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IN VITRO CHARACTERIZATION OF NATURAL MUCOADHESIVE AGENT ISOLATED FROM *OCIMUM AMERICANUM* SEED

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ABSTRACT

Aim of present investigation was to isolate natural mucoadhesive agent from *Ocimum americanum* seeds and characterize through *in vitro* mucoadhesion methods, FTIR and DSC studies. Mucoadhesion force of isolated natural mucoadhesive agents and synthetic polymer Carbopol 934P was determined using *in vitro* mucoadhesion methods viz. Wihelmy's method, falling sphere method and modified physical balance method. The research study reveals that the natural mucoadhesive agent isolated from *Ocimum americanum* seed was shown promising mucoadhesion strength. The formation of hydrogen bond by natural mucoadhesive agent with mucosa was confirmed by FTIR spectra showing carboxyl and hydroxyl groups. Natural mucoadhesive agent may be useful to formulate mucoadhesive drug delivery systems as it bears excellent mucoadhesion property and advantageous over synthetic polymers for less toxicity, biocompatibility, biodegradability and cost.

INTRODUCTION: *Ocimum americanum L.*, is an annual herbaceous plant native to asia and africa. It is an aromatic plant having 0.7 m hight with an erect stem and very green, ovate leaves, grayish green beneath, and white, greenish or purplish pink-white flowers [1].

Seeds are having nutlets with narrowly ellipsoid, punctulate black shape [2]. Volatile oils include methyl cinnamate, methylheptenone, methylnonylketone, d-camphor, citral, Ocimin, methyl chavicol, linalool, nevadensin, salvigenin, beta-sitosterol, betulinic, ursolic, oleanolic acids, flavanoids, pectolinarigenin-7-methylether and nevadensin.

Polysaccharides composed of xylose, arabinose, rhamnose and galacturonic acids are the main chemical constituents [3]. Reported uses of *ocimum americanum are* antimicrobial, antioxidant, anti-helmintic and anti diabetic [4].

Now a day, mucoadhesive agents are thoroughly studied for gastric retention or site specific retention of drug delivery to improve bioavailability, sustain drug release and produce better patient compliance by reducing frequency of administration. Mucoadhesive agents extracted from fruits of *Hakea gibbosa* gum [5], *diospyros peregrine* fruits [6], *Caesalpinia pulchirrima* Swartz. and *Leucaena leucocephala* Lam. Seeds [7], *Lallimantia royalena* seed coat [8], were evaluated for various *in vitro* and *in vivo* mucoadhesion studies.

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In various studies, natural substances were reported mucoadhesive property due to presence of carbonyl group, thiol group, sugars, proteins, carbohydrates, hydroxyl groups, hydrogen bond, amide groups, cations and anions in their composition [9, 10].

Therefore the use of natural mucoadhesive agents for the purpose of keeping the drug for a prolonged period of time in stomach region should be of great interest. Present research work was mainly focused on isolation and characterization of natural mucoadhesive agent using different in vitro mucoadhesion methods.

MATERIALS AND METHODS: Plant material was authenticated and specimens were stored at Botanical Survey of India, Western Regional Centre, Pune, Maharashtra wide voucher number BSI/ WRC/ TECH/ 2011/ ABGOCA5. Chemicals used in the present study were of analytical reagent grade.

Isolation and Purification of mucoadhesive agent from seeds of *Ocimum americanum*: Seeds of *Ociumum americanum* were collected from fields of Nashik, Maharashtra in April month. Seeds were soaked in distilled water for 24 hours. The material was vigorously stirred using heavy duty mechanical stirrer at 2000 rpm and at temp 60°C for two hours. The released mucoadhesive agent was filtered through muslin cloth to obtain clear filtrate. Then double quantity of acetone was added to precipitate the mucoadhesive agent. Precipitated mucoadhesive agent was washed thrice with small quantity of acetone. It was dried in oven at temperature 40°C for six hours and then kept in vacuum desiccator. The dry mucoadhesive agent was reduced to fine powder and stored in an air tight container.

