutes. A reaction mixture consists of various concentrations of plant extract 1000 µL (100-500 µg/ml), 200 µL of egg albumin or 450 µL (5% w/v aqueous solution) bovine serum albumin, 1400 µL of phosphate buffered saline. Distilled water instead of extracts with above mixture is used as a negative control. Afterward, the mixtures is incubated at 37 °C for 15 min and then heated at 70°C for 5 min. After cooling under running tap water, their absorbance's are measured at 660 nm. Acetyl salicylic acid is taken as a positive control. The experiment is carried out and percent inhibition for protein denaturation is calculated using following equations:

% protein inhibition = (Abs control – Abs sample) x 100

Figure.3: Preparation of Sample & Standard Solution

Formulation Development

Table 1: Formulation ethanol extract

Material	F1	F2	F3
The ethanol extract	5%	10%	15%
Sorbitol	10%	10%	10%
Glycerin	10%	10%	10%
Carbopol 934	0.5%	0.5%	0.5%
Triethanolamine	0.75%	0.75%	0.75%
Methyl paraben	0.1%	0.1%	0.1%
Aqua	100%	100%	100%

Table 2: Formulation ethyl acetate fraction

Material	F1	F2	F3
The ethyl acetate extract	5%	10%	15%
Sorbitol	10%	10%	10%
Glycerin	10%	10%	10%
Carbopol 934	0.5%	0.5%	0.5%
Triethanolamine	0.75%	0.75%	0.75%
Methyl paraben	0.1%	0.1%	0.1%
Aqua	100%	100%	100%

Procedure: Weighed all the ingredients, then develop carbopol 934 in distilled water.

Development is carried out for 24 hours. Dissolve methyl paraben in sorbitol. Then mix all ingredients with carbopol, mixing using a mixer with rpm 558. Add triethanolamine to reach a neutral pH.

EVALUATION OF GEL

Physical appearance: The colour of the formulation was checked out against white background. The consistency was checked by applying on skin. The greasiness was assessed by application on skin. The odor of the gel was checked by mixing the gel in water and taking the smell

pH: The pH was determined using digital pH meter by dipping the glass electrode completely in the gel system.

Transparency: Approximately 5 ml of formulated gel was taken in the 10 ml test tube and its transparency was checked visual.

Determination of spreadability: Took one gram of toothpaste, placed on a glass slide (10 x 10 cm) and covered with another glass slide. Then carefully placed two kg weight on covered glass slide (sliding, shall not take place). Measured the spreading (in cm) of the toothpaste after 3minutes. Repeated the experiment and took the average value of three readings.

RESULT AND DISCUSSION

Extraction:

The process of extraction of Mimosa pudica is done by Soxhlet apparatus, the process of extraction generally takes place by immersing the grinded leaves and shoots with 90% alcohol solution in the ratio of 1:4 the mechanism of hot percolation takes place for 24X2 hours. After evaporation final extract weights 230g with the yield of 32.14% as it is mention below in the table.

Table 3: Percentage yield for Ethanolic Extract

No.	Dry weight (grams)	Extract Weight (grams)	The yield (%)
1.	715.6	230	32.14

Phytochemical screening:

The test results of phytochemical extracts and fractions of ethanol extract of mimosa pudica, showed that the positive Mimosa pudica extract contains flavonoids and tannins. while only fraction of ethyl acetate contains flavonoids. Flavonoids and tannin have a property of anti-oxidant and anti-bacterial activity. Thus, the absence of flavonoid in the ethyl acetate fraction may affects the potency of the drug.

Table 4: Phytochemical screening of plant extract

Test material	The ethanolic extract	Ethyl acetate fraction	n-Hexane fraction
Alkaloid	-	-	-
Flavanoid	+	+	-
Tannin	+	-	+
Saponin	-	-	-

Note: (+) Contains secondary metabolites, (-) does not contain secondary metabolites.

Anti-bacterial study

The evaluation of anti-microbial activity of plant extract was determined by the disc diffusion method against a definite micro-organism (streptococcus aurens). These micro-organisms were frequently encountered in infectious dental diseases, this result shows that plant extract exhibits a potent degree of activity against micro-organisms, the effectiveness of anti-bacterial can be seen from zone of inhibition formed. The zone of inhibition explains the classification of bacterial growth inhibition response that were seen based on the diameter of clear zone, which consist four groups a weak response(diameter <5mm) , a medium response(5-10mm diameter) , a strong response(10-20mm diameter), a very strong response (>20mm diameter).

