International Journal of Advances in Pharmaceutics
E-ISSN: 2320-4923; P-ISSN: 2320-4931
Journal DOI: https://dx.doi.org/10.7439/ijap
Journal home page: http://ssjournals.com/index.php/ijap **Research Article**

Formulation and evaluation of herbal tablets containing *Agaricus bisporus* powder

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***Article History:**
Received: 08/02/2017
Revised: 18/02/2017
Accepted: 20/02/2017
DOI: https://dx.doi.org/10.7439/ijap.v6i2.3933

Abstract

Agaricus bisporus is an edible mushroom known for its nutritional and bio-medicinal properties. The methanolic extract of *Agaricus bisporus* for phytochemical screening performed and Alkaloids, Carbohydrate, Glycosides, Protein, Flavonoids, Saponins, Phenolic, Steroids. Some of them are responsible for anti-diabetic activities. In the present study oral administrable dosage form of herbal tablet and evaluated. The result of all test were found to be satisfactory.
Keywords: *Agaricus bisporus*, phytochemical screening, herbal formulation, Anti-diabetic activity.

1. Introduction

Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all culture throughout history. It was an integral part of the development of modern civilization. Herbal medicinal products are defined as any medicinal products, exclusively containing one or more active substances. WHO report 80% of the world population relies on the drug from natural origin. A number of traditional herbal medical practices have been adopted for the diagnosis, prevention and treatment of various diseases. The objective of development of herbal formulation is to provide the synergistic, potentiated, agonistic/ antagonistic pharmacological agents within themselves and work together in a dynamic way to produce maximum therapeutic efficacy with minimum side effects [1]. Therefore treating diabetes mellitus with Ayurvedic medicines which are accessible and do not require laborious pharmaceutical synthesis seems highly attractive. Although oral hypoglycemic agents and insulin is the main stay of treatment of diabetes and are effective in controlling hyperglycemia [2]. Mushrooms have been reported to have hypoglycemic effects and anti hyperglycemic effects. Mushrooms are known to contain compounds which help in proper functioning to the liver, pancrease and other endocrinal gland there by promoting formation of insulin and related hormones which ensure healthy metabolic functioning. Polysaccharides, such as β-glucanscantained in mushrooms have the ability to restore the function of pancreatic tissues by causing increased insulin output by beta-cells which leads to lowering of blood glucose levels [3]. The oral rout of drug administration is the most important method of administrating drugs for systemic effects. Ayurvedic herbal formulations were also administered preferentially by oral route. Designing of oral herbal formulations is till date a challenge in modern pharmaceutics [4].

2. Material & method

2.1 Materials

Agaricus bisporus obtained from local market and authenticated by taxonomist. All chemicals used were of analytical grade. Glucose analyzer and strips, glibenclamide were purchased from local market.

2.2 Methods

2.2.1 Extraction Process-

The preliminary phytochemical screening of the sample involves extraction of the material and identification of the active constituent.[5.6]

2.2.2 Method for extraction

Continuous hot process by using soxhlet apparatus. Following materials are used- Soxhlet apparatus; Methanol; Distilled water; Shade dried coarse powder of *Agaricus bisporus*

Dried powder of *Agaricus bisporus* was taken in conical flask filterd with condenser and methanol was added in the ratio of (1:10). This mixture was heated on water bath for 4 hours and extract was filtered through Whatman No. filter paper. This extract was cooled at room temperature and then allowed to stand overnight foe methanol to evaporate [7]. The resulting phytochemical screening of the sample is show in table-1.

2.3 Preparation of stock solution

Standard stock solution of extract was prepared by dissolving 100 mg of powder of *Agaricus bisporus* in 100 ml methanol in 100 ml volumetric flask & filterd [8].

2.4 Preparation of working standard solution and concentration of standard graph

To construct Beer's law plot for extract, the stock solution was further used to prepare working standard of concentrations ranging from 2 to 10µg/ml different aliquots of working standard solution of extract was transferred separately into a series of 10ml volumetric flask and diluted to 10ml using methanol. The absorbances were measured at $\lambda_{max\ 271nm}$ against methanol as blank. The standard graph for extract was plotted by taking concentration of the extract on x-axis & y-axis at 271nm.

