Characterization, Anti diabetic and Anti-urolithiatic activity of *Cocculus hirsutus* mucilage

Leema Rose Mary D, Lawrance A, Edwina Sherley Felicita A, Marie Arockianathan. P*

PG &Research Department of Biochemistry,

St. Joseph's College of Arts & Science (Autonomous), Cuddalore –607001, Tamil Nadu,India.

Corresponding Authors: Dr .P. Marie Arockianathan *

*PG and Research Department of Biochemistry, St. Joseph's College of Arts and Science, (Autonomous), Cuddalore-607001, Tamil Nadu, India E-mail: marie@sjctnc.edu.in; pmanathan26@gmail.com; Phone: +91 8608340307

Abstract

Plant mucilage has created great deal of interest among the researchers to be explored for numerous biological applications. In the present study, C*occulus hirsutus leaves* mucilage was isolated and tested for its physiochemical properties like WHC, OBC and also structural characteristic using XRD and DSC. The mucilage was also evaluated for its anti-diabetic and anti-urolithiatic activity. The WHC and OBC of the mucilage were 24.2 g/g gm and 14.8gm, respectively. The XRD spectra of the C*occulus hirsutus* mucilage did not show any sharp peaks thereby proving the material was amorphous. The thermal properties of mucilage particles indicated that they were very thermo stable. The anti-diabetic property of the mucilage was determined by α-glycosidase methods. The result showed that the inhibition role of mucilage on diabetic conditions. However, the efficacy of the mucilage can be increased by increasing its concentration as it shows promising results. An anti-urolithiatic activity studied by titrimetric method, the obtained results exhibited the highest activity, and its result was compared with the regular drug cystone.

Keywords: *Cocculus hirsutus*; Mucilage; antidiabetic; anti-urolithiatic activity

INTRODUCTION

Nowadays a large number of plants derived compounds used as pharmaceutical excipients due to their various functional properties. One such plant products was mucilage which has created great attention of interest among the researchers to be explored for numerous applications such as coating, thickening, film forming, disintegrating, emulsifying, stabilizing and gelling agents [1,2]. Its abundance, biodegradable; inexpensive, purely sourced, non-toxic nature are along with its other advantages [3].It forms an necessary component of the cell and executes a range of functions in plants, together with food and water storage and seed germination and it can be originate in various parts such as leaves, stem, buds, bark, roots, fruits and seeds. [4, 5].

Numerous mucilage was isolated and characterized such as *Mimosa pudica* [6], *Abelmoschus Esculentus*(okra) [7], *Opuntia ficus-indicaL*. [8] and *Ocimum basilicum LM.S***.** [9]. The soluble dietary fiber reduce the glycemic level and this activity has been credited to its physicochemical properties such as; delayed gastric emptying, increased viscosity etc., [10]. The discovery of novel sources of dietary fiber was promising area of research, and mucilage was one of the dietary fibers in plant source and less exploited.

Mucilage was traditionally used in the medical field via oral, topical or routes of respiratory, gastrointestinal, musculoskeletal, reproductive and urinary system and also used for skin disorders. Moreover, their use was extended to cancer, immunity stimulation, antioxidant and antimicrobial property. The nutritional fiber of mucilage exhibit their water attraction and gel-forming ability, the lipid portion possesses OHB [11]. Its have abundant functional properties like film forming, emulsifying, thickening, gelling and viscosity modifying properties [12; 13].

The plant *Cocculus hirsutus* was Menispermaceae family, widely spread all over hot and subtropical regions, as well as China, India and central Asia**.** Traditionally, the plant *Cocculus hirsutus* was used in a range of ailments like diuretic, analgesic, laxative [14] wound healing and anti-inflammatory activities [15], hepatoprotectives and anticancer activity [16] due to the occurrence of alkaloids, flavonoids, triterpenoids, saponins and also contains high percentage of mucilage and it was used as a gelling agent for Flurbiprofen drug [17]**.**

The *Cocculus hirsutus* mucilage was isolated and characterized was reported earlier [18]**.** In the present study, the mucilage was obtained from the of *Cocculus hirsutus leaves* were assessed for its physiochemical properties like Water Holding Capacity, Oil Binding Capacity and also seen structural characteristics using XRD and DSC. The obtained mucilage was treated for its anti diabetic and ant-urolithiatic activity.

2. MATERIALS AND METHODS

2.1. Extraction of mucilage from Cocculus hirsutus leaves

First of all collected and separated the leaves from *Cocculus hirsutus* plant in Cuddalore then washed with tap water; air dried for 3 weeks. The dried leaves were reduced in to fine powder; 5gm of the sample was soaked in water for few minutes, boiled for 15 minutes and kept aside for complete release of the mucilage, finally the precipitated mucilage was collected, dried in an oven and then grounded into fine powder then weighed ,stored in an air tight container..

