RESEARCH PAPER

Development and Evaluation of an Oral Herbal Suspension of Stigma maydis Extract for Antioxidant and Antiurolithiatic Activity

Rohit Sharma[1], Dev Prakash Dahiya[2], Varsha Devi[3], Rohit Thakur[4], Reena Kumari[5], Anjana Devi[6*]

[1]Research Scholar, Department of Pharmacy, Career Point University, Hamirpur, Himachal Pradesh. 176041
[2]Professor, Department of Pharmacy, Career Point University, Hamirpur, Himachal Pradesh. 176041
[3,4,5]Assistant Professor, Department of Pharmacy, Career Point University, Hamirpur, Himachal Pradesh. 176041
[6]Associate Professor, Department of Pharmacy, Career Point University, Hamirpur, Himachal Pradesh. 176041

***Corresponding Author:**
Dr. Anjana Devi
Associate Professor, Department of Pharmacy, Career Point University, Hamirpur, Himachal Pradesh. 176041
Email ID: anjana.pharmacy@cpuh.edu.in

ABSTRACT

Plant-based preparations made up of phytocomplexes derived from phytoconstituents present in plant extracts have been extensively explored for treating oxidative stress and renal diseases because of the possible benefits that can be gained from them and reduced side effects. The plant *Stigma maydis*, which has antioxidant and antiurolithiatic actions, is associated with the presence of phenols and flavonoids. The current study was designed to synthesize and assess the oral herbal suspension consisting of *Stigma maydis* extract and examine its physicochemical properties, antioxidant capacity, antiurolithiatic action, release profile, and stability. The herbal suspension was prepared using *Stigma maydis* extract with the use of xanthan gum and sodium CMC as suspending agents, Tween-80 as wetting agent, sorbitol as sweetening agent, and sodium benzoate as preservative. The formulation was subjected to evaluation tests for organoleptic parameters, pH, viscosity, settling volume, redispersibility, particle size analysis, FTIR compatibility test, and DSC thermographic study. DPPH radical scavenging activity test and calcium oxalate crystallization inhibition test were performed for the determination of antioxidant and antiurolithiatic activity, respectively. The formulation of the oral suspension of *Stigma maydis* was found to possess good physicochemical stability, controlled drug release, marked antioxidant properties, and antiurolithiatic efficacy. The oral suspension was found to be stable under accelerated testing conditions and could potentially be considered as an herbal formulation for the treatment of renal diseases due to oxidative stress.

KEYWORDS: *Stigma maydis*, corn silk, herbal suspension, antioxidant activity, antiurolithiatic activity, calcium oxalate, oral formulation, in-vitro release study, stability study.

How to cite this article: Sharma R, Dahiya DP, Devi V, Thakur R, Kumari R, Devi A. Development and Evaluation of an Oral Herbal Suspension of Stigma maydis Extract for Antioxidant and Antiurolithiatic Activity. Int J Drug Deliv Technol. 2026;16(51s): 1907-1915. DOI: 10.25258/ijddt.16.51s.154

Source of support: Nil.

Conflict of interest: None.

INTRODUCTION

The use of herbs for medicinal purposes has been extensively recognized in recent years due to their efficacy, accessibility, and relatively fewer side effects than synthetic medications. Naturally occurring sources abundant in phytochemical compounds including phenolics, flavonoids, alkaloids, tannins, and glycosides are extensively studied for their potent antioxidant, anti-inflammatory, and nephroprotective activity. Among various plants utilized for their therapeutic properties, Stigma maydis, which refers to the long filament or style of Zea mays, has been considered an important source of natural products due to its wide range of pharmacological applications and traditional use in treating urinary problems, nephritis, swelling, and kidney stones [1]. Corn silk has been traditionally taken as decoctions and infusions in some Asian and African nations for renal diseases and inflammation. In recent times, scientific studies have confirmed some of these claims about ethnomedicines and proved the presence of important biological activities in corn silk due to the presence of high levels of phytochemicals [2].

