VOLUME - 11, ISSUE - 01, JANUARY - 2022 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper | Pharmaceutical Science

FORMULATION AND IN-VITRO EVALUATION OF HERBAL TABLET CONTAINING LEAF EXTRACT OF *Jasminum mesnyi FOR THE TREATMENT OF DIABETES MELLITUS*

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ABSTRACT *Jasminum mesnyi hance* also known as primrose jasmine is one of the well- known plant in the field of herbal medicine. It belongs to the family called Oleaceae. It is found in China, Nepal, and India. The drug can be prepared either from whole plant, or from leaves, stem, bark, root, flower, seed etc. The phytochemical derived from *Jasminum mesnyi hance* are Jasminin, Jasmoside, Jasmesoside, Oleuropin, Oleoside, Seccoganin, Sambacoside, Syringin, Alpha-amyrin, manitol, etc. The phytochemical derived from *Jasminum mesnyi hance* can be used to treat much illness such as sunburns, rashes, depression, as well as diabetes. It also show antimicrobial, antioxidant, and wound healing activities.

KEYWORDS : *Jasminum mesnyi hance*; anti-diabetes, antimicrobial; antioxidant; anti-helminthic; wound healing.

1. INTRODUCTION

Genus of Jasminum consists of more than 200 species; most of them are ornamental, while some are used in perfume industry [1]. *Jasminum mesnyi hance* (*Jasminum primulinum*) belongs to family Oleacea, is a native to china but distributed in India and Nepal. It is commonly known as Primrose Jasmine, Unnan-obai in japan [2] found in tropical, sub-tropical and warm temperate regions of Asia. It is an open evergreen, rambling shrub, leaves are opposite, tri-foliate and attached to base of branch lets. Flowers are usually solitary, axillary or rarely terminal, yellow colored, having 6-10 petals [3].Owing to their antioxidant and antimicrobial efficacy, low toxicity and cost, plants are the best source to screen for novel antimicrobial agents with minimal adverse effect that are associated with the currently available antibiotics [4]. Paste of Jasmine flower has been used for treating sunburns, rashes and for wound treatment and also used as an Anti-depressant [5].The crude drug is used in various anti-diabetic formulations like "Pahari Butti" to lower down the blood glucose level especially in Himalayan ranges like in Solan, India [6].

2. MATERIALS AND METHODS

2.1 Collection of Plants: *Jasminum mesnyi hance* leaves were collected in the month of February from Solan, India. The leaves were separated, shade dried, powdered and stored at room temperature in an air tight container till further use.

2.2 Extraction of Plant Extract: Coarsely powdered leaves were defatted with petroleum ether (60-80°C), dried and extracted with 90% methanol using soxhlet apparatus. The plant extract was then dried in rota vacuum evaporator and stored in a desiccator.

2.3 Chemicals And Excipients Used: All the chemicals and excipients were of analytical grade

Table 1: List of Chemicals/ Excipients Used

Sr. No	Chemicals Name	Company Names
1	Starch	Central drug house(P) new Delhi
2	PVP	Central drug house(P) new Delhi
3	Magnesium stearate	SD Fine-chem limited, Mumbai
4	Talc	SD Fine-chem limited, Mumbai
5	Sodium phosphate dibasic heptahydrate	Central drug house(P) new Delhi
6	Sodium phosphate monobasic monohydrate	Central drug house(P) new Delhi
7	Alpha-amylase	Moly-chem, Mumbai
8	Dinitrosalicyclic acid color reagent	Lobachemie pvt.ltd

2.4 Pre-Formulation Studies

2.4.1 Organoleptic Properties: Nature, color, odour, taste of the extracts were determined.

2.4.2 Solubility Studies: The solubility of herbal drug extracts was measured in various solvent. Solubility was determined by talking 10mg of drug sample in 10ml of solvent such as water, methanol, ethanol, 0.1N HCL, phosphate buffer pH 6.8 in small test tubes and well solubilized by shaking. The mixture vials were then kept at 25±1.0°C in an orbital shaker to reach equilibrium. The sample were removed after achieving equilibrium and centrifuged at 3000 rpm for 15 min. The supernatant was taken and filtered through wattman filter paper. The filtrate was solubilized in suitable solvent, diluted and the concentration of drug extracts were determined using UV-Visible spectrophotometer at suitable wavelength.