2.5 Formulation

2.5.1 Preparation of Tablet

Herbal tablets prepared by direct compression method all the Formulation ingredients mentioned in table-3 were weighed according and mixed in a mortar and pestle. This powder blend was then allowed to dry for few moments and then again mixed well and passed through sieve no. 60. Blend was compressed by punching machine [9].

Table 3: Composition of Tablets

S. No	Name of ingredients	Quantity taken						
		F_1	F_2	F_3	F_4	F_5	F_6	F_7
1.	*A. bisporus* powder	200mg	200mg	200mg	200mg	200mg	200mg	200mg
2.	Methyl cellulose	180mg	180mg	180mg	180mg	180mg	180mg	180mg
3.	Lactose	40mg	50mg	60mg	80mg	80mg	100mg	100mg
4.	Magnesium stearate	10mg	10mg	10mg	10mg	20mg	20mg	30mg
	Theoretical weight	430	440	450	470	480	500	510

2.6 Evaluation of tablets

All the formulated tablets were subjected to following evaluation parameters.

2.6.1 Weight variation test-

Every individual tablets in a batch should be in uniform weight and weight variation in within permissible limits. By randomly selecting and weighing 20 tablets, "the average weight was determined"

2.6.2 Hardness & friability

For each formulation, the hardness and friability of 20 tablets each were determined using the Pfizer hardness tester and Electro lab friabilator test apparatus, respectively.

2.6.3 Thickness

The thickness of the tablets was determined using Vernier calipers, 20 tablets from each batch were used and average values were calculated.

2.6.4 Disintegration time

Six tablets were placed in the tubes along with a plastic disk over the tablets. The disk imparts pressure on the tablets. The tubes were allowed to move up and down in the media as 29-32 cycle per minute in water media maintained at 370C. Time required to pass all tablets through the mesh was determined as its disintegration time [9,10]. The resulting parameters of tablets of a formulation is show in table 4.

2.6.5 Dissolution rate study of tablets

Dissolution rate of tablets prepared was studied in 0.1M HCl (900ml) solution employing USB 8 station Dissolution Rate Test Apparatus with a paddle stirrer at 50 rpm. One tablet containing 200mg was used in each test. A temperature 370C was maintained throughout. Samples of dissolution medium (5ml) were withdrawn through a filter at different time intervals and assayed for sample at 271 nm. The resulting dissolution rate study of all formulation (F1, F2, F3, F4, F5, F6, F7) are following.[11]

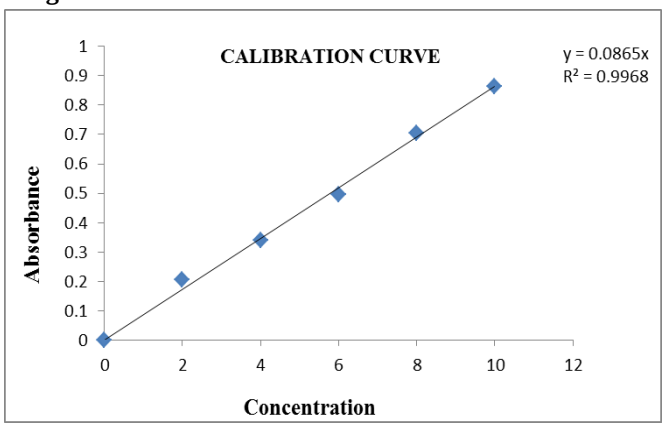
3. Results

Table 1: Phytochemical analysis of *Agaricus bisporus*

S. No	Phytoconstituents	Biochemical Test	Result
1.	Alkaloids	Mayer's Test	+
		Wagner's Test	+
2.	Carbohydrates	Molisch's Test	+
		Fehling's Test	-
		Bendict's Test	+
3.	Glycosides	Legal's Test	+
		Keller-Killani Test	+
4.	Protien	Millon's Test	+
5.	Flavanoids	Alkaline Reagent	+
6.	Saponins	Forthing Test	+
7.	Phenolic	Lead acetate Test	+
8.	Steroids	Salkowski Test	+
9.	Triterpenoids	Salkowski Test	-

