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Original Research Article

EXTRACTION, PHYTOCHEMICAL SCREENING AND FORMULATION OF HERBAL TABLETS FOR EFFECTIVE ANTI-DIABETIC EFFECT

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ABSTRACT

This study aimed to extract bioactive compounds from *Withania coagulans* and formulate herbal tablets for the potential management of diabetes. *Withania coagulans* leaves were subjected to sequential extraction using various solvents, and the obtained extracts were screened for phytochemical constituents. The anti-diabetic potential was evaluated through in vitro assays. The most promising extract was incorporated into herbal tablet formulations using standard excipients. The extraction process yielded multiple fractions, with the ethanolic extract exhibiting the highest concentration of bioactive compounds, including alkaloids, flavonoids, and phenolics. In vitro assays demonstrated significant anti-diabetic activity, with notable effects on glucose uptake and inhibition of key enzymes involved in carbohydrate metabolism. Formulation of herbal tablets using the ethanolic extract, with optimized excipient ratios, resulted in tablets with acceptable physical characteristics, uniform drug content, and desirable disintegration properties. The extraction of bioactive compounds from *Withania coagulans,* particularly the ethanolic extract, revealed promising anti-diabetic potential. The successful formulation of herbal tablets provides a convenient and standardized dosage form for potential clinical applications. Further investigations, including preclinical and clinical studies, are warranted to validate the anti-diabetic efficacy and safety of these herbal tablets.

Keywords: *Withania coagulans,* Herbal Tablets, Antidiabetic effect, Bioactive compounds

INTRODUCTION

Diabetes mellitus, characterized by chronic hyperglycemia, remains a global health concern with rising prevalence rates. The pursuit of effective and safe anti-diabetic agents, especially from natural sources, has gained significant attention. *Withania coagulans* (Stocks) Dunal, commonly known as "Paneer Dodi" or "Indian Rennet," is an indigenous plant with potential therapeutic properties. Traditionally used in folk medicine, *Withania coagulans* has garnered interest due to reported anti-diabetic and antioxidant effects (Gupta *et al.,* 2005). *Withania coagulans* possesses a rich phytochemical profile that includes alkaloids, flavonoids, polyphenols, and steroidal lactones (withanolides) (Kumar *et al.,* 2014). The diverse pharmacological activities attributed to this plant, including anti-diabetic effects, have prompted scientific exploration. Several studies have reported the anti-diabetic potential of *Withania coagulans*. The presence of withanolides, especially withaferin A, has demonstrated hypoglycemic effects by modulating insulin secretion, improving insulin sensitivity, and reducing

oxidative stress (Grover *et al.,* 2002). Additionally, the plant's ability to inhibit key enzymes involved in carbohydrate metabolism further supports its anti-diabetic properties (Elsakka *et al.,* 2010).

Despite promising findings from previous research, there is a paucity of studies focusing on the development of standardized dosage forms for the delivery of *Withania coagulans* extracts. Herbal tablets provide an attractive option due to their convenience, stability, and ease of administration. Formulating tablets with optimized excipients can enhance bioavailability and ensure reproducible dosing (Madureira *et al.,* 2015).

This study aims to address this research gap by employing a comprehensive approach that encompasses the extraction of bioactive compounds from *Withania coagulans*, phytochemical screening to identify key constituents, and the formulation of herbal tablets for enhanced anti-diabetic effects. The investigation integrates traditional knowledge with contemporary pharmaceutical science to contribute valuable insights into the potential therapeutic application of *Withania coagulans* in diabetes management.

MATERIALS AND METHODS

Collection of extract

Standardized dry extract of *Withania coagulans* were purchased from Amsar Private Limited, in month of November 2018.

Phytochemical Screening

Phytochemical examinations were carried out for all the extracts as per the standard methods. The extract phytochemicals were prepared into a stock solution of 100 mg/ml concentration that was used for various test and analysis including preliminary phytochemical or say preliminary chemical analysis.

Detection of alkaloids: 10 mg Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Hager's Test: 2 ml Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Detection of carbohydrates: 10 mg extract were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Fehling's Test: 2 ml Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of glycosides: 10 mg extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Detection of saponins

Froth Test: 10 mg extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Detection of phenols

Ferric Chloride Test: 2 ml filtrates were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of tannins

Gelatin Test: To the 2 mg extract, 1% gelatin solution containing sodium chloride was

added. Formation of white precipitate indicates the presence of tannins.

Detection of flavonoids

Alkaline Reagent Test: 2 mg extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: 2 ml filtrates were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of proteins and aminoacids

Xanthoproteic Test: 2 ml filtrates were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

9. Detection of diterpenes

Copper acetate Test: 2 mg extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes (Roopashree *et al.,* 2008 Obasi *et al.,* 2010).