Wihelmy's method: Wihelmy's method was studied to determine mucoadhesion strength of isolated mucoadhesive agent in terms of detachment weight required to break the mucus-polymer bond against adhesion. Small glass plates of dimension 2×5cm were coated by spreading 1%, 2 % and 3% w/v solution of isolated mucoadhesive agent and 1% w/v Carbopol 934P. These solutions were uniformly coated to small glass plates respectively. Coated plates were dried at 40°C. The mucus gel was collected from goat stomach and kept at 20°C. Thread was attached at one end of coated glass plate. Coated glass plate was dipped into mucus gel while other side of thread was passed through pulley and end of thread was attached to small plastic bag. Plates were dipped into mucus gel for 10, 20 and 30 minutes. Weight was added into small polyethylene bag with small increments of water till the surfaces get pull out and separated vertically from mucus gel. Glass plate weight was minimized from the final detachment weight. Weight in gram required to pull out the glass plate from the mucus gel represents the force required to break the mucus-polymer bond against adhesion. Six times study was performed [11-13].

Falling Sphere method: Falling sphere method was studied to determine mucoadhesive strength in terms of time in second required for mucoadhesive agent coated grain to fall 50 divisions in the burette filled with 10 % mucus solution. 1%, 2%, 3% w/v solutions of isolated mucoadhesive agents and Carbopol 934P respectively were prepared. Mustard grains of diameter 1.0-1.1 mm were taken and then coated with above mucoadhesive agent solutions. Grains were dried at 30°C. Each grain was slowly placed at the top layer of 10 % mucus solution in the burette. Time in seconds taken by the mucoadhesive agent coated grain to fall 50 divisions in the burette was noted. Six times study was performed [14, 15].

Modified Physical Balance method: Modified physical balance method was studied to determine mucoadhesive strength in terms of detachment force. Tablets (thickness 1mm and diameter 8.75 mm) of natural mucoadhesive agent and Carbopol 934P were prepared by direct compression method. Fresh goat stomach mucosa was collected from slaughter house and stored in 0.1 HCl at 37°C before use. Pans of both arms of physical balance were removed. Mucosa was placed on teflon block and tightly fixed using cynoacrylate adhesive.

Mucosa fixed block was placed in a beaker. To the beaker 0.1 N HCl was added up to surface of mucosa for moistening. Prepared tablet was stick to plastic bottle closure surface using cynoacrylate adhesive and was attached to one arm of modified physical balance using thread, below which mucosa attached block was affixed. On the opposite side of balance arm, small polyethylene bag was attached and both arms were balanced.

Block with attached mucosa in a beaker was raised up to tablet surface. Tablet was slightly pressed on mucosa to allow mucoadhesion. Then, small increments of water added in polyethylene bag to detach the surfaces [16]. The weight in gram required to detach the surfaces noted. Six times study was performed. The force of adhesion (kgm/s^2) was calculated by, Force of Adhesion = Weight required for detachment (kg) x acceleration (m/s^2).

Fourier Transform Infrared Spectroscopy Studies (FTIR): Mucoadhesive agents were dried at 40°C for 24 hours and then kept in vacuum desiccator for 3 days. 20 mg of the samples were grounded with KBr and pellets were formed under a hydraulic pressure of 10 tones/cm^2. FTIR spectra of mucoadhesive agents were taken at room temperature using FTIR (Bruker) and obtained spectrum was studied for identification of functional groups [17, 18].

Differential Scanning Calorimetric Studies (DSC): Mucoadhesive agents were dried at 40°C for 24 hours and then kept in vacuum desiccator for 3 days before DSC study. Mucoadhesive agent was weighed into aluminum crucible and sample was analyzed by differential scanning calorimeter (Mettler Toledo, DSC 823e) Sample was heated at scanning rate of 10°C/ min over a temperature range 20-300°C under nitrogen flow of 40 ml/min. DSC curve was studied for powder properties [19].

RESULT AND DISCUSSION: Natural mucoadhesive agent isolated from *Ocimum americanum* seed was characterized for mucoadhesion properties and was compared with Carbopol 934P. 1%w/v, 2%w/v and 3 %w/v solutions of isolated mucoadhesive agent and 1 %w/v solution of Carbopol 934P were prepared. These solutions were studied for mucoadhesive property using Wihelmy's method, Falling sphere method and Modified physical balance method. Results are shown in **Table 1**.