2.4.3 Fourier Transformation Infrared Spectrophotometer (FTIR): The FTIR studies were carried for the drug, the polymers and the drug-polymer physical mixture in the ratio 1:1 were mixed separately with IR grade KBr in the ratio of (100:1) and corresponding discs were prepared by applying 5.5 metric ton of pressure in a hydraulic press using FTIR spectrophotometer. The disks were scanned over a wave number range (4000-400 cm).

2.4.4 Maximum Wavelength of *Jasminum Mesnyi hance*: Stock solution (1000 µg/ml) was prepared in 90% methanol. This solution was diluted with same solvent to obtain concentration of 100µg/ml.

The resultant solution was scanned in the range of 200-800 nm on double beam UV-spectrophotometer. The resultant was plotted in chart in the range of 200-800nm.

2.4.5 Preparation of Standard Plots

2.4.5.1 Preparation of Standard Plot of *Jasminum mesnyi hance* In 6.8 Phosphate Buffer: 100 mg of *Jasminum mesnyi hance* extract was weighed accurately and transferred into 100 ml volumetric flask. 100ml 6.8 phosphate buffer solution was added in volumetric flask to make-up the volume. This gives a concentration of 1000μg/ml to stock solution. This stock was again diluted to 10 times by diluting 1ml of stock solution with 10ml of 6.8 phosphate buffer solutions. From the above stock solution 1, 2, 3, 4, 5ml, was pipette out and diluted to 10ml give final concentration of 10, 20, 30, 40, and 50μg/ml respectively. Measured the absorbance of prepared samples at 375nm wavelength and plotted the graph by taking concentration (μg/ml) on X-axis and absorbance on Y-axis.

2.5 Evaluation of *In-Vitro* Anti-diabetic activity of Extract

2.5.1 α-Amylase Inhibition Assay: Appropriate dilution of extract (50μl) and (500μl) of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing Hog pancreatic □-amylase were incubated at 25°C for 10 minutes. Then 500μl of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube. The reaction mixture was incubated at 25°C for 10 minutes and stopped with 1ml of dinitosalicylic acid color reagent. Then the mixture was incubated in a boiling water bath for 5 minutes and cooled to room temperature. The reaction mixture was then diluted by adding 10ml of distilled water and absorbance measured at 540 nm in a spectrophotometer. The results were expressed as % enzyme inhibition using the formula:

$$\text{Inibition}(\%) = (\text{Abs}_{\text{ref}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{ref}} \times 100$$

$\text{Abs}_{\text{ref.}}$ Abs without sample,
$\text{Abs}_{\text{sample.}}$ Abs of extracts [7].

2.6 Formulation of Herbal Tablet: Various batches of tablet *Jasminum mesnyi hance* were prepared by direct compression technique with each batch containing 50 tablets with 1000mg of drugs. All the ingredients were thoroughly mixed. Then the powder was passed through sieve mesh #20 to get uniform size of particles. Then it was lubricated by adding magnesium stearate and talc. The above powder was compressed with the help of tablet punch machine, by keeping average weight 1.15g. After compression the tablet were evaluated for weight variation, hardness, thickness, friability, disintegration and dissolution. The composition of each formulation is given in Table 2.

