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Pharmacognostic, Isolation and Physicochemical Characterization of American Aloe (*Agave americana*) Cellulose Powder

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Abstract

This study investigates the pharmacognostic properties, isolation, and physicochemical characterization of cellulose powder derived from *Agave americana* (American aloe). Pharmacognostic evaluations included macroscopic observations, geographical distribution, and botanical authentication. Isolation and purification methods focused on delignification and elimination of impurities using sodium hydroxide and bleaching agents. Purified cellulose powder was characterized for phytochemical and physicochemical parameters such as solubility, thermal stability, swelling index, water uptake capacity, and micromeritic properties. Phytochemical tests revealed the absence of lignin and the presence of cellulose post-purification. Physicochemical properties indicated improved thermal stability, porosity, and flow properties upon the addition of glidants. This comprehensive study highlights the potential applications of purified cellulose in pharmaceutical formulations.

Keywords: *Agave americana*, cellulose powder, pharmacognostic study, physicochemical characterization, delignification, micromeritics

Introduction

Natural polymers such as cellulose have found extensive applications in pharmaceutical and industrial sectors due to their biocompatibility, renewability, and functional versatility [1,2]. Among natural sources, *Agave americana* (American aloe), a perennial plant belonging to the Agavaceae family, has gained attention for its cellulose-rich stalk [3]. The present study focuses on isolating and characterizing cellulose from *A. americana*, aiming to assess its potential as a pharmaceutical excipient.

Pharmacognostic studies, including macroscopic observations and botanical identification, provide a comprehensive understanding of plant properties and their medicinal value [4,5]. In this study, cellulose extraction involved delignification and purification processes to ensure removal of lignin, hemicelluloses, and impurities, enhancing its suitability for pharmaceutical applications [6-8].

Post-purification characterization of American aloe cellulose powder (AACP) involved phytochemical and physicochemical evaluations to determine its suitability for use in various formulations. Phytochemical tests confirm the presence of carbohydrates and cellulose, while physicochemical assessments reveal properties such as swelling index, water retention capacity, and flow properties critical for pharmaceutical utility [9-11].

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Material and Method

Pharmacognostic study

Macroscopic examinations

The Pharmacognostic examination of plant for its habitat, height, fruit, leaf and flower with colour, weight and dimensions etc. and information about geographical distribution, local name of plant is collected by taking help of botanist and local peoples.

Collection, Identification and Authentication of Plant

The herb specimen was collected from Bhopal, Madhya Pradesh and then it was treated with 1 % v/v formalin solution followed by poising by dipping itin a saturated solution of mercuric chloride and ethanol and mounted on a herbarium sheet as per the procedure of Botanical survey of India. Then it was sent for identification and authentication with required data.

Collection, Isolation and Purification of *American aloe cellulose* powder

Collection and Isolation

Dried stalk of *American aloe was* collected in the month of March, dried under the sun for 24 hrs cut into small spices; removed the inner portion of the stalk by removing outer portion. This inner portion again dried under the sun for 24hrscut into small pieces, weighed and kept in the hot air oven for drying at 100 ^{0}C for 5 hrs. Then its powder was prepared using mixer and powder was passed through sieve No. 04 to get fine powder less than 4.75 mm particle size.

Purification of cellulose powder

About 50 g of fine powder was treated with 500 ml of a 2% w/v aqueous solution of NaOH for delignification, in a stainless steel vessel immersed in a water bath set at 100 ^{0}C for 3 hrs. Complete of the removal of lignin and to eliminate low molecular weight carbohydrates such as pectin's, proteins, hemicelluloses, and some mineral components, carried out by further digesting the material with 400 ml of a 17.5% aqueous solution of NaOH for 1 hrs at 80 ^{0}C. After thorough washing with distilled water, the remaining solids filtered off and dry in an oven at 60 ^{0}C for 16 hrs. The product (unbleached cellulose) bleached with 62.5 ml of a 3.2% aqueous solution of sodium hypochlorite in the stainless-steel vessel at 40 ^{0}C for 1.5 hrs. The bleached sample was thoroughly washed with distilled water until obtaining a neutral pH,filtered and then dried in the oven at 60 ^{0}C for 16 hrs. Then the purified powder passedthrough 120 mesh sieve to obtain uniform particle size and stored in air tight container. Yield of Cellulose powder was calculated from initial and final weight of cellulose powder

Characterization of purified *American aloe cellulose* powder

Phytochemical characterization

The Purified *American aloe cellulose* powder was tested for presence of various phyto-constituents like alkaloids, amino acids, carbohydrates, fats and oils, flavonoids, glycoside, mucilage, proteins saponins, tannins, terpenoids, volatile oils and heavy metals with reference to standard procedures listed in Table No. 1 in accordance with Indian Pharmacopeia and reported methods.The powder was obtained from inner portion of stalk (wood) of *American aloe* which made up of support tissues supposed to presence of lignin and cellulose. The presence of lignin and cellulose in raw and purified cellulose powder was confirmed by following tests.