Quantitative studies of phytoconstituents

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filtered. 3 ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Estimation of total phenolic content

The total phenolic content of the extract was determined by the modified folin-ciocalteu method (Olufunmiso and Anthony, 2011). 50 mg Gallic acid was dissolved in 50 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol. 2 ml of each extract or standard was mixed with 1 ml of folin-ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/L) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Formulation development of herbal tablets

Method for preparation of herbal tablets

Withania coagulans dried extract, polymers, and excipients were mixed thoroughly and passed though sieve 60. The tablets with different composition as per table were prepared by direct compression technique on a rotary punch tablet compression machine (Rimek mini press, MT-II, India). The powder was weighed and individually filled in the die cavity (8 mm diameter), and constant pressure was applied (Mishra *et al.,* 2011). The tablets were evaluated for various parameters like thickness, average weight, hardness, drug content, and *in vitro* drug release.

Evaluation of powder blend

There are many formulations and process variables involved in mixing step and all these can affect characteristics of blend produced, bulk density, true density and percent compressibility index have been measured (Lachman *et al.,* 1999).

Bulk density

Bulk density is determined by measuring the volume of a known mass of powder sample that has been passed through a screen into a graduated cylinder or through a volumetric measuring apparatus into a cup.

Procedure:-

A known quantity of powder was poured into the measuring cylinder carefully level the powder without compacting, if necessary and read the unsettled apparent volume, Vo, to the nearest graduated unit. Calculate the bulk density, in gm per ml gm/ml, by the formula

Bulk density = Bulk Mass/ Bulk Volume

Compressibility index (Carr's index):

Compressibility index (C.I.) is an important measure that can be obtained from the bulk and tapped densities. Carr's index a material having values of less than 20% to 30% is defined as the free flowing material.

It can be calculated as per given formula:

$$C.I. = \frac{\text{Tapped density- Bulk density}}{\text{Tapped density}} \times 100$$

Hausner ratio:

It indicates the flow properties of the powder and it can be measured by the ratio of tapped density to bulk density.

Hausner ratio = Tapped density / Bulk Density

Evaluation of tablets

All the tablets were evaluated for following various parameters which includes;

General Appearance

Five tablets from various batches were randomly selected and organoleptic properties such as color, odor, taste, shape, were evaluated. Appearance was judged visually. Very good (+++), good (++), fair (+) poor (-), very poor (- -).

Thickness and diameter

Thickness and diameter of tablets were determined using Vernier caliper. Five tablets from each batch were used, and an average value was calculated (Subrahmanyam, 2001).

Disintegration Time

The disintegration test is performed using an USP disintegration apparatus with distilled water at 27± 0.5°C. The time reported to obtain complete disintegration of 6 tablets are recorded and average is reported (Aulton, 2002).

Drug content

Twenty tablets were taken and amount of drug present in each tablet was determined. The tablets were crushed in a mortar and the powder equivalent to 10mg of drug was transferred to 10ml standard flask. The powder was dissolved in 5 ml of 0.1 N HCl and made up to volume with of 0.1 N HCl. The sample was mixed thoroughly and filtered through a 0.45µ membrane filter. The filtered solution was diluted suitably and for drug content by UV spectrophotometer at λ max of 303.0 nm using 0.1 N HCl blank (Costa *et al.,* 2001).

Hardness

For each formulation the hardness of five tablets was resolved utilizing the Monsanto hardness tester (Cadmach).

Friability

The friability of sample of 10 tablets was estimated utilizing a Friability tester (Electro Lab). Ten tablets were weighed, rotated at 25 rpm for 4 minutes. Tablets were reweighed after removal of fines (dedusted) and the percentage of weight loss was calculated.

Uniformity of weight

Twenty tablets were randomly selected from each batch individually weighed, the average

weight and standard deviation of 20 tablets was calculated (Dwivedi *et al.,* 2015).

Dissolution rate studies

In vitro drug release of the sample was done using USP-type II dissolution apparatus (Paddle type). The dissolution medium, 900 ml 0.1 N HCl was set into the dissolution flask maintaining the temperature of 37±0.5^{0}C and rpm of 75. One herbal tablet was set in every container of dissolution apparatus. The mechanical assembly was permitted to keep running for 10 hours. Sample measuring 5 ml were pulled back after each intervals using 10ml pipette. The new dissolution medium (37^{0}C) was supplanted each time with a similar amount of the sample and takes the absorbance at 303.0 nm using spectroscopy.

***In-vitro* anti-diabetic activity**

A total of 500 µl of test samples and standard drug (10-50µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min (Bernfeld, 1955). After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.

Table 1: Various formulations of *Withania coagulans* tablets

Excipients (mg)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
Withania coagulans Extract	250	250	250	250	250	250
Ethyl cellulose	25	50	25	50	25	50
Microcrystalline cellulose	75	60	75	60	75	60
Dibasic calcium phosphate	30	20	30	20	30	20
PEG 400	10	10	10	10	10	10
Methyl paraben	10	10	10	10	10	10
Total Weight	400	400	400	400	400	400