2.2. Physiochemical Characterization

The mucilage of *Cocculus hirsutus* was subjected to physiochemical analysis. The physical characters considered in the presence study included organoleptic parameters such as WHC and OBC and also structural characteristic like XRD and DSC.

2.2.1 Water Holding Capacity

WHC was determined by Thanatcha and Pranee method. Prepared 1% mucilage in distilled water mixed thoroughly then centrifuged at 3000rpm for 30 min. Supernatant from the sample was discarded and precipitate was weighed. The formula used for the WHC is

$$\text{WHC} = \frac{\text{Wt. of Wet Sample} - \text{Wt. of dry Sample}}{\text{Wt. of dry Sample}}$$

2.2.2 Oil Binding Capacity

OBC was determined according to the methods of Chau et al. 1 % mucilage (w/v) in coconut oil was mixed by vortex mixture for 1 min and kept 37^0C for 30 mins, then centrifuged at 3000 rpm for 30 mins and then the supernatant was dicarded and weighed. The OBC was calculated by the formula,

$$OBC = \frac{\text{Oil Absorbed Sample Wt.} - \text{Wt. Of Dry Sample}}{\text{Wt. Of Dry Sample}}$$

2.2.3 XRD:

XRD is an analytical tool, mainly used for the identifying crystalline materials and measure the purity of sample. X-ray diffractometer was measured by BRUKER USA D8 advance, Davinci model. The results were recorded in the 2theta ranging from 10^0-80^0 to determine the level of crystallinity, after the extraction of mucilage from *Cocculus hirsutus*.

2.2.4 Differential Scanning Calorimetry

It is thermal device used to determine the physical properties of sample change, along with temperature against the time. A DSC study was performed on a TA Instruments Inc. model Discovery (New Castle, DE).The curve obtained was used to study the transition of the sample like, melting point, crystallization, glass transition and polymorphic transformations

2.3. Anti-Diabetic Activity

α- glucosidase inhibitory assay:

The alpha glucosidase inhibition activity of was performing with small modification. 70µl 50mM of phosphate buffer, maintains pH at 6.8, 10µl (0.5mM) test sample, consequently adding of 10µl α-glucosidase enzyme, in the total volume of 100µl of reaction mixtures. The samples were mixed, reinsulated for 10 min at 37^0C & pre-analyze at 440nm. The reaction was begined by the addition of 10µl of 0.5mM substrate PNPG. After 30 mins of isolation at 37° C, the yellow colour was developed due to the formation of P-nitro phenol was measured by using synergy HT (biotek, USA) 96, well plate reader at 400nm. In this above experiment, acarbose was as positive control; the entire tests were performed in triplicates. [21] The % inhibition was calculated by

Inhibition (%) = (abs of control - abs of test / abs of control × 100)

2.4 Anti-Urolithiatic Activities:

Preparation of Calcium oxalate and Egg membrane

The protocol about renal stones i.e. artificially synthesized calcium oxalate by homogenate precipitation. Calcium chloride dehydrates and sodium oxalate dissolved in equimolar solution of

water, additionally added 0.2N H_2SO_4, mixed well and allowed to stand for few hours. The calcium oxalate precipitate was obtained. Then the precipitate were collected and rinsed with ammonia solution to free from H2So4, again rinsed with distilled water. Finally, the dried calcium oxalate (artificial stone) utilized for this experiment. [22].