Note * (+) Presence & (-) Absence

Fig 1: Standard curve for the estimation in methanol

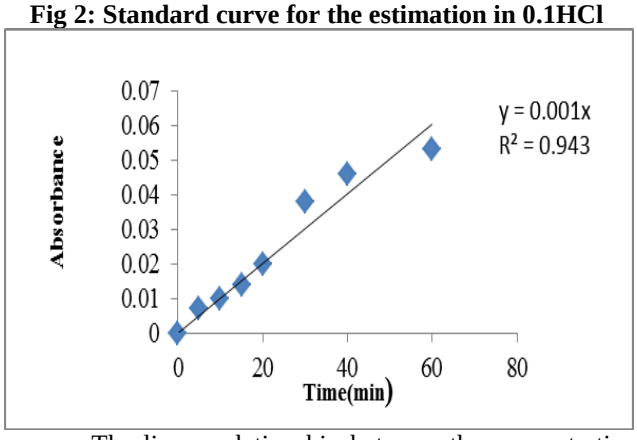


The linear relationship between the concentration of extract and the corresponding absorbance values was shown by-

y= 0.0865x.

A positive correlation between the concentration of extract and corresponding absorbance values was observed (correlation coefficient, r=0.996). The amount of extract in all the formulation was calculated using the linear relationship as given above or directly from the standard graph as show in Fig.1.

Fig 2: Standard curve for the estimation in 0.1HCl



The linear relationship between the concentration of extract and the corresponding absorbance values was shown by-

y= 0.001x.

A positive correlation between the concentration of extract and corresponding absorbance values was observed(correlation coefficient r=0.94) The amount of extract in all the formulation was calculated using the linear relationship as given above or directly from the standard graph as show in Fig.2.

Table 4: Evaluation of tablets

S.N	Parameter	Tablets						
		F_1	F_2	F_3	F_4	F_5	F_6	F_7
1.	Weight variation	429±1.732	435.6±4.35	451±3.338	470.3±0.57	481±1.73	495.6±7.50	509±0.005
2.	Thickness	3.86±0.01	4.00±0.005	3.56±0.005	4.01±0.013	3.79±0.005	3.66±0.023	3.96±0.001
3.	Friability	0.45±0.005	0.40±0.011	0.46±0.005	0.41±0.011	0.43±0.017	0.35±0.051	0.36±0.062
4.	Hardness	2.96±0.05	3.3±0.17	3.0±0.28	3.06±0.23	3.5±0.113	3.13±0.011	3.13±0.011
5	D.Time	5.32±0.011	5.42±0.005	5.42±0.005	5.38±0.011	5.33±0.011	5.54±0.39	5.45±0.005

3.1 Dissolution test

Formution-1

Time (min)	Absorbance	Conc (ppm)	Conc (mcg/ml)×DF	Amt in 900 ml	% DR	Log % DR	% DRE	Log % DRE
0	0	0	0	0	0	-	100	2
5	0.005	5	0.025	22.5	11.25	1.051153	88.75	1.948168
10	0.009	9	0.045	40.5	20.25	1.306425	79.75	1.901731
15	0.013	13	0.065	58.5	29.25	1.466126	70.75	1.849726
20	0.018	18	0.09	81	40.5	1.607455	59.5	1.774517
30	0.023	23	0.115	103.5	51.75	1.71391	48.25	1.683497
40	0.03	30	0.15	135	67.5	1.829304	32.5	1.511883
50	0.038	38	0.19	171	85.5	1.931966	14.5	1.161368
60	0.042	42	0.21	189	94.5	1.975432	5.5	0.740363