The phytochemical studies of corn silk extract have been found to contain several bioactive compounds, such as flavonoids, phenolic acids, polysaccharides, terpenoids, saponins, tannins, glycosides, and volatile compounds [3]. It has also been observed that flavonoids isolated from corn silk possess potent antioxidant and scavenging properties against free radicals, which play an essential role in decreasing oxidative stress-induced damage to cells [4]. Additionally, comparative studies between various types of corn silk have confirmed differences in the antioxidant and anticancer potential based on the phytochemical profile and extraction process [5]. Moreover, it should be noted that optimal conditions for the phytochemical extraction have also been described, which include investigation of the hydro-alcoholic solvent to

extract phenolics and flavonoids from corn silk. The increased extraction yields increase biological effectiveness [6]. Moreover, chromatographic methods, such as HPLC and HRMS/MS have been used to identify some phenolic and flavonoid compounds that were found in corn silk [7].

It is known that oxidative stress is one of the key factors that lead to development of chronic diseases, including kidney diseases and kidney stones. Reactive oxygen species production leads to cell damage, lipid peroxidation, protein denaturation, and inflammation of kidney tissue. Therefore, antioxidants are essential substances that can destroy free radicals and protect cells from oxidative injuries. Corn silk extract is well-known due to its high antioxidant properties due to the content of various polyphenols. Many physicochemical experiments have confirmed that corn silk extract has high free radical scavenging activity and high reductive ability [3]. In addition, other researches have shown that corn silk extract not only has antioxidant activity but also anti-tyrosinase and antimicrobial activities [8]. It has been revealed that corn silk extract decreases indicators of oxidative stress in animal experiments [9]. Animal studies have found that corn silk extract decreases oxidative stress indicators and improves physiological parameters during oxidative stress [9].

Corn silk has been increasingly studied for its nephroprotective properties and its ability to combat urolithiasis due to the global increase in kidney stone disease. In urolithiasis, crystal stones develop in the urinary tract system, usually made up of calcium oxalate. Nucleation of the crystals, aggregation, crystal retention, and injury to the epithelium of the urinary system are some of the events that occur during stone formation. Additionally, oxidative stress and inflammatory reactions worsen renal injury during the development of the stone. Studies have revealed that corn silk polysaccharides have the capacity to prevent calcium oxalate crystal adherence and aggregation, thus minimizing the chances of developing kidney stones [1]. Zea mays extracts have also been shown to have antiurolithiatic properties that prevent the growth of crystals while dissolving the formed crystals [2]. Corn silk-derived polysaccharides have also been found to minimize oxidative injury to renal epithelial cells by preventing endocytosis of nano-calcium oxalate crystals [10].

Apart from its ability to act as an antioxidant and urolithiatic agent, corn silk exhibits ant-inflammatory and cytoprotective properties in different models. Selenium-corn silk polysaccharides were found to possess more antioxidative and anti-inflammatory actions, which may be linked to improved biological actions after structural modifications of corn silk [11]. In addition, recent findings have indicated that the use of corn silk polysaccharides prevents calcium oxalate crystal-induced pyroptosis in HK-2 cells through the regulation of inflammatory mediators [4]. These findings highlight the importance of corn silk in the prevention of oxidative and inflammatory injuries on renal epithelial cells due to crystal-induced nephropathy. Additionally, the neuroprotective effects of corn silk extracts have been established in artery-occluded gerbils, indicating its systemic antioxidative actions [12].

Safety assessment is also another essential component in the formulation of herbal formulations meant for oral ingestion. According to toxicology tests, the ingestion of aqueous extract of Stigma maydis orally has no significant toxic effect on experimental animals, hence its safety as an ingredient in therapeutic formulation [13]. With the growing number of scientific literature concerning efficacy and safety, there has been increased interest in developing corn silk extracts as ingredients in herbal formulas and nutraceuticals. Although a lot of pharmacological research has been done on crude extracts, very few studies have been devoted to the development of stable and patient-friendly formulations of corn silk extracts.