Table 1: *In vitro* Mucoadhesive Characteristics of *Ocimum americanum* seed and Carbopol 934P

Mucoadhesive agent	Mucoadhesion Method*					
	Conc. of mucoadhesive agent	Wihelmy's method [Detachment Weight (g)]	Conc. of mucoadhesive agent	Falling sphere method [Time (sec.)]	Modified physical balance method	
					Detachment Weight (g)	Force of adhesion kgm/s^2
Ocimum americanum seed	1 % w/v	1.964 ± 0.101	1 % w/v	8.653 ± 0.836	47.306 ± 1.042	0.464
	2 % w/v	3.856 ± 0.135	2 % w/v	17.475 ± 1.23		
	3 % w/v	7.205 ± 0.102	3 % w/v	42.928 ± 1.247		
Carbopol 934P	1 % w/v	4.03 ± 0.14	1 % w/v	18.135 ± 1.106	85.519 ± 1.216	0.838

* Average and standard deviation of six observations

In Wihelmy's method weight in grams required to pull out the plate from mucus gel was determined. It was observed that mucoadhesive bond formation between isolated mucoadhesive agent and mucus gel opposes the plate to move upward and thus was required greater weight to pull out coated plates at higher concentrations of isolated mucoadesive agent. Carbopol 934P was shown better mucoadhesive force than the mucoadhesive agent isolated from *Ocimum americanum* seed. Force required to detach glass plate caoted using 1, 2 and 3 %w/v isolated mucoadhesive agent was shown 1.964, 3.856 and 7.205 g respectively while 1% w/v Carbopol 934P was shown 4.03 g of mucoadhesion force. Isolated mucoadhesive agent was shown comparable mucoashesion force at 2% w/v concentraion and better mucoadhesive force at 3 %w/v concentration than 1%w/v of Carbopol 934P. It is shown in **figure 1**.

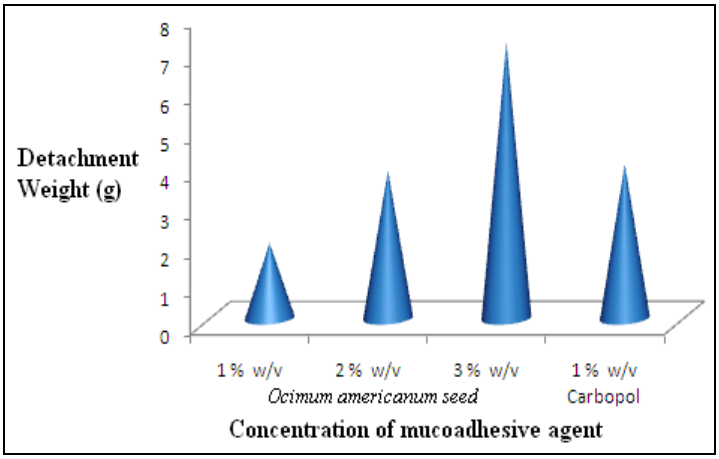

FIGURE 1: DETACHMENT FORCE OF MUCOADHESIVE AGENTS BY WIHELMY'S METHOD

In falling sphere method mustard grains of size 1.0 to 1.1 mm were coated with 1%w/v, 2%w/v and 3 % w/v solutions of isolated mucoadhesive agent and 1 % w/v solution of Carbopol 934P.

Coated grains were allowed to move top to bottom in 50 ml burettee conating 10% mucus solution. Mucoadhesion time was found to be 8.653, 17.475 and 42.928 sec for coated grains using 1%w/v, 2%w/v and 3%w/v isolated mucoadhesive agent. Grain coated using 1%w/v Carbopol 934P was shown 18.135 sec mucoadhesion time. Isolated mucoadhesive agent was shown comparable mucoadhesion time at 2%w/v concentation and better mucoadhesion time at 3 % w/v concentation than 1%w/v of Carbopol 934P. It is shown in **figure 2**.