Fig 3: Drug release in Zero order

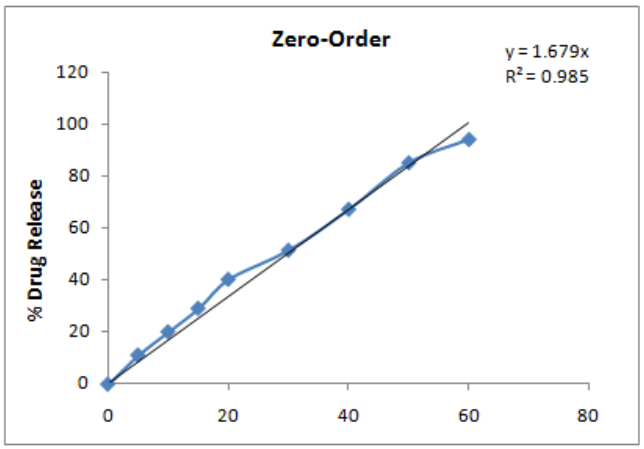
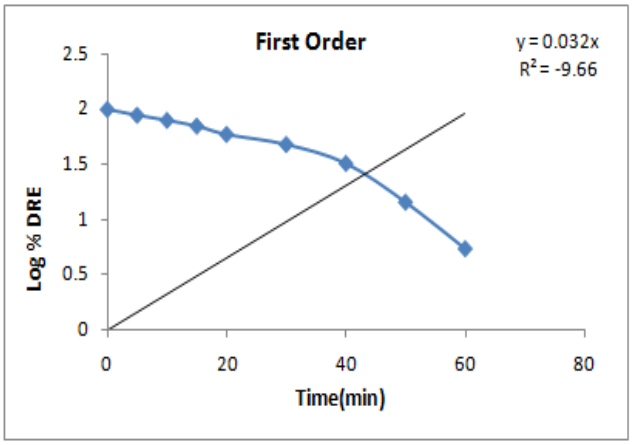


Fig 4: Drug release in First order



Formulation F_2

Time (min)	Absorbance	Conc (ppm)	Conc (mcg/ml)×DF	Amt in 900 ml	%DR	Log%DR	%DRE	Log% DRE
0	0	0	0	0	0	-	100	2
5	0.005	5	0.025	22.5	11.25	1.051153	88.75	1.948168
10	0.009	9	0.045	40.5	20.25	1.306425	79.75	1.901731
15	0.013	13	0.065	58.5	29.25	1.466126	70.75	1.849726
20	0.018	18	0.09	81	40.5	1.607455	59.5	1.774517
30	0.023	23	0.115	103.5	51.75	1.71391	48.25	1.683497
40	0.03	30	0.15	135	67.5	1.829304	32.5	1.511883
50	0.038	38	0.19	171	85.5	1.931966	14.5	1.161368
60	0.042	42	0.21	189	94.5	1.975432	5.5	0.740363

Fig 5: Drug release in Zero order

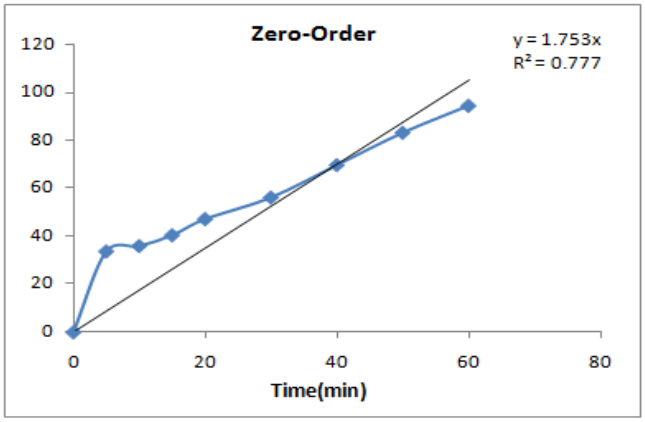
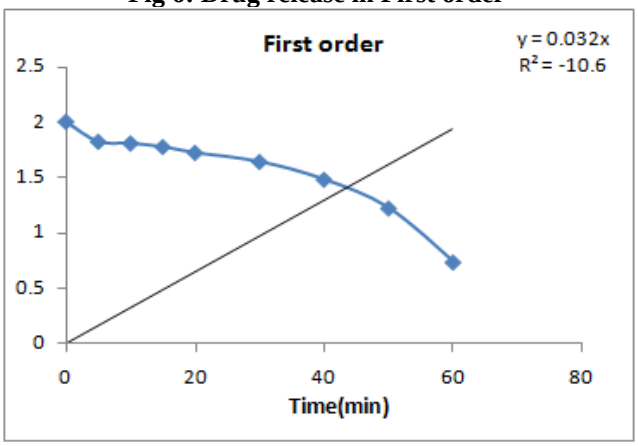