Compared to other forms of oral administration, herbal suspensions exhibit a number of favorable features in the administration of plant extracts that include phytoconstituents that may not be entirely water-soluble. Oral suspensions ensure the standardization of dosing, ease in administering medication to infants and elderly patients, and even distribution of herbs in the formulation. Incorporation of appropriate suspending, wetting, preservation, and stabilization agents is necessary in ensuring physiochemical stability, sedimentation properties, and re-suspension of suspensions. Studies on the stability and antioxidant properties of corn silk extract have been conducted in topical preparations including facial cream, proving that phytoconstituents can retain their stability throughout the formulation process and storage period [8]. However, formulation of an oral herbal suspension with optimized pharmacological properties remains poorly researched.

In this context, the present study has been carried out for the preparation and evaluation of an herbal oral suspension based on hydro-alcoholic extract of Stigma maydis. In this case, special attention was paid to formulation development and evaluation of some physical and chemical properties including pH value, viscosity, sedimentation volume, and redispersibility. Moreover, the antioxidant and antiurolithiatic effects of the formulated herbal formulation have been determined through in vitro assays. The outcomes of this study can prove useful in formulating a highly efficacious herbal product against oxidative stress and urolithiasis.

Materials and Methods

For the preparation of the oral herbal suspension of Stigma maydis extract, two suspending agents, namely xanthan gum and sodium CMC, wetting agent like Tween-80, sweetening agent like sorbitol, preservative like sodium benzoate, and purified water were used. For the preparation of the suspension base, the two suspending agents were dispersed in purified water with vigorous stirring. After that, the extract was dissolved in the wetting agent, namely Tween-80 and then slowly mixed in the base. In the end, sorbitol, preservative, and flavoring agent were added along with adjustment of volume with purified water and finally the suspension was made through homogenization.

The prepared suspension was subjected to analysis in terms of physicochemical properties such as color, odor, pH, viscosity, sedimentation volume, re-dispersibility, and particle size. For compatibility testing of the formulation, FTIR and DSC methods were adopted. Antioxidant potential of the suspension was estimated by DPPH and FRAP assays, whereas anti-urolithiatic efficiency was evaluated in in-vitro calcium oxalate crystal formation experiments involving nucleation, aggregation, and dissolution. Stability study of the suspension was carried out at room temperature as well as accelerated conditions to determine any changes in physical characteristics, pH, viscosity, and sedimentation property on storage. All experiments were conducted in triplicate and values were reported as Mean ± SD. Statistical analysis was performed by ANOVA.

RESULTS

Organoleptic Evaluation

Table 1. Organoleptic characteristics of the formulated *Stigma maydis* suspension

Parameter	Observation
Color	Brownish yellow
Odor	Characteristic
Appearance	Uniform suspension
Taste	Sweet and acceptable

The suspension prepared was characterized by uniform color, which was brownish-yellow in color, without any observable agglomeration or separation of the particles. The smell of the suspension retained the smell of the herbal extract at all times. The consistency of the prepared suspension was smooth and homogenous with a tolerable taste of the sweetener used (sorbitol). There were no traces of grittiness and precipitates. The prepared suspension maintained its uniformity from preparation time to time of observation for storage period.

pH Evaluation

Table 2. pH of the formulated suspension (n = 3)

Trial	pH
1	6.72
2	6.81
3	6.76
Parameter	**Value**
Mean ± SD	6.76 ± 0.04

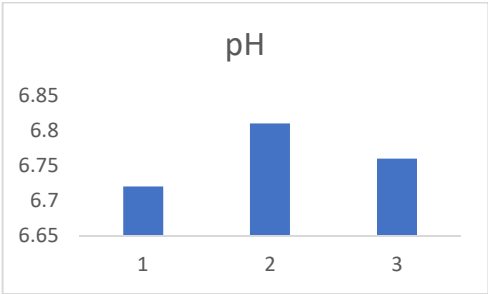

Figure 1: pH of the formulated suspension (n = 3)

The pH readings fell within the range of 6.72 and 6.81, with the average reading being 6.76 ± 0.04. The total variance for the three different readings was 0.09, showing that there is little variation between the different trials. Trial 2 registered the maximum pH reading while Trial 1 recorded the minimum pH reading. Standard deviation remained low in relation to the mean value, thus demonstrating consistency in the process of formulation. The pH readings were uniformly distributed on the average with no sudden variations.