Test for the Presence of Lignin

To 100 mg of obtained powder placed on a glass slide and moistened with con. HCl, two drops of Phloroglucinol were added and heated, until the liquid content was completely evaporated. The slide was examined under light microscope for any coloration.

Test for Cellulose

0.5ml of 5% w/v of KOH solution was added to 50 mg of powder placed in a test tube and heated for few minutes and was observed for any canary yellow coloration.

Table No. 1: Tests performed for phytochemical characterization

Sr. No.	Class of Compounds	Test Performed
i.	Alkaloids	Mayer's Test
ii.	Amino acids	Ninhydrin test
iii.	Carbohydrate	Molisch's Test

iv.	Fats & fixed oil	Saponification Test
v.	Flavonoids	Alkaline reagent test
vi.	Glycosides	Borntrager Test
vii.	Mucilage	Ruthenium red test
viii.	Protein	Biuret Test
ix.	Saponins	Froth formation Test
x.	Tannins	Ferric chloride test
xi.	Terpenoids	Libermann-burchard Test
xii.	Volatile oil	Sudan III test
xiii.	Heavy metals	Limit test

Physicochemical Characterizations

The purified AACP was subjected to various physicochemical characterizations such as organoleptic properties, solubility, loss on drying, ash value, pH, melting point, swelling index of #60 mesh passed AACP in distilled water and 0.1 N HCl, Water retention/absorption capacity in distilled water and 0.1 N HCl in accordance with Indian Pharmacopeia[11] and reported methods. In addition to these AACP was evaluated for following physicochemical parameters

Water uptake study

A capillary tube of 3.14 mm diameter was filled with powder sample and immersed in a container containing 20 ml amaranth solution. The rise of solution in the capillary tube was measured at predetermined time intervals and repeated with or without tapping of capillary tube to a constant height. Water uptake study for micro- Crystalline cellulose (MCC) was similarly performed.

Micromeritic properties

The fundamental micromeritic properties particle Size distribution by measuring size of about 300 particles using Binocular Microscope (Make Olympus) and number of particles present in each size range were calculated and the particlesize data distribution was represented. Surface area of mucilage powder was determined by N2 gas adsorption method. Derived properties such as Angle of repose, Bulk density and True density by liquid displacement method, Carr's index and Hausner's ratio were determined in triplicate with reference to standard procedures in accordance with reported methods[15-22].

Results and Discussion

Pharmacognostic Study

Macroscopic Examinations

It is evergreen perennial plant growing at a slow rate in light (sandy) and medium (loamy) soils. Leaves grow every year, having length about 200-250 meter, width 20 cm and thickness 3-5 cm. The leaves grow in clusters. The stalk was initially green in colour and after fully mature it turns into dark brown, grown at the center of the cluster in straight upside, grown up to 10 to 12 feet in height (up to 15 feet with flower) and 20 cm in diameter as shown in Figure No. 2. Stalk blooming in summer and are very high heat tolerant. The inflorescence was grown on top of stalk in December- January month. The flowers are grown in bunch initially green and turn yellow color petals and bottom side of flowers converts into fruit. Mature fruits are dark brown colors. The outer surface of the stalk was the very stuff while the inner portion was very smooth.

