

STANDARDIZATION AND *IN-VITRO* ANTI UROLITHIATIC ACTIVITY OF *STIGMA MAYDIS*

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ABSTRACT

Stigma maydis (*Zea mays*) is yellowish thread-like strand found inside the husks of corn. Corn stigma measure 4-8 inch (10-20 cm) long and are collected for medicinal use before the plant is pollinated. In present study, various standardization parameters of *Stigma maydis* like macroscopical, microscopical characters, physicochemical parameters have been evaluated. Behaviour on treatment with different chemical reagents of the dried *Stigma maydis*, were studied to fix some pharmacognostic parameters that vary from region to region. Preliminary phytochemical studies on different extracts were also reported. In this study the antiurolithiatic activity of aqueous extract of *Stigma maydis* was also investigated by *in vitro* model of urolithiasis. In this method test tubes were divided in three experimental groups and test group received 200mg/ml conc. of test sol., standard group received 200mg/ml conc. of standard drug (cystone) and control group was without any drug. At the end of the study precipitates of calcium oxalate of the all three groups were collected and the percentage inhibition of each group was calculated.

Keywords: Anti Urolithiatic activity, Kidney stone, Supersaturation, Aggregation of calcium containing crystal, *cornsilk*, Poaceae

1. INTRODUCTION

The drug consists of the stigmas of the female flowers harvested during the flowering period. The pale yellowish or brownish stigmas are filamentous, 0.1-0.2 mm thick, and upto 20 cm long. It is native to Central America, but now days cultivated worldwide. The drug is imported from the former USSR, Bulgaria, Albania, and former Yugoslavia and it is also obtained from the USA. Corn has sweetish taste and its odour is faint, used in cystitis, rheumatism and arthritis [1]. The plant is reported to possess antiviral [2], antifungal [3], diuretic [4] and antitumour [5] activity. It contains fixed oil, essential oil, flavonoids, saponins, bitter substances, tannin-like polyphenols, reducing sugars, mucilage. Its utilization by the Peruvian Indians as an intoxicant is supposed to be based on the presence of alkaloids, which after being inhaled cause psychic stimulation [6].

2. MATERIAL AND METHODS

2.1. Plant material

2 kg of *stigma maydis* were collected from local market in rainy session. The *stigma maydis* was dried at room temperature (24 to 27°C) or shade dried [7]. The dried *Stigma maydis* was then grounded to coarse powder. *Stigma maydis* was identified and authenticated by

botanist Dr. S.K. Mahajan, retired professor of P.G. College, Khargone MP, India. Plant authentication voucher number obtained was 213/Bot/PGC/11 for *stigma maydis*.

2.2. Processing of plant material

After authentication, *Stigma maydis* was dried at room temperature until they were free from the moisture and subjected to physical evaluation for different parameters.

2.3. Reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade.

2.4. Methods

The organoleptic characters including colour, odour, taste and external features of *stigma maydis* were observed and the results were recorded in Table 1. The extractive values were determined successively starting from petroleum ether, chloroform, ethyl acetate,

ethanol and water by using soxhlet extraction apparatus. The extractive values were obtained after evaporation of solvent under reduced pressure using vacuum rotary evaporator. The behaviour of the powdered flower heads with different chemical reagents was studied and Preliminary phytochemical tests of different extracts were performed with specific reagents [7, 8].

2.5. In-vitro method of mineralization

To evaluate the inhibition of calcium oxalate mineralization by *stigma maydis*; we used simultaneous flow static model (S.S.M.). We used *stigma maydis* conc. 200 mg/ml, calcium acetate (0.1 M) and sodium oxalate (0.1 M) (for calcium oxalate) in three separate burettes (25 ml) and were allowed to fall simultaneously into a 250 ml beaker with a slow and steady pace. After 30-40 min, the mixture was kept in a hot water bath for 10 min, cooled to room temperature and the precipitate was collected into a pre-weighed centrifuge tube. Supernatant fluid was discarded. Then, these tubes were dried in a hot air oven at 120 °C, cooled to room temperature and weighed till constant weight was achieved. Weight of the precipitates was calculated.