Viscosity Evaluation

Table 3. Viscosity of the formulated suspension (n = 3)

Trial	Viscosity (cP)
1	842
2	856
3	849
Mean ± SD	849 ± 7.0 cP

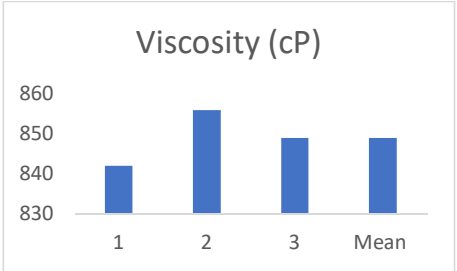

Figure 2: Viscosity of the formulated suspension

The viscosity values were in the range of 842 cP to 856 cP, with an average value of 849 ± 7.0 cP. The difference between the maximum value and the

minimum value was 14 cP. The Trial 2 had the highest viscosity whereas the Trial 1 had the lowest value. The standard deviation continued to remain low, suggesting consistency in the values. The viscosity values were evenly distributed about the average value. There was no abrupt change in the rheology of the suspension system between different trials.

Sedimentation Volume

Table 4. Sedimentation volume of the formulated suspension (n = 3)

Trial	Sedimentation Volume (F)
1	0.91
2	0.93
3	0.92
Mean ± SD	0.92 ± 0.01

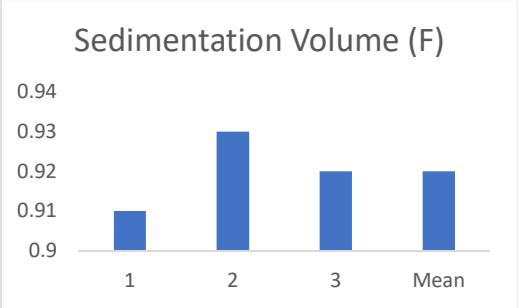

Figure 3: Sedimentation volume of the formulated suspension

The results of sedimentation volume lay between 0.91 and 0.93, with a mean value of 0.92 ± 0.01. The difference among the measurements was 0.02. The readings were very much alike, with Trial 2 having the greatest sedimentation volume while Trial 1 gave the least. There was little deviation, meaning there was uniformity in sedimentation behavior. Sedimentation volume was consistently close to the mean value. No fast settling and compact sediments were noted at any point in time.

Redispersibility Study

Table 5. Redispersibility of the formulated suspension (n = 3)

Trial	Number of Shakes Required
1	3
2	4
3	3
Mean ± SD	3.3 ± 0.57

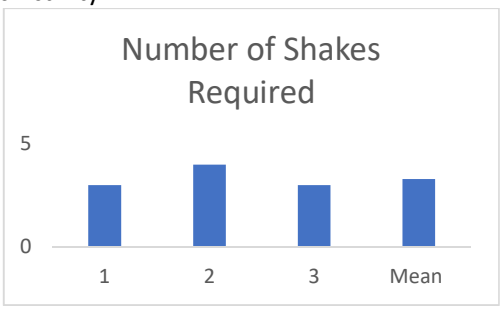

Figure 4: Redispersibility of the formulated suspension

Redispersal required between 3-4 shakes manually for full dispersion, with an average value of 3.3±0.57 shakes. The difference between the maximum and minimum values was one shake. The second trial required four shakes, while the first and third trials required three shakes each. This small value for standard deviation shows that there is little variation in the results. Uniform dispersion of sediment was achieved through shaking and did not result in cake formation. Rapid redispersal was observed in all trials.