Fig 6: Drug release in First order



Formulation F_3

Time (min)	Absorbance	Conc (ppm)	Conc (mcg/ml)×DF	Amt in 900 ml	%DR	Log%DR	%DRE	Log% DRE
0	0	0	0	0	0	-	100	2
5	0.001	1	0.005	4.5	2.25	0.352183	97.75	1.990117
10	0.003	3	0.015	13.5	6.75	0.829304	93.25	1.969649
15	0.008	8	0.04	36	18	1.255273	82	1.913814
35	0.015	15	0.075	67.5	33.75	1.528274	66.25	1.821186
45	0.024	24	0.12	108	54	1.732394	46	1.662758
60	0.032	32	0.16	144	72	1.857332	28	1.447158

Fig 7: Drug release in Zero order

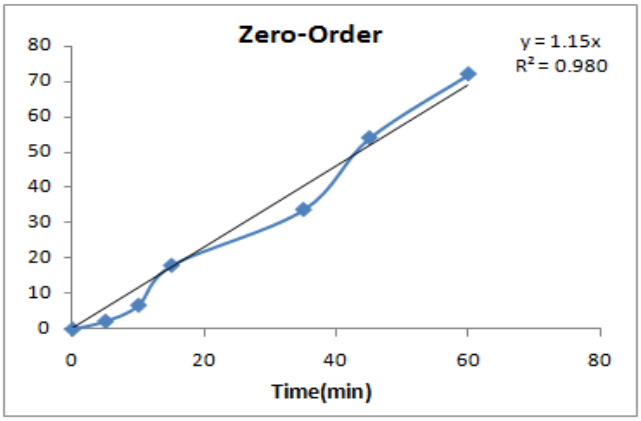
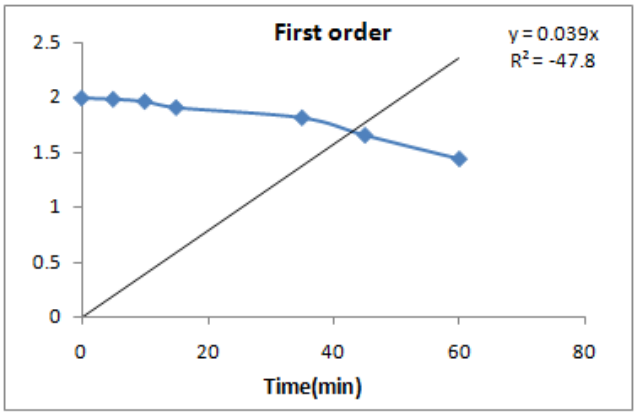


Fig 8: Drug release in First order



Formulation F_4

Time (min)	Absorbance	Conc(ppm)	Conc(mcg/ml) ×DF	Amt in 900 ml	%DR	Log%DR	%DRE	Log% DRE
0	0	0	0	0	0	-	100	2
5	0.019	19	0.095	85.5	42.75	1.630936	57.25	1.757775
10	0.021	21	0.105	94.5	47.25	1.674402	52.75	1.722222
15	0.025	25	0.125	112.5	56.25	1.750123	43.75	1.640978
25	0.029	29	0.145	130.5	65.25	1.814581	34.75	1.540955
35	0.034	34	0.17	153	76.5	1.883661	23.5	1.371068
45	0.039	39	0.195	175.5	87.75	1.943247	12.25	1.088136
60	0.044	44	0.22	198	99	1.995635	1	0