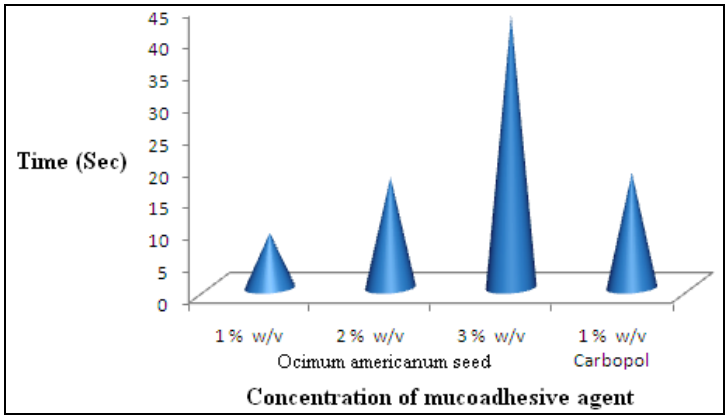

FIGURE 2: MUCOADHESION TIME OF MUCOADHESIVE AGENTS BY FALLING SPHERE METHOD

In modified physical balance method, tablets of thickness 1 mm and diameter 8.75 mm were prepared using isolated mucoadhesive agent and Carbopol 934P. The mucoadhesion force required for the detachment of tablet suface from the mucosal surface was determind using modified physical balnce method. Isolated mucoadhesive agent isolated from *Ocimum americanum* seed was shown 47.306 g mucoadhesion force and 0.464 Kg.m/s^2 mucoadhesion strength. Carbopol 934P was shown 85.519 g mucoadhesion force and 0.838 Kg.m/s^2 g mucoadhesion strength.

FTIR spectrum of mucoadhesive agent isolated from *Ocimum americanum* seed: FTIR spectrum is shown in **figure 3**. Characteristic absorption occurs as various peaks (functional groups) 1192.91 cm^{-1} (R-OH stretch), 1378.67 cm^{-1} (C-H stretch), 1720.19 cm^{-1} (C=O Stretch), and 3260.44 cm^{-1} (O-H stretch) indicated that isolated mucoadhesive agent form *Ocimum americanum* may forms hydrogen bond with mucosa to adhere the mucosal membrane.