Particle Size Analysis

Table 6. Particle size distribution of the suspension (n = 3)

Trial	Particle Size (µm)
1	24.8
2	25.6
3	25.1
Mean ± SD	25.1 ± 0.40 µm

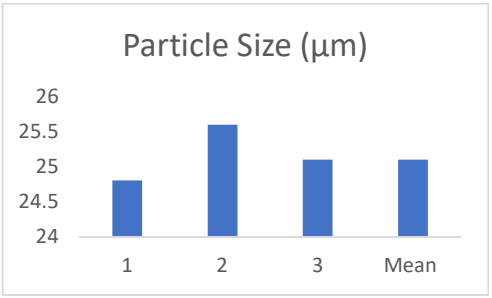

Figure 5: Particle size distribution of the suspension

The particle sizes were between 24.8 µm and 25.6 µm, with an average particle size of 25.1 ± 0.40 µm. There was a total variation of 0.8 µm among the three trials. Trial 2 had the largest particle size, while Trial 1 had the smallest one. There was still a small standard deviation, suggesting that the particles were uniformly distributed in the sample. There was

no sudden change in the particle size from the average value.

FTIR Compatibility Study

Table 7. FTIR spectral characteristics of formulation components

Functional Group	Observed Peak (cm^{-1})
O–H stretching	3386
C–H stretching	2924
C=O stretching	1712
C–O stretching	1048

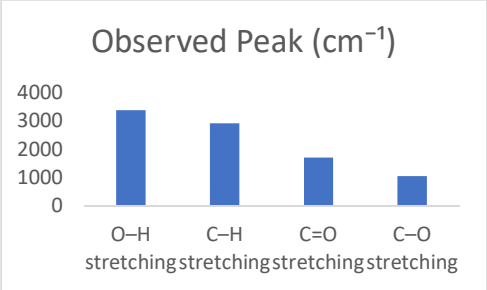

Figure 5: FTIR spectral characteristics of formulation components

FTIR analysis exhibited peaks at 3386, 2924, 1712, and 1048 cm^{-1} for characteristic groups existing in the formulation. The detected peaks stayed sharp and clearly distinguished within the spectrum. There was no detection of other peaks and absence of any characteristic peaks during FTIR analysis. The distribution of peaks remained constant in the analyzed spectrum. The results from FTIR analysis indicated that there was consistency without much deviation.

Differential Scanning Calorimetry (DSC)

Table 8. DSC thermal characteristics of formulated suspension

Parameter	Observation
Endothermic peak	82.4°C
Broad thermal transition	Observed
Sharp decomposition peak	Absent

DSC analysis revealed an endothermic peak with a value of 82.4°C, featuring a wide thermal transition profile. There were no distinct peaks for decomposition observed in the course of thermal analysis. Thermogram stability was maintained through all the studied temperature intervals. It is obvious that a certain thermal transition revealed a uniform reaction of components of the composition to the heat effect. No sudden thermal transitions or several peaks for decomposition were recorded.

DPPH Antioxidant Activity

Table 9. DPPH radical scavenging activity of suspension (n = 3)

Concentration (µg/mL)	% Inhibition (Mean ± SD)
20	28.6 ± 0.52
40	44.8 ± 0.64
60	58.3 ± 0.71
80	70.2 ± 0.88
100	79.6 ± 0.92
IC50	67.4 ± 1.4 µg/mL