3. RESULTS AND DISCUSSIONS

The dried *stigma maydis* was subjected to standard procedures for the determination of various physicochemical parameters. The following parameters were determined:

3.1. Ash values

The determination of ash value is meant for detecting low-grade drugs, exhausted drugs, sandy or earthy matter. The results of ash values were recorded in Table 2.

3.2. Extractive values

This method determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for materials for which as yet no suitable chemical or biological assay exists. The air dried, accurately weighed drug was treated with solvents: petroleum ether, benzene, chloroform, ethyl acetate and methanol. The values were recorded in Table 3.

3.3. Phytochemical screening

The plant material was subjected to preliminary phytochemical screening for the detection of various

plant constituents. The extracts obtained from successive solvent extraction were subjected to qualitative test for the identification of various plant constituents like alkaloids, flavonoids, glycosides, proteins and amino acids, saponins, mucilages, fixed oils and fats [9, 10]. The results were recorded in Table 4.

3.4. Powdered drug reaction with various chemical reagents

The powdered drug was treated with various chemicals like concentrated hydrochloric acid, sulphuric acid, nitric acid, 10% NaOH, picric acid, iodine solution, methanol, ethanol, acetic acid, chloroform, petroleum ether, ferric chloride, ammonia solution. The behavioural change of the drug was determined [11]. The results were recorded in Table 5.

Table 1: Organoleptic character of the *Stigma maydis*

Organoleptic Characters	Observations
Type	Simple
Colour	Pale yellowish or Brownish
Odour	Characteristic
Taste	Sweet
Size	0.1-0.2 mm thick, upto 20 cm long
Shape	Filamentous

Table 2: Physicochemical parameters of *Stigma maydis*

Parameters	Values obtained (% w/w)
Total ash	6.5
Acid insoluble ash	1.65
Water soluble ash	3.6
Swelling index	2.5
Loss on drying	9.8

Table 3: Extractive values of *Stigma maydis*

Solvents	Alcohol soluble extractive value (%w/w)	Water soluble extractive value (% w/w)
Petroleum ether	6.4	10
Chloroform	6.0	11
Ethyl acetate	4.4	3.6
ethanol	5.6	5.2
water	5.6	12

Table 4: Preliminary phytochemical screening of *Stigma maydis*

Phyto constituents	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aq.extract
Alkaloids	-	+	-	+	+
Glycosides	-	+	+	+	+
Proteins and Amino acids	++	++	-	-	-
Flavanoids	+	-	+	+	+
Tannins	+++	+++	+++	-	+++
Fats and Fixed oils	+	+	-	+	-
Saponins	-	+	+	-	+
Gums and mucilages	+	+	+	+++	-

Table 5: Behavioural analysis of powdered *Stigma maydis* with various chemical reagents

Procedure	Observations
Powder	Limepeel
Powder + conc. HCl	Limepeel
Powder + conc. H ₂ SO ₄	Pumpenickel 2824
Powder + conc. HNO ₃	Montego Bay 2814
Powder + Acetic acid	Great canyon 2076
Powder + Picric acid	Ra Gold 2042
Powder + 5% iodine solution	Terracotta 6049
Powder + 5% NaOH	Limebright 2671
Powder + FeCl ₃	Burnished Gold 2707
Powder + Methanol	Golden fleece 2706
Powder + Ethanol	Cinnamon tea 2084
Powder + Ammonia solution	Burnished gold 2707
Powder + Chloroform	Terracotta 6049