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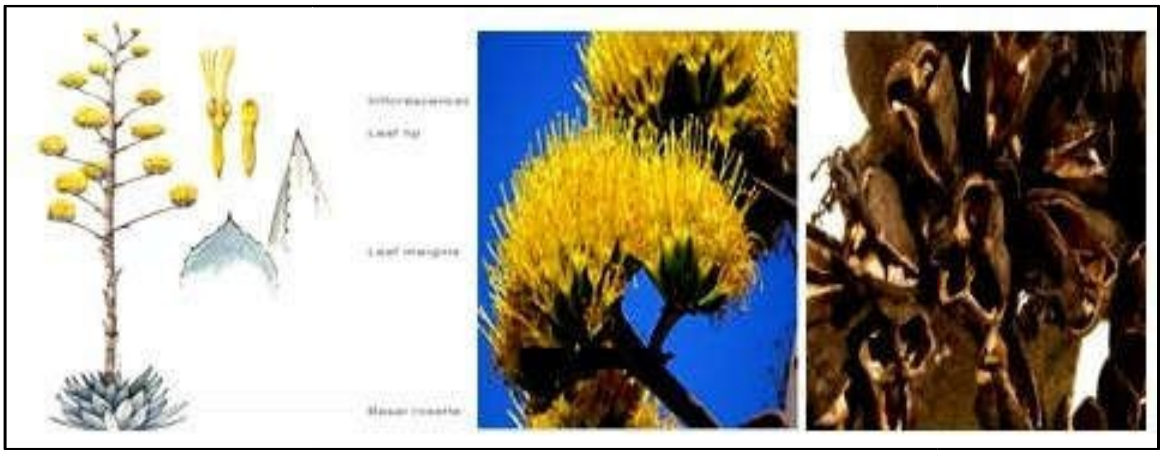

Figure No.1: Morphology of *American aloe Linn.*

Identification and authentication of plant:
The prepared herbarium sheet was authenticated by Botanical survey of India Pune. Herbarium sheet and voucher specimen were preserved in the herbarium record in Pharmaceutics Laboratory, School of Pharmacy for further reference shown in Figure No. 3 and the botanical description of identified plant given in Table No.2

Figure No. 2: Herbarium sheet of *Agave Americana*

Table No. 2: Botanical description of authenticated plant

Botanical Name	***American aloe Linn.***
Kingdom	*Planate*
Division	*Magnoliophyta*
Class	*Liliopsida*
Order	*Asparagales*
Family	*Agavaceae*
Genus	*Agave*
Species	*Agave ameriecana*

Collection, Purification of *American aloe cellulose* powder

Collection of raw *American aloe cellulose* powder

The dark brown slurry of raw *American aloe* cellulose powder (AACP) wasobtained from dried stalk of *American aloe* shown in figure No.4

Figure No.3: Collection of *American aloe cellulose* powder

Purification of raw *American aloe cellulose* powder

American aloe cellulose powder was purified by washing with distilled water until complete removal of brown colour which indicates complete removal of lignin and low molecular weight carbohydrates and some minerals (delignification process). The light yellow powder was obtained as shown the figure No. 5.

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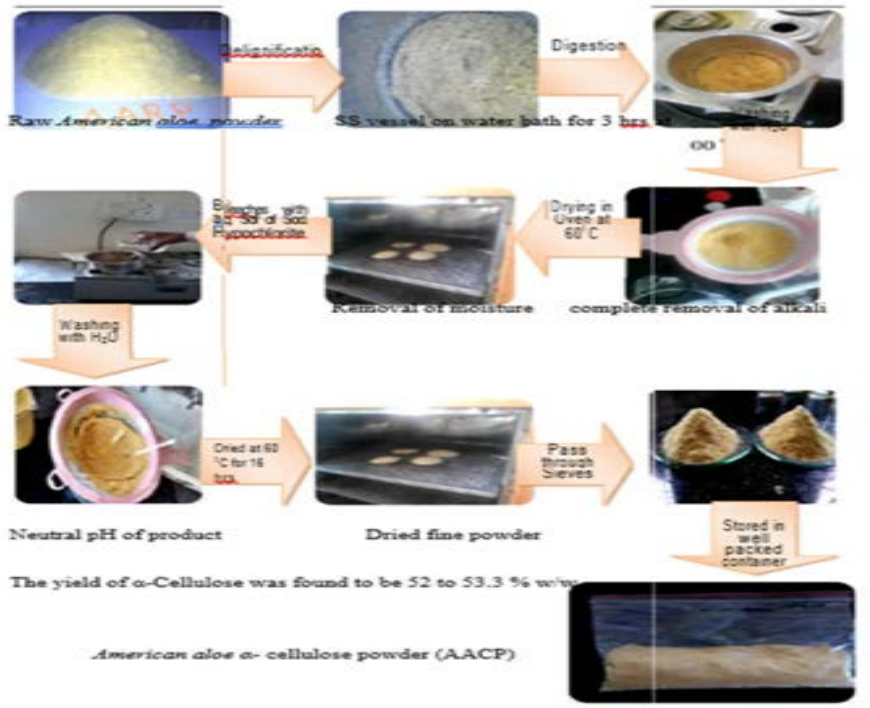

Figure No.4: Purification of *American aloe* cellulose powder (AACP)

Characterization of *American aloe cellulose* Powder

Phytochemical Characterization

Results of preliminary phytochemical characterization of purified *American aloe cellulose* powder were shown in Table No.3.