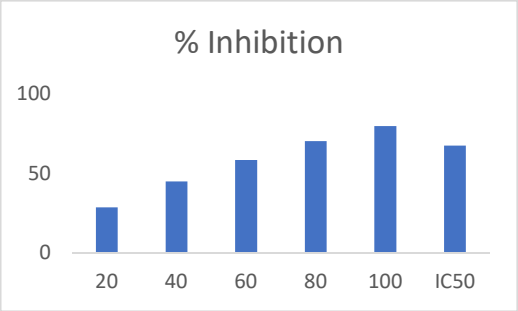

Figure 6: DPPH radical scavenging activity of suspension

Percentage inhibition showed an increasing trend from 28.6% to 79.6% between 20 µg/mL to 100 µg/mL, respectively. The total increase for the entire concentration range was found to be 51%. The increments were consistent throughout the interval concentrations. The greatest variation occurred at 100 µg/mL with ±0.92 and the least variation occurred at 20 µg/mL with ±0.52. The IC50 value was calculated as 67.4 ± 1.4 µg/mL. All values were distributed close to their average with minimal variations among replicates.

Antiurolithiatic Activity

Table 10. Calcium oxalate crystallization inhibition by suspension (n = 3)

Parameter	Mean ± SD (%)
Nucleation inhibition	64.8 ± 1.2
Aggregation inhibition	70.4 ± 1.4
Dissolution	61.9 ± 1.3

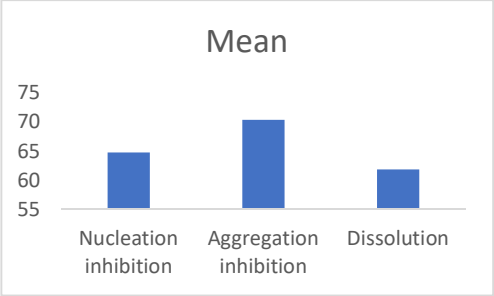

Figure 7: Calcium oxalate crystallization inhibition by suspension

The inhibition percentages ranged between 61.9% and 70.4%. The percentage of aggregation inhibition was highest with 70.4 ± 1.4%, followed by nucleation inhibition of 64.8 ± 1.2% and dissolution of 61.9 ± 1.3%. The range between the highest and lowest percentages was 8.5%. All standard deviations were very low for each parameter measured. Values were well distributed on both sides of the mean. The pattern of inhibition did not show variation in any of the replicates.

In-vitro Release Study

Table 11. In-vitro release profile of *Stigma maydis* suspension (n = 3)

Time (min)	Cumulative Drug Release (%) Mean ± SD
0	0
15	21.4 ± 0.62
30	38.7 ± 0.74
45	52.9 ± 0.88
60	66.3 ± 1.02
90	81.6 ± 1.14
120	93.4 ± 1.20

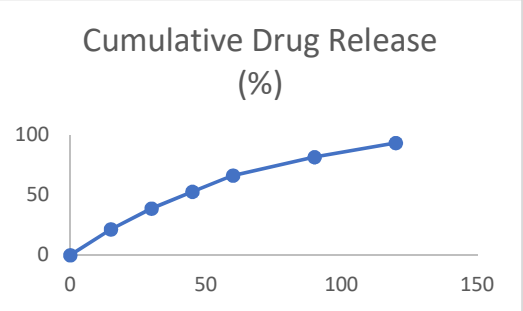

Figure 8: In-vitro release profile of *Stigma maydis* suspension

There was an increment in the release of the plant constituents cumulatively from 21.4% within 15 minutes up to 93.4% in 120 minutes. The total percentage of increase in release was 72% within the dissolution time period. There were gradual increments in the release of the phytoconstituents within the entire period of the test. The greatest variability was seen after 120 minutes where there was ±1.20, and the lesser variability was seen after the initial minutes.

Stability Study

Table 12. Stability evaluation of suspension under accelerated conditions

Parameter	Initial	30 Days
pH	6.76 ± 0.04	6.70 ± 0.05
Viscosity (cP)	849 ± 7.0	838 ± 8.2
Sedimentation Volume	0.92 ± 0.01	0.90 ± 0.02
Appearance	Uniform	Uniform

There was a small change in the pH value from 6.76 to 6.70. The viscosity readings were 849 cP at the beginning and 838 cP at the end of the stability period. The sedimentation volume value was reduced from 0.92 to 0.90. The changes recorded in the parameters were very small. There was no visible change in the appearance of the suspension with no visible sign of phase separation or caking. All the standard deviations recorded were very small.