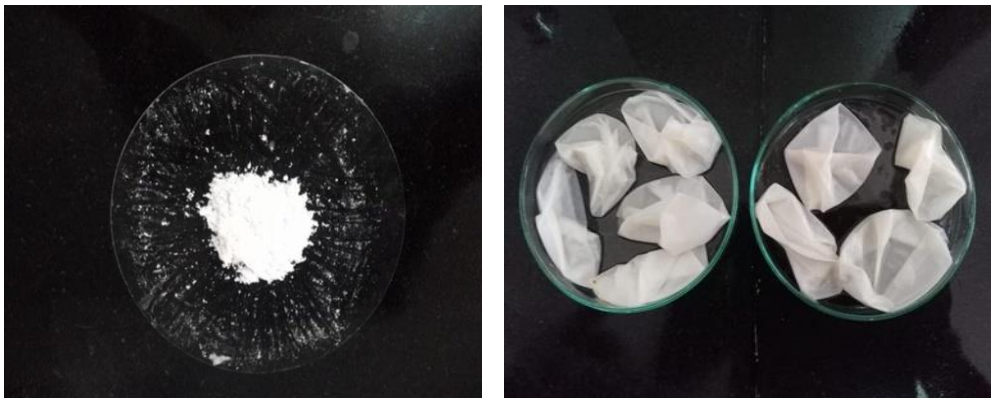

Fig. (1&2). Precipitation of calcium oxalate & Prepared egg membrane

In this experiment, prepare the semi-permeable membrane by using the chicken eggs. The eggs were covered with 2M Hcl solution for decalcification at half of day (12hrs). After the decalcification, puncturing hole at the top of the eggs and remove the content of eggs were carefully. Then the collected membrane sac, rinsed with ammonia solution consequently again rinsed with distilled water. The prepared membrane sac was preserved at 4° C for further use.

Estimation of calcium oxalate by titrimetric method

About 1mg of prepared calcium oxalate and 10, 20, 30, mg/ml of samples was packed in the semi permeable membrane. The membrane stitched at one end and positioned in a conical flask, that contain 100 ml of 0.1N tris-buffer. The calcium oxalates alone serve as negative control without the extract, while cystone act as a positive control. Then pre-incubate the conical flask in the pre-incubate chamber at 37° C for 8 hours. Subsequently incubation, the contents were collected into the test tube from the sac membrane. Moreover, added 2ml of 1N sulphuric acid in the content and titrated against the 0.94N KMnO4 until the observance of pale pink colour. In this titrimetric method, 1ml of 0.9N KMnO4 is equivalent to be 0.1898mg of calcium. The calcium oxalate of percentage dissolution was calculated using the amount of undissolved calcium oxalate. [23]

3. RESULTS AND DISCUSSION

The plant mucilage obtained from *Cocculus hirsutus* contains phytochemicals like carbohydrates, flavonoid, tannin, saponins, alkaloid and glycosides. These phytochemicals showed wide range of applications.

Water holding capacity (WHC) was an important attribute in food technology because of its consistency, yield, texture, and sensory assessment. 1 gm of Cocculus hirsutus mucilage could absorb 24.2 g/g of water, the high WHC of mucilage .indicated that it can be used as a functional ingredient or texture modifier like dropping calories, thickness and evading syneresis of food, whereas WHC for *Cordia myxa* mucilage was 14.94 g/g and Opuntia dillenii mucilage was 4.00 g/g [24]**.**

The food products' texture was enhanced by their high OHC (Oil holding capacity) values. Additionally the pore size, which was increased with an increase in temperature, also impacted the OHC value [25], 1 gm of *Cocculus hirsutus* mucilage could absorb 14.8 g of oil, whereas OHC were observed in basil seed mucilage and chia seed mucilage were 8.37 g oil/g and 12.97g oil/g respectively. [26].The good OHC values improved the texture of food products and also enhanced the food flavors.

The DSC spectra described the endothermic and exothermic peaks due to temperature transfer. The exothermic and endothermic peaks connected to the humidity departure and degradation of the substance. The initial endothermic peak was observed at 73.39°C i.e. between 32.30°C to 149.98°C, this temperature was related to the removal of water from mucilage. Followed by the second endothermic transition temperature was observed at 265.65°C in between 226.83°C and 296.41°C. This peak temperature was attributes to the release of bound water. The vaporization of the water represents the occurrence of hydrophilic groups in the sample. An exothermic peak at 265.65°C indicates the decomposition of polysaccharide structure. ΔH value of endothermic peak was higher than exothermic peak. This was mainly due to the high energy exceeded to release water from the de-crystallization and breaking of H bonds in the sample.[27]