Fig 9: Drug release in Zero order

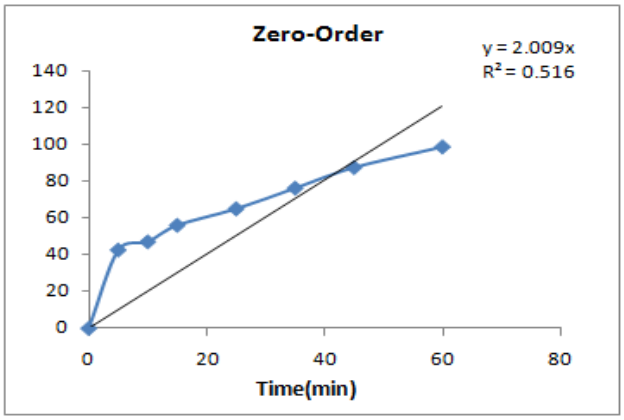
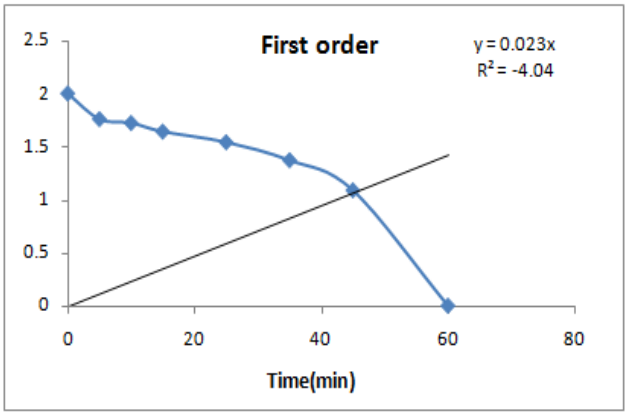


Fig 10: Drug release in First order



Formulation-5

Time (min)	Absorbance	Conc (ppm)	Conc (mcg/ml)×DF	Amt in 900 ml	%DR	Log%DR	%DRE	Log% DRE
0	0	0	0	0	0	-	100	2
5	0.002	2	0.01	9	4.5	0.653213	95.5	1.980003
10	0.005	5	0.025	22.5	11.25	1.051153	88.75	1.948168
15	0.009	9	0.045	40.5	20.25	1.306425	79.75	1.901731
20	0.014	14	0.07	63	31.5	1.498311	68.5	1.835691
30	0.021	21	0.105	94.5	47.25	1.674402	52.75	1.722222
40	0.03	30	0.15	135	67.5	1.829304	32.5	1.511883
50	0.038	38	0.19	171	85.5	1.931966	14.5	1.161368
60	0.043	43	0.215	193.5	96.75	1.985651	3.25	0.511883