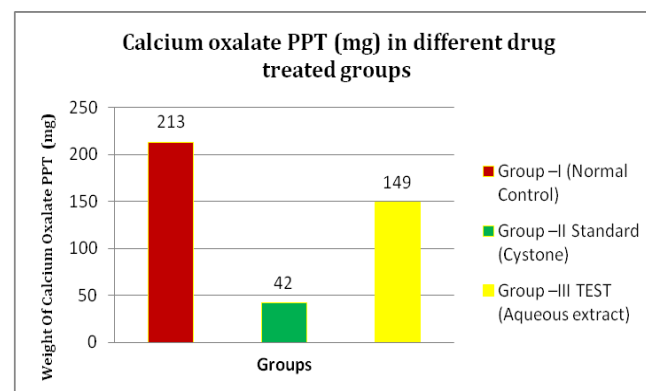
3.5. In-vitro anti urolithiatic activity

The stone formation in urinary system is called urolithiasis. The stone formation may happen in kidney, ureter and urinary bladder or urethra. The stone formation is major problem of the urinary tract. The approximately 12% of global population is affected by the stone formation in urinary system and the occurrence rate is high in man than woman [12]. Various synthetic and natural drugs are used for treatment of kidney stone [13]. In kidney stone the major portion is calcium [14]. The stone of kidney are passing out by urine stream easily without showing the symptoms. The risk factor can be depends upon the size of stone [15]. In India the 5-7 million people suffer from kidney stone [16]. The beneficial effect on kidney stone which exerted by the herbal medicine contain several phytoconstituents [17]. The immediate treatment is bed

rest. The NSAIDS are used for reduce the pain and the alternative medicine are used if pain is not decreased [18].

Table 6: Weight of calcium oxalate precipitate in different groups

Groups	Weight Of Calcium Oxalate PPT (mg)
Group –I (Normal Control)	213
Group –II Standard (Cystone)	42
Group –III TEST (Aqueous extract)	149

**Fig.1: Calcium oxalate ppt in treated groups****Table 7: Anti urolithiatic effect of aqueous extract of *stigma maydis***

Parameter	Percentage(%) inhibition of calcium oxalate PPT
Group –I (Normal control)	00 %
Group –II Standard (Cystone)	80.28%
Group –III TEST (Aqueous extract)	34%

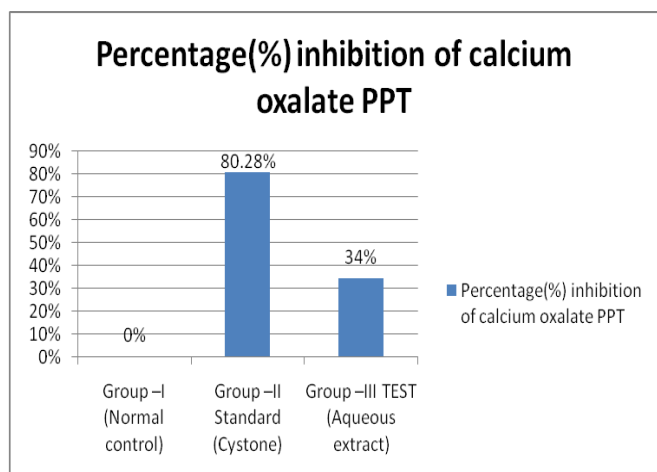


Fig. 2: Percentage inhibition of calcium oxalate ppt.

4. CONCLUSION

The result indicates that aqueous extract of *stigma maydis* showed 34 % inhibition of calcium oxalate precipitate. In the kidney when there is imbalance between promoters and inhibitors it may lead to urolithiasis. If patients who have formed one stone are more likely to form another indicate a serious problem. All standard pharmaceutical drugs which are used to prevent urolithiasis may not be effective in all patients and many have adverse effects that compromise their long-term use. Therefore Herbal medicine is mostly used in the treatment of kidney stone. In the present work *stigma maydis* shown a possible therapeutic potential for treatment of kidney stone & also on the other side of study generated data can be used for determining correct identity and purity of plant parts and for the detection of adulteration. Botanical authentication and physicochemical parameters will give an idea about the quality of drug. All these parameters which are being reported could be useful in identification of distinctive features of the drug.

5. REFERENCES

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