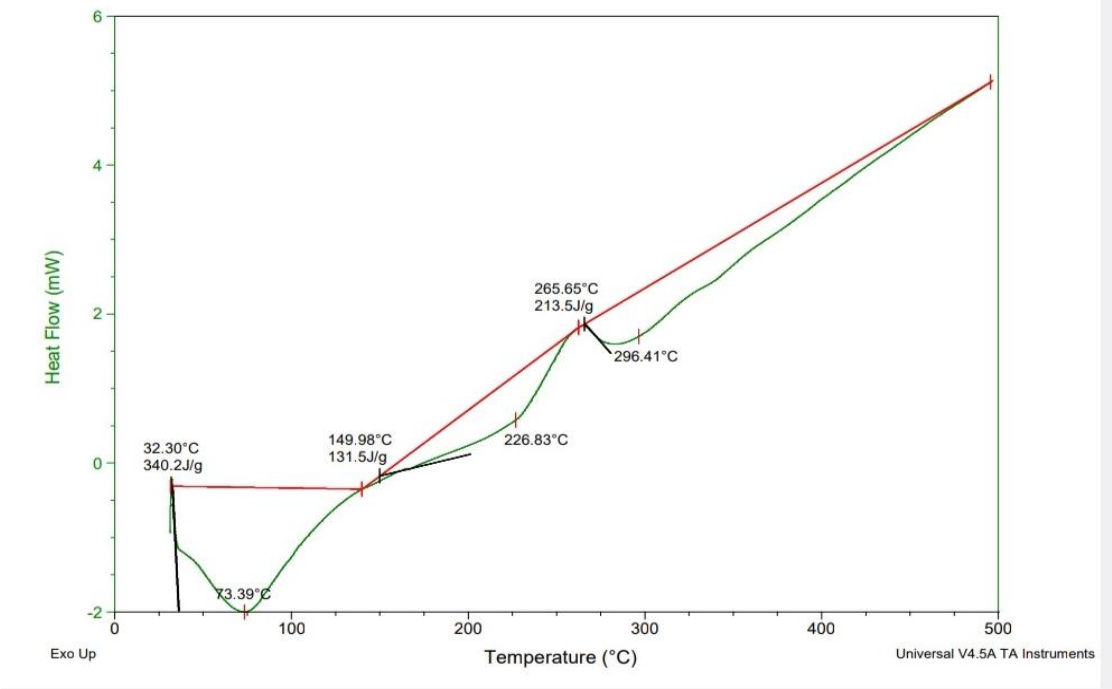

Fig. (3). DSC thermogram of *Cocculus hirsutus* leaves mucilage

XRD

XRD used as a technique for structural analysis and also evaluate different materials in crystalline nature. The crystalline structure was analyzed by X-ray diffractometer. The braggs refection were observed in the XRD pattern at 2Θ (theta) values in Cocculus hirsutus mucilage showed peaks at different angles 21.20^{o}, 21.40^{o}, 21.59^{o}, 22.25^{o}, 22.62^{o},23.66^{o},24.16^{o},26.66^{o} and 30.21°. Mainly the peaks at 21.59°, 24.40°,26.89° showed no sharp peaks in the diffraction spectrum, indicating that the mucilage was completely amorphous in nature.[28]