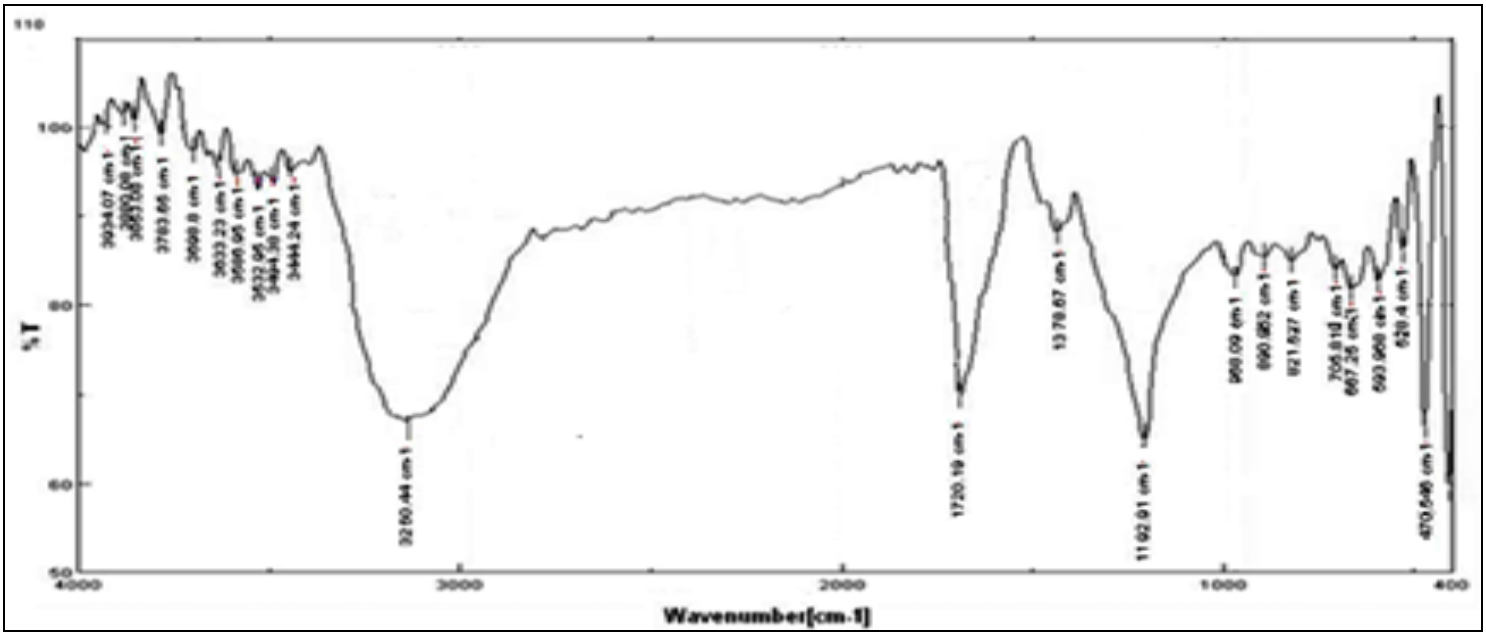

FIGURE 3: FTIR SPECTRUM OF MUCOADHESIVE AGENT ISOLATED FROM *OCIMUM AMERICANUM* SEED

Diffrential Scanning Calorimetric studies: The DSC thermogram of mucoadhesive agent isolated from *Ocimum americanum* seed is shown in **figure 4**. It showed two broad endothermic peaks at temperature 92.91°C and 258.17°C.

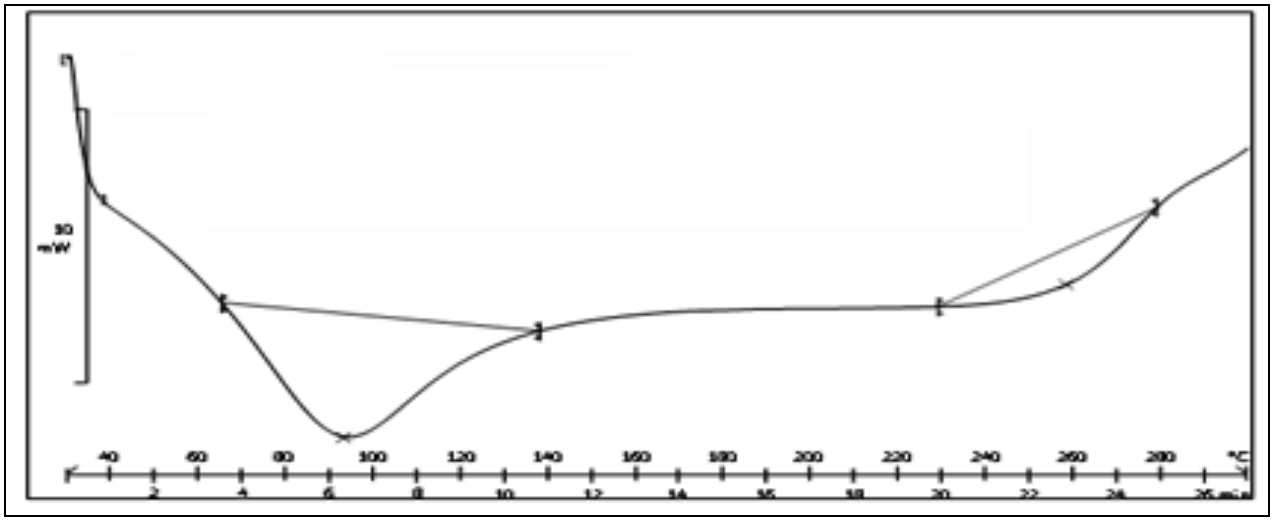

FIGURE 4: DSC CURVE OF MUCOADHESIVE AGENT ISOLATED FROM *OCIMUM AMERICANUM* SEED

CONCLUSION: Natural mucoadhesive agent isolated from the *Ocimum americanum* seed was found comparable in mucoadhesive property when compared against Carbopol 934P. It is also confirmed by presence hydroxyl and carbonyl functional groups in FTIR spectrum which are responsible for mucoadhesion. Natural mucoadhesive agent is advantageous over Carbopol 934P, synthetic polymer owing to its less toxicity.

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