Table 2: Formulation of various batches of Tablets (F1-F4)

Formulation code	Plant extract (mg)	Starch (mg)	PVP (mg)	Mg stearate (mg)	Talc (mg)
F1	1000	160	30	5	5
F2	1000	150	40	5	5
F3	1000	140	50	5	5
F4	1000	130	60	5	5

Figure 1: Tablet of *Jasminum mesnyi hance*

2.7 Pre-Compression Studies

2.7.1 Bulk Density

The powder sample under test equivalent to 10gm was accurately weighed in a 50ml graduated cylinder. Powder was leveled and the unsettled volume, VO was noted. The bulk density was calculated in g/cm3 by the formula:

$$\text{Bulk Density } (\rho 0) = M/V0 \text{ [8]}$$

Where, M= mass of powder taken.
V0= apparent unsettled volume.

2.7.2 Tapped Density

The powder sample under test equivalent to 10g was filled in 50ml graduated cylinder. The mechanical tapping of the cylinder was carried out using tapped density apparatus at a constant rate according to pharmacopoeia. Volume was considered as tapped volume Vf. The tapped density was calculated in g/cm3 by the formula,

$$\text{Tapped Density } (\rho t) = M/Vf \text{ [9]}$$

Where, M=mass of powder taken.
Vf = tapped density.

2.7.3 Angle of Repose

Angle of repose was determined by using funnel method. The accurately weighed blend was taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touches the apex of the heap or head of blend. The drug excipient blend was allowed to flow through the funnel freely on the surface. The diameter of the powder cone was measured and angle of repose was calculated using the equation:

$$\text{Tan}\Theta = h/r \text{ [10]}$$

Where, h =height of pile.R = radius of pile.
Relationship between the angle of repose and powder flow was determined as per pharmacopoeial standards.

2.7.4 Compressibility Index (Carr´S Index) [9]

Based on the bulk density and tapped density, the % compressibility index of the granules was computed using the equation:

$$\text{Compressibility (Car´s) Index} = \text{Tapped Density-Bulk Density/ Tapped Density} \times 100$$

2.7.5 Hausner´s Ratio

It was determined using the formula:

$$\text{Hausner´s Ratio: Tapped Density/Bulk Density}$$

2.8 Post-Compression Studies

2.8.1 General Appearance: The general appearance of all tablets, its visual identity and overall elegance is essential for consumer acceptance. The formulated tablets were evaluated for size, shape. The diameter and thickness of the tablet were measured by vernier calipers [11].

2.8.2 Weight Variation: The weight variation test was run by weighing 20 tablets individually and compared individual weight to average weight. The tablets meet the USP test, if no more than two tablets are outside the percentage limit and if no tablet differs by more than 2 times the percentage limit [11].

2.8.3 Hardness: Hardness indicates the ability of a tablet to withstand mechanical shocks while handling randomly 5 tablets to be tested were held between a fixed and a moving jaw of hardness test apparatus (Monsanto) and reading of the indicator was adjusted to zero. The screw knob was moved forward until the tablet breaks and the force required breaking the tablet was noted. Average hardness was determined [12].

2.8.4 Friability: Friability test was performed using Roche friabilator. Ten tablets were weighed and placed in the friabilator, which was then operated for 25 revolutions per minute. After 100 revolutions the tablets was dusted and reweighed. The percentage friability was determined using the formula [12].

$$\text{Percentage Friability} = \text{Initial Weight-Final Weight/Initial Weight} \times 100$$

2.8.5 Disintegration Test for Tablets: The disintegration test was performed using disintegrating apparatus. One tablet was placed in each of the six tubes of the basket and the apparatus was maintained at 37±0.50°C of the immersion liquid (phosphate buffer (pH6.8)). The time required for complete disintegration of tablet was noted. The tablets are disintegrated when no particles remain above the gauge, when readily has passed through 10# mesh screen.

2.8.6 *In-Vitro* Dissolution Studies: dissolution studies of formulation was carried out using USP type 2 dissolution apparatus for 1 hours in 900ml of phosphate buffer(pH 6.8) at 100 rpm maintaining the temperature at 37±0.5°C. Sample of 5ml of each were collected over a period of 1hr at an interval of every 5min. the withdrawn sample was immediately replaced by equal volume of fresh buffer. Collected samples were analyzed spectrophotometrically on a UV-Visivle spectrop hotometer (Systronics double beam) at measured wavelength of *Jasminum mesnyi hance* and cumulative percent drug release was calculated. A plot of cumulative % drug release v/s time in min was plotted [12].