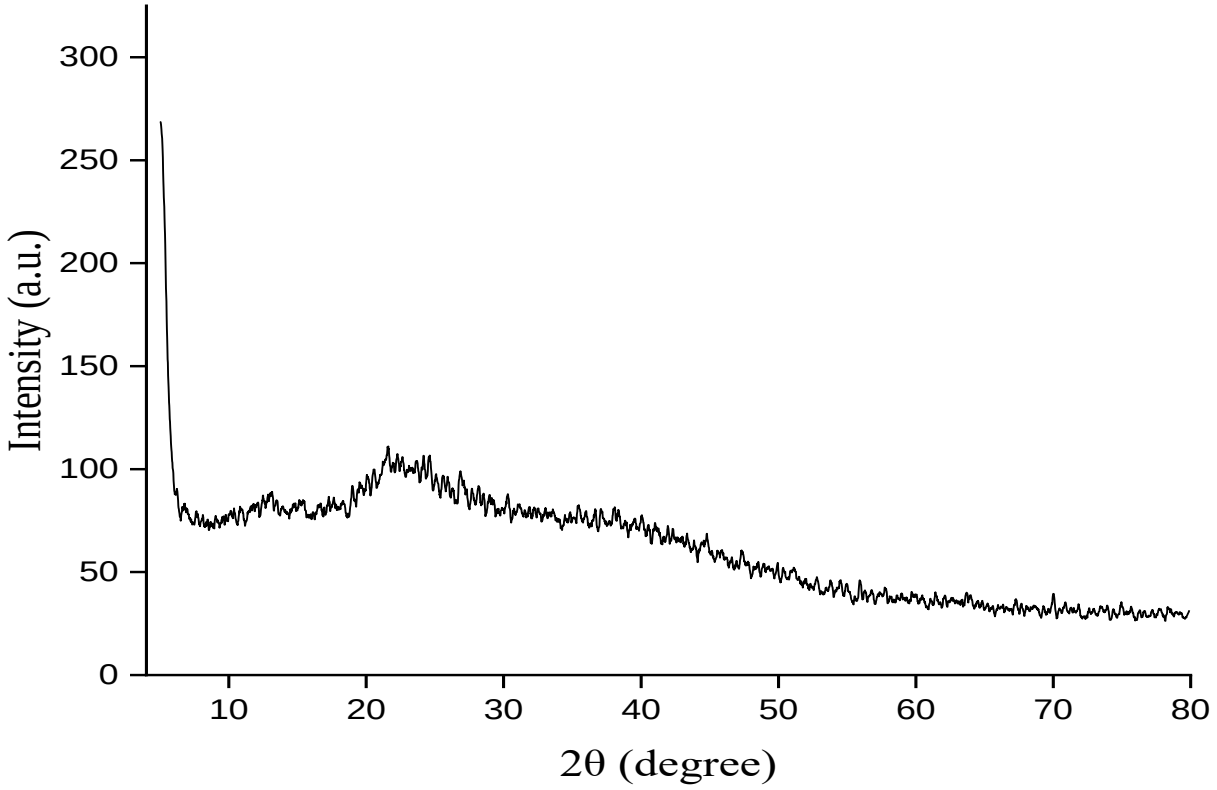

Fig. (4). An XRD spectrum of *Cocculus hirsutus*. Leaves mucilage

Anti-diabetic Activity

The bioactive compounds present in the natural products were respect to reduce the glucose production in the gut. The membrane bound enzyme α –glucosidase located on the small intestine and catalyzing the cleavage of disaccharides in to glucose. So the inhibition activity of α –glucosidase was considered to be valuable tool to control the sugar level. This is the most effective method to prevent the hyperglycemic condition [29]. The mucilage extracted from *Cocculus hirsutus* exhibited potential anti diabetic effect in dose dependant manner which correlated with the anti diabetic effect of aloe vera mucilage [30], okra mucilage [31] and chia mucilage [32].

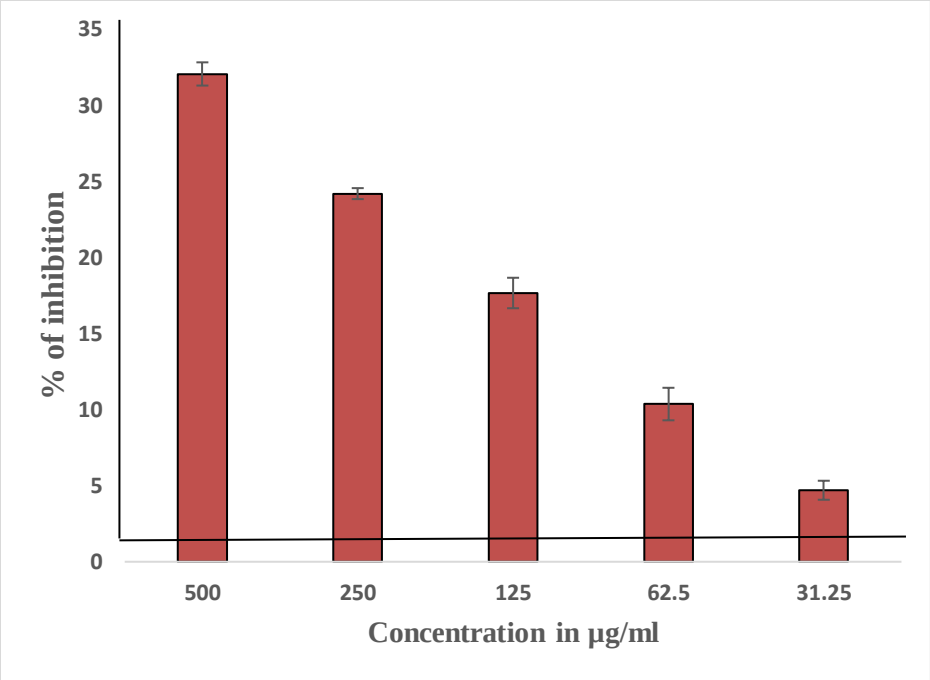

Fig. (5). Anti-diabetic activity of α-glycosidase Method:

Anti urolithiatic Activity

The prepared artificial stone like calcium oxalate dissolved by various concentrations of *Cocculus hirsutus* mucilage was measured by titrimetric method. This is compared to the standard antibiotic drug Cystone. The current allopathic treatment was not effective in dissolving the renal stones as compared to plant medicine. So the herbal remedies only provided better results for stone treatment due to the presence of some bioactive compounds in plants and plant products. Recently it was reported that flavonoids reduce CaOx crystallization in human urine and in animal models and also saponins have an anticrystallization character by disaggregating the suspension of mucoprotein as the activators of crystallization response [33, 34]. So the antiurolithiatic action considered in the present study was due to the synergetic effect of these bioactive compounds present in mucilage of *Cocculus hirsutus.*