Table No.3: Preliminary Phytochemical characterization of AACP powder

Sr. No.	Class of Compound	Test Performed	Result
i.	Alkaloids	Mayer's test	-
ii.	Amino Acid	Ninhydrintest	-
iii.	Carbohydrates	Molisch'stest	+
iv.	Fats and Oils	Saponification test	-
v.	Flavonoids	Alkaline Reagent test	-
vi.	Glycosides	Brontragertest	-
vii.	Mucilage	Ruthenium Red test	-

viii.	Protein	Biuret test	-
ix.	Saponins	Forth formation test	-
x.	Tannins and Phenolic	Ferric Chloride test	-
xi.	Terpenoids	Libermann- Burchardtest	-
xii.	Volatile Oils	Sudan III test	-
xiii.	Heavy Metals	Limit test	-

(+ Present, - Absent)

The results phyto-chemical test shows that only carbohydrate was present in the purified *American aloe cellulose* powder (AACP) while other phytoconstituents were absent.

Table No. 4: Test for Carbohydrates

Test	Observation	Inference
Molisch's test	Reddish violet ring at the junction between two liquids	Carbohydrate present

The presence or absences of lignin and cellulose in raw and purified cellulose powder was confirmed and results were shown in Table No. 5.

Table No. 5: Test for Lignin and Cellulose

Samples	Test for Lignin		Test for Cellulose	
	Observation	**Inference**	**Observation**	**Inference**
Raw AACP Powder	Faint brown coloration	Presence of lignin	Faint yellow coloration	Cellulose present
Purified AACP Powder	No color observes	Absence of lignin	Faint yellow coloration	Cellulose present

The lignin and cellulose were present in raw powder, whereas only cellulose present in purified powder and the removal of lignin in purification of powder was confirmed.

Physicochemical characterizations

Organoleptic properties

The purified AACP powder was observed using sense for its physical appearance, odor and taste and results is shown in Table No. 6.

Table No.6: Organoleptic properties of AACP

Sr. No.	Property	Observation
i.	Color	Light yellow
ii.	Odor	No characteristic odor
iii.	Taste	Tasteless
iv.	Nature/ State	Coarse and Smooth fracture
v.	Texture	Irregular

Solubility

The Cellulose powder was slightly soluble in DMSO, Very slightly soluble and swells in NaOH and also in water. Practically insoluble in phosphate buffer pH 1.2 to pH 7and organic solvent like acetone, benzene, chloroform, ethanol, methanol and acetonitrite are summarized in following Table No. 7.

Table No. 7: Solubility of AACP

Solvent	Observation
Acetone	Practically insoluble
Benzene	Practically insoluble
Chloroform	Practically insoluble
DMSO	Slightly soluble
NaOH	Very Slightly soluble and Swells
Water	Very Slightly soluble and Swells
Ethanol	Practically insoluble
Methanol	Practically insoluble
Acetonitrite	Practically insoluble
Phosphate Buffer pH 1.2 to pH 7	Practically insoluble

The Physicochemical properties of AACP are as summarized in Table No.8. The swelling index of AACP powder in distilled water was doubles that of in 0.1 N HCl at room temperature. AACP absorb more distilled water (Neutral pH) then 0.1 N HCl (acidic).Water uptake capacity of AACP cellulose powder was more as compared to MCC because of less dense and porous nature of AACP. Melting point was 350 ^{0}C which also confirms its thermal stability shown in results of thermal analysis.

Table No. 8: Physicochemical characterizations of AACP

	Parameters		Observed
c.	Loss on Drying		15±0.01 % w/w
d.	Ash Values	Total Ash	3.15±1.14 % w/w
		Acid Insoluble Ash	2.25±0.07.7 % w/w
		Water-soluble Ash	3.36±0.046 % w/w
e.	Water Uptake	AACP	10.8±1.82 cm
		MCC	7.03±0.246 cm
f.	pH		9.96±0.29
g.	Melting Point		400±4.24 ^{0}C
h.	Swelling Index	Distilled Water	50±0.01 % w/v
		0.1 N HCl	30±0.07 % w/v
i.	Water Retention / Absorption Capacity	Distilled Water	86±0.01 ml
		0.1 N HCl	96.33±3.19 ml

Mean ± SD, n=3

Micromeritic properties

The flow properties of AACP powder were assessed by determining bulk, tapped and true densities, compressibility index, Hausner ratio and angle of repose and shown in Table No.9.The porosity value of sample based on its bulk density and true density was 80.3 % which was due to presence of higher inter-particle space or voids, pores and cracks in the in loose packed powder bed.