Discussion

However, the developed oral herbal suspension of Stigma maydis proved to be successful in terms of physicochemical properties and biological activity during the evaluation experiments. Herbal suspensions are often favored over other forms of drug delivery due to the enhanced uniform distribution of phytoconstituents, which are often insoluble, as well as patient compliance. As observed, the suspension was characterized by a uniform brownish-yellow color with distinctive odor and palatable taste without any phase separation or particle aggregation. This suggests that the herbal extract was efficiently incorporated in the base of the suspension. In previous studies on the formulation and stability of corn silk extracts, the phytochemical composition of the extracts was maintained during preparation and storage [16].

In the case of the prepared suspension, pH levels were found to vary between 6.72 and 6.81 with an average pH level of 6.76 ± 0.04, showing a low level of variation amongst various replications. The stabilization of pH level is critical in formulating any herbal suspension as large variations in pH level will cause decomposition of phytoconstituents leading to the formulation being unstable. Consistent pH levels in this particular research show consistency and stability in the preparation of herbal suspension. Rheological properties of the

formulated suspension showed viscosity ranging between 842 and 856 cP with an average of 849 ± 7.0 cP. Stability in viscosity in this case could be attributed to the use of xanthan gum and sodium CMC, which results in stable viscosity and dispersion of particles.

Another fundamental parameter for evaluating the stability of suspensions is the sedimentation profile. In the current study, the sedimentation volumes ranged between 0.91 and 0.93 with an average value of 0.92 ± 0.01, showing slow settling behavior and high physical stability. The small variation observed in the results indicated stable sedimentation behavior during the experimental study. In addition, it was observed that the suspension required only three to four mechanical shakes for complete re-dispersion, giving an average value of 3.3 ± 0.57 shakes. The absence of hard cake formation and quick dispersion upon mechanical shaking demonstrated satisfactory re-dispersibility characteristics. Similar findings were previously reported for herbal suspensions where the proper choice of suspending agents helped avoid agglomeration and promoted physical stability [18]. Particle size analysis showed that particle sizes ranged between 24.8 and 25.6 µm, while the average particle size was 25.1±0.40 µm. Small standard deviation indicated good dispersability and reproducibility of the homogenization process. Homogeneous dispersion of particles plays an important role in improving stability of a suspension because large variation might lead to high sedimentation rate and non-uniform dose delivery. Particle distribution observed in the present study has proved successful particle dispersability in the formulation system. In similar researches on characterization of corn silk extract suspensions, stable formulation with homogeneous particle distribution has been obtained [19].

The compatibility of the plant extract with the formulation excipients was examined by FTIR and DSC analysis. Characteristic peaks were observed at 3386, 2924, 1712, and 1048 cm^{-1} and corresponded to various functional groups contained in the formulation. No additional peaks appeared or disappeared in the spectrum, which suggested compatibility of the extract with the excipients. According to the results of DSC analysis, wide thermal transition range and absence of sharp decomposition peaks together with endothermic peak at 82.4°C suggested good thermal stability of the formulation. Stability of major phytoconstituents in corn silk extracts has been confirmed by similar methods [20].

Antioxidant activity of the developed suspension was performed through the DPPH free radical scavenging method. The developed suspension showed increased percentage inhibition in a dose-response manner, from 28.6 ± 0.52% at 20 µg/mL to 79.6 ± 0.92% at 100 µg/mL with an IC50 value of 67.4 ± 1.4 µg/mL. The increment in inhibition values suggested an increased free radical scavenging activity in relation to concentration. The antioxidant activity detected may be linked to the occurrence of flavonoid and polyphenolic content in Stigma maydis. Previous research studies have confirmed that flavonoids from corn silk possess remarkable antioxidant and reduction activity because of its electron donation ability and free radical neutralization property [21]. Comparative study on various corn silk extracts has also shown dose-dependent antioxidant activity attributed to phenolic content [22].