Table 5: Disc Diffusion Method Result

Plant Extract	Concentration	Zone of Inhibition (mm)
Ethanolic extract	1ml	15.55
Ethyl acetate	1ml	10.12
Ciprofloxacin	25ug/disc	25.30

In vitro anti-inflammatory assay

For the result of the study ethanolic extract effectively inhibit protein denaturation (Albumin) caused by heat. The inhibitory effect of different concentration of herbal preparation on protein denaturation as shown in table below, herbal preparation (100- 1000ug/ml) shows significant inhibition of denaturation of egg albumin in concentration dependent manner. The effect on protein denaturation contribute to the in vitro anti-inflammatory activity of herbal preparation used in our study.

Table 6: Inhibition of Protein Denaturation Assay

Concentration (ug/ml)	Sample		Standard	
	Absorbance	% inhibition	Absorbance	%inhibition
100	0.260	31.57	0.12	68.42
200	0.20	47.36	0.106	72.10
400	0.13	65.78	0.087	77.10
800	0.11	71.05	0.074	82.50
1000	0.08	78.94	0.056	85.26s
Control Reading: 0.380				

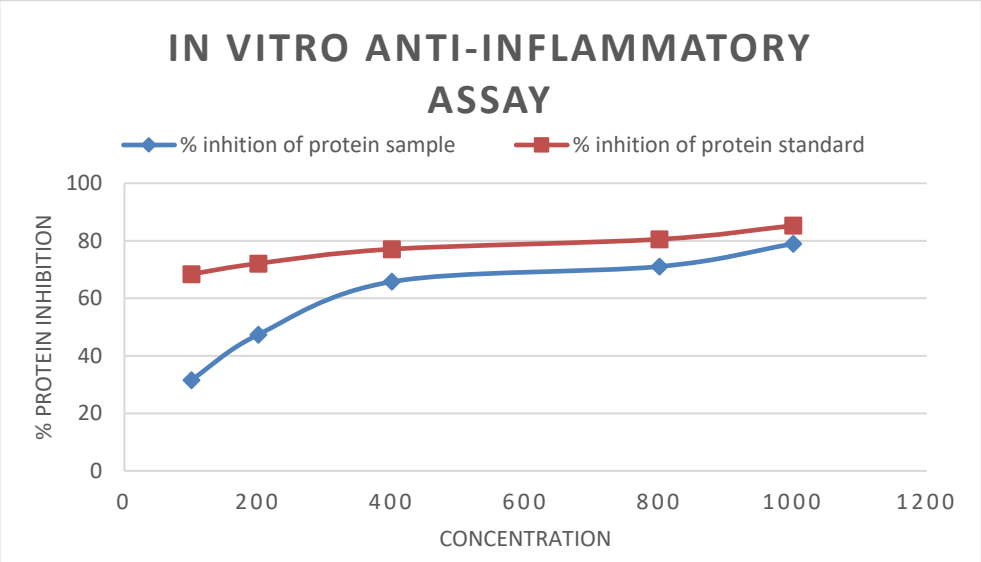

Figure 4 : In Vitro Anti-inflammatory Assay

Evaluation of Gel

Table.7: Evaluation of Ethanolic Extract Dental Gel

Formulation	Colour	consistency	greasiness	pH	Transparency	Spreadability (in cm)
5%	Greenish	smooth	Non- greasy	6.7	Transparent	8.28
10%	Greenish	smooth	Non- greasy	6.7	Opaque	8
15%	Greenish	smooth	Non- greasy	6.9	Opaque	7.9

Table 8: Evaluation of Ethyl acetate Fractionated Dental Gel

Formulation	Colour	consistency	greasiness	pH	Transparency	Spreadability (in cm)
5%	Yellowish-green	smooth	Non- greasy	6.8	Transparent	8.7
10%	Yellowish-green	smooth	Non- greasy	6.8	Transparent	8.5
15%	Yellowish-green	smooth	Non- greasy	6.9	Transparent	8.2

CONCLUSION

The effectiveness of antibacterial activity can be seen from the zone of inhibition is formed. Based on the above research shows that the ethanolic extract from had inhibitory zone greatest value is 15.55 mm compared to the extract formed by fraction of ethyl acetate. Now our study will be directed to explore more convenient and effective formulations composed of Mimosa pudica to treat dental caries and related problems.

SOURCE OF SUPPORT: Nil

CONFLICT OF INTEREST: None declared

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