Fig 11: Drug release in Zero order

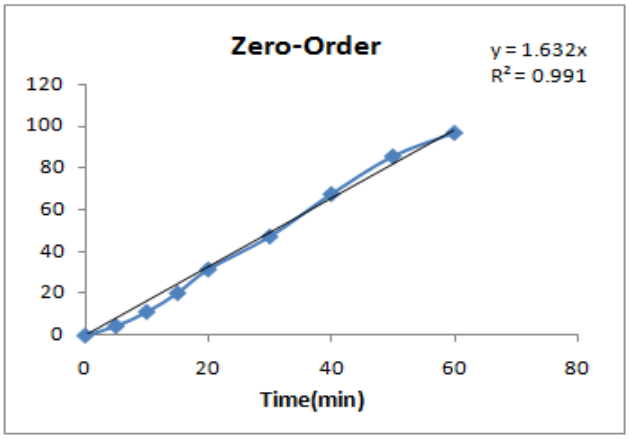
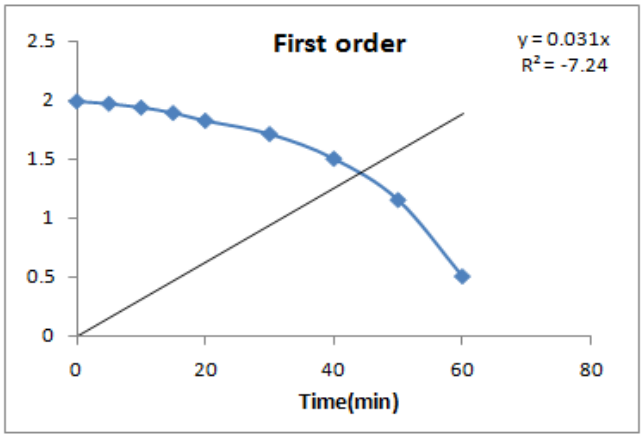


Fig 12: Drug release in First order



Formulation F_6

Time (min)	Absorbance	Conc (ppm)	Conc (mcg/ml)×DF	Amt in 900 ml	%DR	Log%DR	%DRE	Log% DRE
0	0	0	0	0	0	-	100	2
5	0.007	7	0.035	31.5	15.75	1.197281	84.25	1.92557
10	0.009	9	0.045	40.5	20.25	1.306425	79.75	1.901731
15	0.011	11	0.055	49.5	24.75	1.393575	75.25	1.876507
20	0.014	14	0.07	63	31.5	1.498311	68.5	1.835691
30	0.018	18	0.09	81	40.5	1.607455	59.5	1.774517
40	0.025	25	0.125	112.5	56.25	1.750123	43.75	1.640978
50	0.032	32	0.16	144	72	1.857332	28	1.447158
60	0.041	41	0.205	184.5	92.25	1.964966	7.75	0.889302

Fig 13: Drug release in Zero order

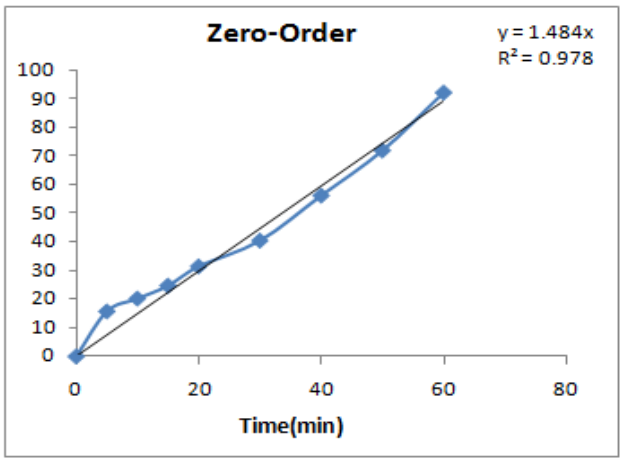
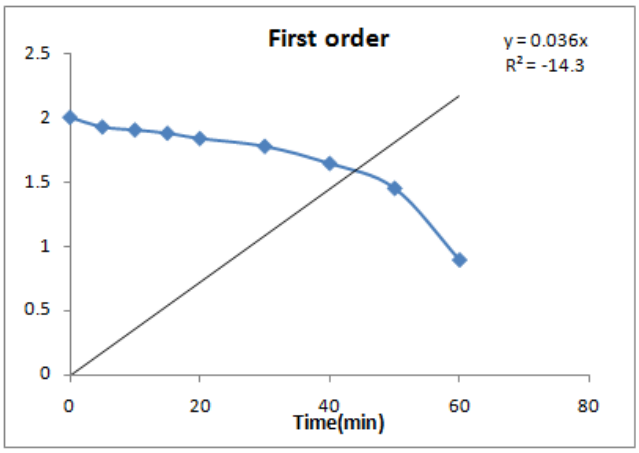