3. RESULT AND DISCUSSION

3.1 Pre-Formulation Studies

3.1.1 Organoleptic Properties: The observed organoleptic properties are summarized in Table 3.

Table 3: Organoleptic Properties of Extract

Nature	Amorphous
Color	Greenish
Odour	Characteristic
Taste	Bitter

3.1.2 Solubility studies: Solubility of J*asminum mesnyi hance:* phosphate buffer > methanol > distilled water > 0.1HCl maximum solubility was found to be in phosphate buffer pH 6.8.

Table 4: Solubility profile of *Jasminum mesnyi hance*

Sr No	Solution	Solubility(µg/ml)
1	Distilled Water	484.05
2	0.1N HCl	401.42
3	Methanol	515.31
4	Phosphate Buffer 6.8	550.52

3.1.3 Fourier Transfer Infrared (FTIR) Studies: Plant extract-excipients compatibility studies were confirmed by carrying out FTIR studies. There was none of the extra peak found in the graph. Thus, FTIR studies showed that there was no plant extract-excipient interaction.

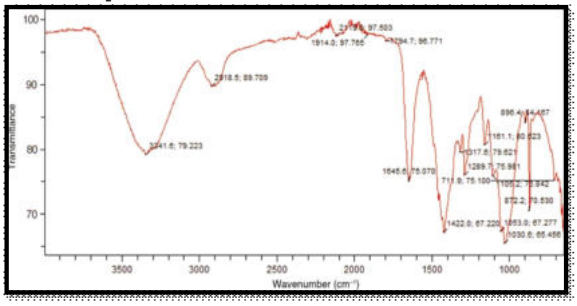

Figure 2: FTIR Spectra Data of *Jasminum mesnyi hance*

Table 5: FTIR Spectra Data of *Jasminum mesnyi hance*

Sr No	Vibration Frequency cm^{1}	Major Peak cm^{1}	Molecular Vibration
1	3357	3600-3200	O-H stretching
2	2929	3600-2500	Carboxylic acid OH stretching
3	1626	1680-1600	C=C alkene
4	1380	1400-1300	NO_2 stretching
5	1022	1400-1300	C-OH stretching

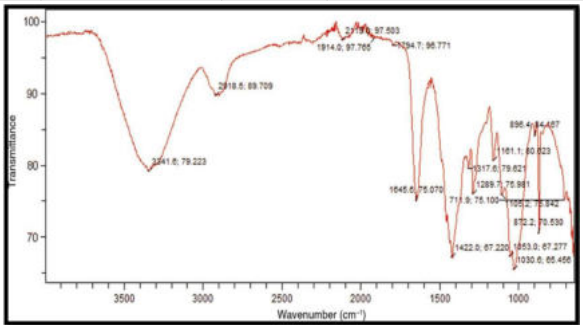

Figure 3: FTIR Spectra of PVP

Table 6: FTIR Spectral Data of PVP

Sr No	Vibration Frequancy Cm^{1}	Major Peak Cm^{1}	Molecular Vibration
1	3341	3600-3200	O-H Stretching
2	2918	2950-2840	C-H Stretching
3	1645	1680-1600	C=C alkene
4	1422	1480-1440	CH_2 bend
5	1317	1400-1300	NO_2 Stretching
6	1030	1200-1020	C-OH Stretching