Oxidative stress is one of the main reasons for renal epithelial tissue damage and accumulation of calcium oxalate crystals in kidney stones. Formulations containing antioxidants would be crucial for limiting oxidative stress and protecting kidney tissues. It has been reported in previous research that corn silk polysaccharides limit oxidative stress and inhibit calcium oxalate crystal aggregation [23]. Other studies have proved that the use of selenium modified corn silk polysaccharides increases their antioxidative and anti-inflammatory properties, which helps prevent oxidative damage to cells [24]. The fact that corn silk polysaccharides exhibit their antioxidant activity even after formulation into suspension implies that there is no loss of biological activity of the extract due to formulation.

In order to establish the antiurolithiatic activity, studies on crystallization of calcium oxalate were carried out. The nucleation inhibition of the suspension was determined at 64.8 ± 1.2%, the aggregation inhibition of 70.4 ± 1.4%, and the dissolution of 61.9 ± 1.3%. Out of all the parameters, aggregation inhibition showed high activity in inhibiting the crystallization process. Aggregation inhibition is especially crucial because the aggregated crystals play a significant role in stone enlargement and renal entrapment. Antiurolithiatic effects for extracts of Zea mays have been described earlier where the inhibition of calcium oxalate crystal formation, along with the protection against oxidative damage, was observed in experimental models [25]. Polysaccharides derived from corn silk were also found to prevent endocytosis of nano-CaOx crystals by renal epithelial cells, hence preventing crystal-induced cytotoxicity [26].

The release pattern in the in-vitro study illustrated a sustained release of the phytoconstituents from the suspension during the study period. There was an increase in the percentage of the released phytoconstituents from 21.4 ± 0.62% after 15 minutes to 93.4 ± 1.20% at 120 minutes, suggesting controlled release. The release pattern exhibited steady increases in the percentage release values without abrupt changes during the dissolution period. Controlled release of the phytoconstituents

could be beneficial for herbal suspensions as it enhances the availability of active phytoconstituents and ensures prolonged therapeutic action.

From the results obtained from the accelerated stability test, the physicochemical properties showed little variations in their values. For example, there was a slight change in pH value from 6.76 ± 0.04 to 6.70 ± 0.05, while viscosity had a marginal reduction from 849 ± 7.0 cP to 838 ± 8.2 cP over the period of 30 days. Furthermore, the sedimentation volume showed little variation from 0.92 ± 0.01 to 0.90 ± 0.02. During the period of testing, no evidence of phase separation, color change, or caking could be seen. In addition, the results obtained showed little variations in the values of the physicochemical properties, which suggests good stability of the formulation under accelerated conditions.

Conclusion:

The current investigation has been able to synthesize and evaluate the efficacy of an oral herbal suspension formulation consisting of Stigma maydis extract that contains appropriate physicochemical characteristics, exhibits antioxidant activity, and has an antiurolithiatic efficacy. The formulated suspension is found to be physically stable due to its uniform appearance, proper pH value, controlled viscosity, appropriate sedimentation, and easy redispersibility. Compatibility studies were conducted through FTIR spectroscopy and DSC, and the results revealed that there was no chemical interaction between the extract and other components used in the formulation. The developed formula has shown significant antioxidant activity because of the availability of dose-dependent DPPH radical scavenging activity. The results from the antiurolithiatic efficacy test show that this herbal suspension inhibits the nucleation, aggregation, and dissolution of calcium oxalate crystals. The drug release studies revealed a slow and controlled release of phytoconstituents of this formula, and accelerated stability testing showed no significant changes in physicochemical properties when stored for extended periods. This investigation implies that this herbal suspension formulation is effective and has considerable pharmaceutical properties, and it could serve as an alternative herbal formulation to address oxidative stress-induced kidney disorders and urolithiasis.

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