Table No.9: Micromeritic properties of AACP

Parameters	Observation
Bulk Density	0.2±0.01g/ml
Tapped Density	0.238± 0.04g/ml
Compressibility Index	37.53±0.48%
Hausner's Ratio	2.38±0.013
Angle of Repose	53^0.95" ±0.715
True Density	0.6078±0.03 g/ml

(Mean ± SD, n=3)

The compressibility index and Hausner ratio values of sample were higher which indicates poor flow also the angle of repose values was $53^0.95^{"}$, which indicatespoor flow of AACP Powder which suggest the requirement of improvement in the flow property of powder by the addition of suitable glidant.

Flow property of powder was improved by decreasing the angle of repose valueby addition of 0.4% w/w of Aerosil as a glidant. Further increase in glidant % increases angle of repose and decrease the flow properties as shown in Table No. 10.

Table No.10: Effect of glidant on flow property of AACP

Sr. No.	Glidant % w/w	Angle of Repose	Remark
1	0	$53^0.95$" ±0.715	Passable
2	0.1	$52^0.93$"±1.655	Passable
3	0.2	$50^0.88$"±1.576	Passable
4	0.3	$47^0.82$"±3.391	Fair
5	0.4	$47^0.33$"±2.695	Fair
6	0.5	$49^0.4$"±2.240	Fair

(Mean ± SD, n=3)

Therefore, AACP with improved flow properties by addition of 0.4% w/w Aerosil as a glidant was used in further Study.

Particle size distribution

The different sizes of AACP particle were observed due to its coarse nature under the 45x magnification. The particle size distribution and mean diameter (dav) expressed in various ways based on different size and frequency indexes shown in Table No. 11.

The Arithmetic mean diameter, Surface length mean diameter (dsm), Volume surface mean diameter (dvm), Weight moment mean diameter (dwm) Surface number mean diameter (dsn) and Volume number mean diameter(dvn) values are 166.1666 µm, 230.877 µm, 271.3676 µm, 262.072 µm, 195.8383 µm and 218.3322 µm respectively indicative of polydispersity and asymmetric nature of AACP

Table No. 11: Representation of particle size distribution of AACP

Size In µm	Mean size (d	No. of particles (n)	Nd	Log d	n Log d	nd2	nd3	nd4	No. less than maximum of size group	% of Particles in each size group	% of particles less than maximum size of group
10-50	30	12	360	1.4771	17.7252	10800	324000	9720000	12	4	4
50-100	75	58	4350	1.8750	108.75	326250	24468750	1835156250	70	19.333	23.333
100-150	125	70	6875	1.0969	76.783	1093750	136718750	17089843750	140	23.333	43.666
150-200	175	61	10675	2.2430	136.823	1868125	326921875	57211328125	201	20.333	66.999
200-250	225	50	11250	2.3521	117.605	2531250	569531250	128144531250	251	16.666	83.665

250 - 300	275	22	6050	2.4393	53.6646	1663750	452540000	124448500000	273	7.333	90.995
300 - 350	325	11	3575	2.5118	27.6298	1161875	377609375	122723046875	284	3.666	94.661
350 - 400	375	7	2625	2.5740	18.018	984375	369140625	138427734375	291	2.333	96.994
400 - 450	425	6	2550	2.6283	15.7698	1083750	460593750	172523437500	297	2	98.994
450 - 500	475	2	950	2.6766	5.3592	451250	214343750	101813281250	299	0.666	99.66
500 - 550	575	1	575	2.7596	2.7596	330625	190109375	109312890625	300	0.333	99.993
Total No. of particles		**∑300**	**49835**		**580.8872**	**11505800**	**312230150000**	**818268376250**			

Conclusion

The study successfully isolated and characterized cellulose powder from *Agave americana*. Pharmacognostic evaluations confirmed its identity and purity, while physicochemical analyses demonstrated its suitability as a pharmaceutical excipient. The cellulose powder exhibited excellent thermal stability, water absorption, and swelling properties, with potential for enhancing drug formulation processes. Further studies on its application in drug delivery systems could solidify its utility in the cosmeceutical and pharmaceutical industries.

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