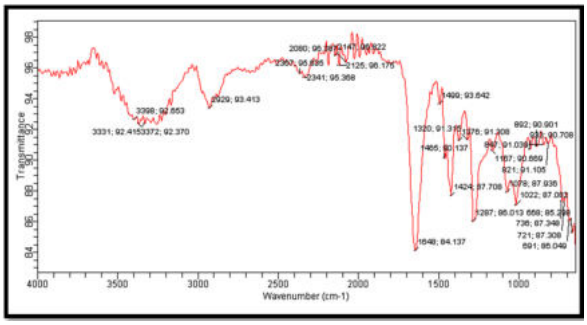

Figure 4: FTIR of Plants Extract And Excipient

3.1.4 Determination Of Maximum Wavelength: Maximum wavelength (λmax) of Jasminum mesnyi hance was found to be 375 nm.

3.1.5 Standard Plot of *Jasminum mesnyi hance*:

Table 7: Absorbance of extract of Jasminum mesnyi hance at Different Concentrations

Concentration(µg/ml)	Absorbance
10	0.069
20	0.089
30	0.096
40	0.132
50	0.140

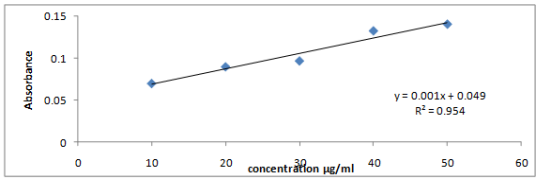

Figure 5: Standard Plot of *Jasminum mesnyi hance* in Phosphate Buffer pH 6.8

3.2 Evaluation of *In-Vitro* Anti-diabetic activity of Extract

3.2.1 α –Amylase Inhibition Assay: From the study, it was concluded that the *Jasminum mesnyi hance* extract showed α-amylase inhibition at 500µg/ml conc. It was compared with standard Acarbose 500µg/ml as shown in Table 8. From the results, we concluded that we can use *Jasminum mesnyi*

hance extract in diabetes treatment as it showed maximum inhibition of α-amylase.

Table 8: % Inhibition by Jasminum mesnyi hance & Acarbose at Different Concentration

Sr No	Concentration (µg/ml)	% Inhibition by *Jasminum mesnyi hance* Extract	% Inhibition by Acarbose
1	300	21.05	30.5
2	350	25	45.7
3	400	28.29	61.3
4	450	64.82	77.5
5	500	86.13	91

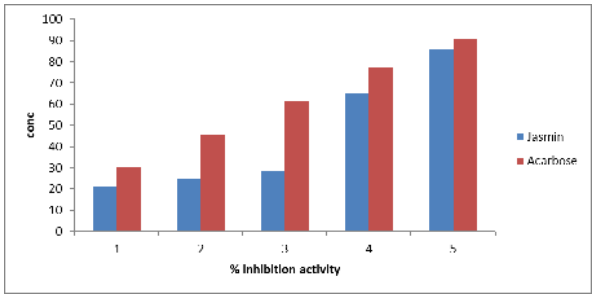

Figure 6: Comparison Of α-Amylase Inhibition Activity of *Jasminum mesnyi hance* & Acarbose

3.3 Evaluation of Pre-Compression Parameters:

3.3.1 Angle of repose: Angle of repose was determined by fixed funnel method. Angle of repose was found to be in range of 27.47°-33.80° for different powder blend batches, which indicates good powder flow. Results are shown in Table 9.

3.3.2 Bulk and Tapped Density: Pre-compressional blend was evaluated for bilk and tapped density by using tapped density. Bulk and tapped density was found to be 0.41-0.46gm/ml and 0.50-0.54gm/ml shown in Table 9. This shows good repacking ability of powder blend.

3.3.3 Carr´s Index and Hausner´s Ratio: Carr´s index and hausner´s ratio was calculated from bulk and tapped density. The carr´s index of the ingredients was found to be in the range of 8.00-17.64% and hausner´s ratio in the range of 1.08-1.31. These findings proves that the composition of ingredients for compression possess good compression property and good flow property. Results are shown in Table 9.