Fig 14: Drug release in First order



Formulation F_7-

Time (min)	Absorbance	Conc(ppm)	Conc(mcg/ml)×DF	Amt in 900 ml	%DR	Log%DR	%DRE	Log% DRE
0	0	0	0	0	0	-	100	2
5	0.007	7	0.035	31.5	15.75	1.197281	84.25	1.92557
10	0.01	10	0.05	45	22.5	1.352183	77.5	1.889302
15	0.014	14	0.07	63	31.5	1.498311	68.5	1.835691
20	0.02	20	0.1	90	45	1.653213	55	1.740363
30	0.025	25	0.125	112.5	56.25	1.750123	43.75	1.640978
40	0.032	32	0.16	144	72	1.857332	28	1.447158
50	0.039	39	0.195	175.5	87.75	1.943247	12.25	1.088136
60	0.043	43	0.215	193.5	96.75	1.985651	3.25	0.511883

Fig 15: Drug release in Zero order

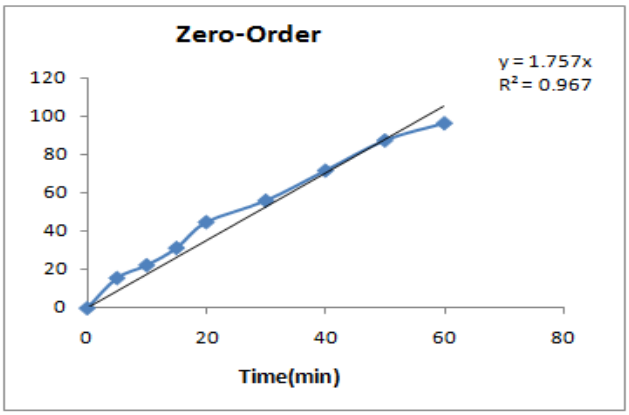
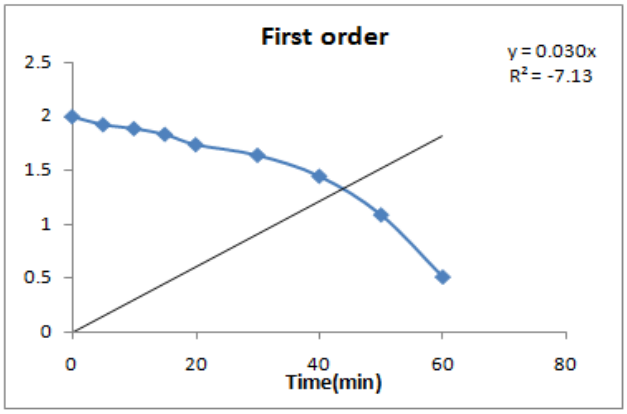


Fig 16: Drug release in First order



4. Discussion

Based on the result obtained from the present study it can be concluded *Agaricus bisporus* have the anti diabetic activity. The result of preliminary phytochemical analysis are identify the bioactive compound viz alkaloids, carbohydrate, glycoside, protein, Flavonoids, Saponins, Phenolic, steroids and they are reported to have a wide range of pharmaceutical properties, such as anti-diabetic effects. Oral herbal dosage form of *Agaricus bisporus* like tablet showed good elegance. The herbal tablets were prepared by direct compression method. Tablets were prepared using Methyl cellulose & Lactose was used as a binder in varying concentration & magnesium stearate as lubricant. Seven batches of the tablets were prepared & micromeritic, properties were determined for all physical mixture of *Agaricus bisporus*. The physical properties of all tablets were determined.

The results of the Uniformity of weight, Hardness, Friability, Disintegration time and Dissolution rate was found all batches acceptable but batch 7 are the optimized.

5. Conclusion

Based on the results obtained from the present study, it can be concluded that the Agaricus bisporus had anti-diabetic activity. The herbal formulation of Agaricus bisporus as tablets were no noticeable changes physicochemical parameters when stability studied were performed at different temperature, indicating that developed the herbal formulation in tablets form are stable and acceptable & make importance in phytopharmaceutical.

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