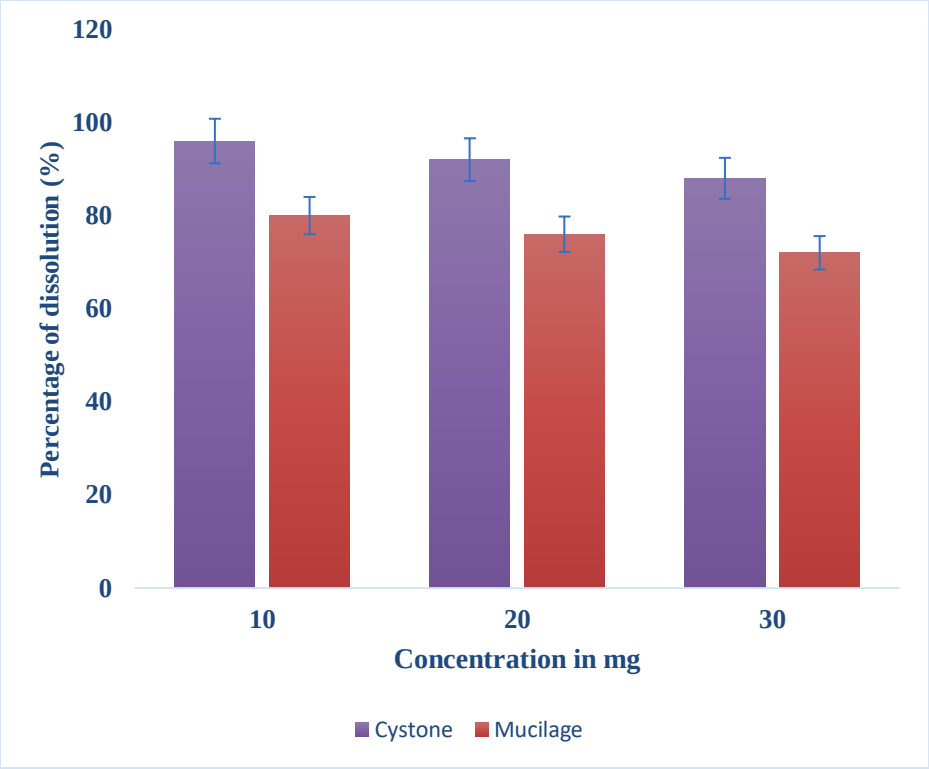

Fig. (6). Effect of Cocculus *hirsutus* mucilage on calcium oxalate

4. CONCLUSION

In this study, the mucilage was obtained *Cocculus Hirsutus* leaves. The mucilage contains many important *phytoconstituents* apart from sugars. They possess characteristic physiochemical properties like WHC, OHC etc., It also exhibited amorphous structure apart from showing characteristic endothermic and exothermic peaks. This mucilage also exhibited its anti diabetic and anti-urolithiatic activity. Thus, this mucilage from *Cocculus hirsutus* can be exploited for various medical applications like anti diabetic and anti-urolithiatic and anticancer activity.

5. REFERENCES

1. Kuldeep Singh, Ashok Kumar, Naresh Langyan. (2009). Evaluation of Mimosa pudica seed mucilages sustained-release excipient. AAPS Pharm Sci Tech, 10(4):44-9. https://doi.org/10.1208/s12249-009-9307-1.

2. Prajapati, V.D., Jani, G.K., Moradiya, N.G., Randeria, N.P.(2013).Pharmaceutical applications of various natural gums, mucilage and their modified forms. Carbohydr. Polym. 92, 1685–1699. https://doi.org/10.1016/j.carbpol.2012

3.M.M. Tosif, A. Najda, A. Bains, R. Kaushik, S.B. Dhull, P. Chawla, M. WalasekJanusz, (2021). A comprehensive review on plant-derived mucilage: characterization, functional properties, applications, and its utilization for nanocarrier fabrication, Polymers (Basel) 13-1066, https://doi.org/10.3390/polym13071066

4. Amid, B. T.; Mirhosseini, H. (2012) Effect of different purification techniques on the characteristics of hetero polysaccharide-protein biopolymer from durian (Duriozibethinus) seed. *Molecules, 17*(9),10875–10892.PMCID: PMC6268219DOI: 10.3390/molecules170910875

5. Akbari, S. M. Ghoreishi, N. Habibi. (2014).Generation and precipitation of paclitaxel nanoparticles in basil seed mucilage via combination of supercritical gas anti solvent and phase inversiontechniques,JSuperscriptFluids.94,182-188. http://dx.doi.org/10.1016/j.supflu.2014.07.007.