Table 9: Evaluation of Physical Properties of Powder Blend of All Formulation

Formulation Code	Angle Of Repose (Θ)	Bulk Density (gm/Ml)	Tapped Density (gm/Ml)	Carr´s Index (%)	Hausner´s Ratio
F1	30.96	0.45	0.52	13.46	1.15
F2	27.47	0.41	0.54	15.07	1.31
F3	33.80	0.42	0.51	17.64	1.11
F4	32.2	0.46	0.50	8.00	1.08

3.4 Post formulation studies

3.4.1 General Appearance: The formulated tablets were evaluated for general appearance such as color, size and shape and are summarized in Table 10.

Table 10: Size, Shape and Color of Different Formulations (F1-F4)

Formulation Code	Size (Cm)	Shape	Color
F1	1.5	Round	greenish
F2	1.5	Round	Greenish
F3	1.5	Round	Greenish
F4	1.5	round	greenish

3.4.2 Post Compression Parameters: The formulated tablets were evaluated for general appearance such as color, size and shape and are summarized in Table 10.

Table 11: Post Compression Parameters of Different Formulations (F1-F4)

Formulation Code	Wight Variation (%)	Hardness (Kg/Cm^2)	Thickness (mm^2)	Friability (%)	Disintegration Time
F1	2.71	4.0	3.4	0.87	10min 35sec
F2	2.21	4.0	3.6	0.76	13min 30sec
F3	2.60	4.1	3.7	0.79	11min 25sec
F4	2.51	4.2	3.5	0.69	9min 50sec

3.4.3 *In-Vitro* Dissolution Studies: Dissolution studies of formulation were carried out and collected samples were analyzed spectrophotometrically on a UV-Visible spectrop hotometer (Systronics double beam) at measured wavelength of *Jasminum mesnyi hance* and cumulative percent drug release was calculated. A plot of cumulative % drug release v/s time in min was plotted. Comparative study of different formulation (F1-F4) is shown in the Figure 7. The in-vitro release of formulation F4 was found to be maximum.

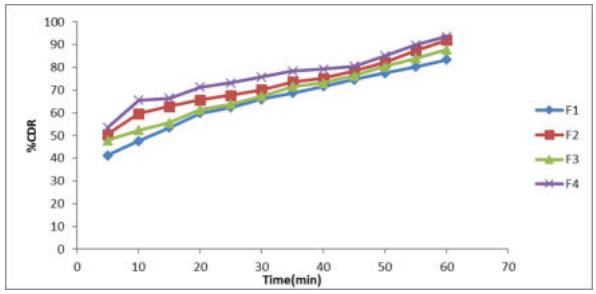

Figure 7: Comparison of % CDR of Different Formulation (F1-F4)

3.4.4 Analysis Of Drug Release Mechanism: Regression coefficient of *Jasminum mesnyi hance* for zero order release plot was found to be 0.9462, for Higuchi plot was found to be 0.9605 and for Korsmeyer-Pappas model was found to be 0.9582. Thus, from the results the formulation showed Higuchi release kinetics. Thus formulated tablet follows Higuchi release rate:

Table 12: Results of Model Fitting of Formulation F4

Model	Parameter	F4
Zero Order Plot	Slop	0.5993
	Intercept	56.541
	R^2	0.9462
Higuchi Plot	Slop	6.234
	Intercept	42.052
	R^2	0.9605
Kosermeyer-Peppas Plot	Slop	0.1982
	Intercept	1.594
	R^2	0.9582

4. CONCLUSION:

Herbal tablet was formulated containing *Jasminum mesnyi hance* for the treatment of diabetes mellitus. Herbal plant extract showed α-amylase inhibition activity. F4 formulation showed the best result among all the formulations. *In-vitro* release profile indicated that there was Higuchi release of optimized formulation.

5. Acknowledgement

We acknowledge School of Pharmacy, Abhilashi University and Himalayan Institute of Pharmacy, Kala- Amb for supporting the research work.

6. CONFLICT OF INTEREST

There is no Conflict of interest.

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