6. Ahuja, M., Kumar, A., Yadav, P., & Singh, K. (2013). Mimosa pudica seed mucilage: Isolation; characterization and evaluation as tablet disintegrant and binder. International Journal of Biological Macromolecules, 57, 105–110. http://dx.doi.org/10.1016/j.ijbiomac.2013.03.004.

7. Gemede, H.F.; Haki, G.D.; Beyene, F.; Rakshit, S.K.; Woldegiorgis, A.Z. (2018) Indigenous Ethiopian Okra (Abelmoschus Esculentus) Mucilage: A Novel Ingredient with Functional and Antioxidant Properties. Food Sci. Nutr., 6, 563–571. [CrossRef] [PubMed]

8. Fox, D. I., Pichler, T., Yeh, D. H., & Alcantar, N. A. (2012). Removing heavy metals in water: The interaction of cactus mucilage and arsenate (As(V)). Environmental Science & Technology,46,(8),4553-4559. https://doi.org/10.1021/es2021999

9. Hosseini,M.S., and M.R. Nabid. (2020). Synthesis of chemically cross-linked hydrogel films based on basil seed (Ocimumbasilicum L.) mucilage for wound dressing drug delivery applications, International Journal of Biological Macromolecules. https://doi.org/10.1016mac.2020.06.252.

10. Kay, B. A., Trigatti, K., MacNeil, M. B., Klingel, S. L., Repin, N., Douglas Goff, H., & Duncan, A. M. (2017). Pudding products enriched with yellow mustard mucilage, fenugreek gum or flaxseed mucilage and matched for simulated intestinal viscosity significantly reduce postprandial peak glucose and insulin in adults at risk for type 2 diabetes. Journal of Functional Foods, 37, 603–611. 10.1016/j.jff.2017.08.017.

11. Chiang, J. H., Ong, D. S. M., Ng, F. S. K., Hua, X. Y., Tay, W. L. W., & Henry, C. J. (2021b). Application of chia (Salvia hispanica) mucilage as an ingredient replacer in foods. Trends in Food Science and Technology, 115, 105– 116. 10.1016/j.tifs.2021.06.039.

12.Cakmak, H., Mama, M., & Yilmaz, S. M. (2021). Determination of the effects of flaxseed (Linum usitatissimum L.) sprouts powder on the gluten-free cupcake quality. Journal of Food Processing and Preservation, 45(5), e15435. 10.1111/jfpp.15435.

13. Rostamabadi, M. M., Falsafi, S. R., Nishinari, K., & Rostamabadi, H. (2022). Seed gum-based delivery systems and their application in encapsulation of bioactive molecules. Critical Reviews in Food Science and Nutrition, 0(0), 1–24. 10.1080/10408398.2022.2076065.

14. Ganapaty S., Dash G.K., Subburaju T., Suresh P. (2002). Diuretic, laxative and toxicity studies of *Cocculus hirsutus* aerial parts. *Fitoterapia.*73, 28–31.https://doi.org/10.1016/S0367-326x(01)00345-8

15.Ranjan P.B., Mohanta S.R., Das G.K.(2009). Preliminary phytochemical analysis and wound healing activity from the leaves of *Cocculus hirsutus* Diels. *Int. J. Pharm. Sci. Nano technol.* 2, 675–678. https://doi.org/10.37285/ijpsn.2009.2.3.13.

16. Thavamani, B. S., Mathew, M., & Palaniswamy, D. S. (2014). Anticancer Activity of Cocculus hirsutus against Dalton's lymphoma ascites (DLA) Cells in Mice. Pharmaceutical biology, 52(7), 867-872. https://doi.org/10.3109/13880209.2013.871642

17. K..Mallikarjuna Rao, K. Gnanaprakash, A.V. Badarinath, C. Madhusudhana Chetty, M.Alagusundaram,(2010). Preparation and evaluation of flurbiprofen gel; mucilage of Cocculus hirsutus leaf powder as gel base, Int. J. PharmTech Res. 2,1578–1583. [CrossRef] [Google Scholar]

18. Devasahayam Leema Rose Mary, Antonysamy Lawrance, Arokiadoss Edwina Sherley Felicita & Pushpam Marie Arockianathan(2024). Characterization and antioxidant effect of mucilage in leaves from Cocculus hirsutus , Bioinformation 20(5): 439-448. DOI: 10.6026/973206300200439

19. Thanatcha, R.; Pranee, A. (2011). Extraction and characterization of mucilage in ziziphus mauritiana lam. Int. Food Res. J. 18, 201–212.

20. C.-F. Chau, P. C. K. Cheung, and Y.-S. Wong,(1997). "Functional properties of protein concentrates from three chinese indigenous legume seeds," Journal of Agricultural and Food Chemistry, vol. 45, no. 7, pp. 2500–2503..

21. Dewi, R.T., Y.M. Iskandar, M. Hanafi, L.B.S. Kardono, M. Angelina, I.D. Dewijanti and S.D.S. Banjarnahor (2007). Inhibitory effect of Koji *Aspergillus terreus* on α-glucosidase activity and postprandial hyperglycemia. Pak. J. Biol. Sci., 10: 3131-3135.

22. Mary DLR, Arockianathan PM, Lawrance A and Felicita ASE (2021) In-vitro study of anti-diabetic, anti-cancer and anti-urolithiatic activity of different extract of Prosopis juliflora seeds. Int J Pharm Sci & Res; 13(5): 2101-08. doi: 10.13040/IJPSR.0975-8232.13(5).2101-08

23. Sankaranarayanan C and Vijayarani V (2018). Anti-urolithiatic activity of whole plant aqueous andethanolic extract of Coriandrum sativum L. - An in-vitro approach. Malaya Journal of Biosciences; 5(1): 2348-36.

24. Alpizar-Reyes, E.; Carrillo-Navas, H.; Gallardo-Rivera, R.; Varela-Guerrero, V.; Alvarez-Ramirez, J.; Pérez-Alonso, C (2017). Functional properties and physicochemical characteristics of tamarind (Tamarindus indica L.) seed mucilage powder as a novel hydrocolloid. *J. Food Eng*. *209*, 68–75**.** https://dx.doi.org /10.1016/j.jfoodeng.2017.04.021

25. Abir Mokni Ghribi .; Ines MakloufGafsi .; Assaâd Sila.;Christophe Blecker .; Sabine Danthine .; Hamadi Attia .; Ali Bougatef .; Souhail Besbes.(2015). .Effects of enzymatic hydrolysis on conformational and functional properties of chickpea protein isolate. *Food Chemistry*. *15(*187), 322-30. PMID: 25977033.https://doi.org/ 10.1016/j.foodchem.2015.04.109

26. Coorey, R.; Tjoe, A.; Jayasena, V.(2014). Gelling properties of chia seed and flour. *J. Food Sci.***,** *79,* E859–E866. PMID: 24734892. https://doi.org /10.1111/1750-3841.12444

27.SnehPunia ; Sanju Bala Dhull. Chia seed (Salvia hispanica L.) mucilage (a hetero polysaccharide (2019**).** Functional, thermal, rheological behaviour and its utilization**.** *Int J Biol. Macromol .1*(140),1084-1090.ttps://doi.org/10.1016/j.ijbiomac.2019.08.205. PMID: 31465801.

28**.** Kurd, F.; Fathi, M.; Shekarchi Zadeh, H. (**2017**). Basil seed mucilage as a new source for electrospinning: production and physicochemical characterization. *Int. J. Biol. Macromol*. *95,* 689-695. http://dx.doi.org/10.1016/j.ijbiomac.2016.11.116

29. Ubeyitogullari, A.; Ciftci, O.N.(2020). Fabrication of bioaerogels from camelina seed mucilage for food applications. Food Hydrocoll. 102, 105597. [CrossRef]

30. Sefi, M., Chaâbane, M., Rafrafi, M., & Zeghal, N. (2019). Hypoglycemic and hypolipidemic activities of aloe vera leaf mucilage in alloxan-induced diabetic rats. Pharmaceutical and Biomedical Research, 5(3), 29–34. 10.18502/pbr.v5i3.2116

31.Uddin Zim, A. F. M. I., Khatun, J., Khan, M. F., Hossain, M. A., & Haque, M. M. (2021). Evaluation of in vitro antioxidant activity of okra mucilage and its antidiabetic and antihyperlipidemic effect in alloxan-induced diabetic mice. Food Science and Nutrition, 9(12), 6854–6865. 10.1002/fsn3.2641

32. Tamargo, A., Martin, D., Navarro del Hierro, J., Moreno-Arribas, M. V., & Muñoz, L. A. (2020). Intake of soluble fibre from chia seed reduces bioaccessibility of lipids, cholesterol and glucose in the dynamic gastrointestinal model simgi®. Food Research International, 137, Article 109364. 10.1016/j.foodres.2020.109364.

33. Noorafshan A, Karbalay DS and Karimi F: (2013). Diosmin reduces calcium oxalate deposition and tissue degeneration in nephrolithiasis in rats: astereological study. Korean Journal of Urology; 54(4): 252-57.

34. Gurocak S and Kupeli B (2006). Consumption of historical and current phytotherapeutic agents for urolithiasis: a critical review. The Journal of Urology ,176(2): 450-55