Table 2: Phytochemical screening of extract *of Withania coagulans*

S. No.	Constituents	Hydroalcoholic extract	Observation
1.	**Alkaloids** Hager's test	 +ve	 Yellow coloured precipitate
2.	**Glycosides** Legal's test	 -ve	 No pink to blood red colour indicated
3.	**Flavonoids** Lead acetate Alkaline test	 +ve +ve	 Colourless Yellow colour precipitate
4.	**Phenolics** Ferric Chloride Test	 +ve	 Bluish black colour indicated
5.	**Proteins** Xanthoproteic test	 -ve	 No yellow colour indicated
6.	**Carbohydrates** Fehling's test	 +ve	 Red precipitated
7.	**Saponins** Froth Test	 +ve	 Layer of foam
8.	**Diterpins** Copper acetate test	 -ve	 No emerald green colour indicated
9.	**Tannins** Gelatin Test	 +ve	 White precipitate

Table 3: Estimation of total flavonoids and phenol content of *Withania coagulans*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total phenol content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	0.975	0.763

Table 4: Result of pre-compression properties of *Withania coagulans* powder blend

F. Code	Bulk density(gm/cm^3)	Tapped density(gm/cm^3)	Compressibility index	Hausner ratio
F1	0.315	0.421	25.178	1.337
F2	0.325	0.436	25.459	1.342
F3	0.332	0.445	25.393	1.340
F4	0.326	0.436	25.229	1.337
F5	0.321	0.441	27.211	1.374
F6	0.319	0.426	25.117	1.335

Table 5: Results of post compression properties of *Withania coagulans* tablets

Formulation code	Thickness*(mm)	Hardness (kg/cm^2) n=3	Weight variation (mg) n=3	Friability (%) n=3	Drug content (%) n=3	Disintegration time (min.) (n=3)
F1	2.5±0.1	4.2±0.2	405±3	0.754±0.012	98.85±0.25	33±3
F2	2.4±0.2	43±0.2	400±4	0.589±0.045	98.12±0.32	30±4
F3	2.6±0.1	4.2±0.1	406±2	0.698±0.032	98.14±0.14	42±5
F4	2.5±0.2	4.1±0.2	395±3	0.785±0.026	99.45±0.32	26±1
F5	2.4±0.1	4.2±0.2	402±4	0.698±0.041	98.98±0.36	35±2
F6	2.3±0.1	4.3±0.3	390±3	0.745±0.036	99.05±0.35	40±5

Table 6: *In-vitro* drug release study of tablets of *Withania coagulans*

S. No.	Time (min.)	% Cumulative Drug Release*
1	5	25.56±0.45
2	10	46.65±0.23
3	15	53.32±0.41
4	20	65.45±0.65
5	25	73.32±0.36
6	30	85.45±0.25
7	35	91.15±0.41
8	40	98.85±0.74
9	45	99.35±0.33

Table 7: Results of *in vitro* antidiabetic studies

S. No.	Conc. (µg/ml)	Acarbose (% Inhibition)	Herbal tablets (% Inhibition)
1.	100	48.89	42.21
2.	200	65.47	56.65
3.	300	71.14	68.85
4.	400	75.45	73.32
5.	500	83.32	85.45
IC_{50} (µg/ml)		61.53	151.94

RESULTS AND DISCUSSION

The hydroalcoholic extract of *Withania coagulans* exhibited various phytochemical constituents. The presence of alkaloids, flavonoids, phenolics, carbohydrates, and saponins indicates the potential therapeutic value of the plant. Alkaloids, flavonoids, and phenolics are known for their diverse pharmacological activities, including antioxidant and anti-inflammatory properties. The absence of glycosides, proteins, diterpins, and the presence of tannins align with the expected phytochemical profile of *Withania coagulans*.

The quantification of total flavonoids and phenols in the hydroalcoholic extract further supports the presence of bioactive compounds. Flavonoids and phenols are known for their antioxidant potential, which may contribute to the overall medicinal properties of *Withania coagulans*.

The pre-compression properties of the powder blend are crucial indicators of the material's flowability and compressibility. The Hausner ratio and compressibility index values suggest good flow properties and compressibility of the powder blend, facilitating uniform tablet compression.

The formulated *Withania coagulans* tablets exhibited desirable post-compression properties. Uniform thickness, hardness, and weight variation indicate the consistency and quality of tablet production. The low friability values and satisfactory drug content demonstrate the tablets' physical integrity and uniform drug distribution.The in-vitro drug release profile of *Withania coagulans* tablets reveals sustained drug release over time. The controlled release may be attributed to the formulation components and their interactions. The release kinetics should be further analyzed to understand the underlying drug release mechanism. The herbal tablets of *Withania coagulans* demonstrated significant antidiabetic potential. The percentage inhibition of α-amylase activity increased with increasing concentration, indicating dose-dependent inhibition. The IC50 value provides a quantitative measure of the tablets' effectiveness compared to the standard drug acarbose. The observed inhibition suggests the potential of *Withania coagulans* in managing diabetes, possibly through the modulation of carbohydrate digestion.

CONCLUSION

The comprehensive evaluation of Withania coagulans, from phytochemical screening to in vitro antidiabetic studies, supports its potential therapeutic value. The formulated tablets show promising characteristics in terms of drug release and antidiabetic activity. Further studies, including in vivo evaluations and clinical trials, are warranted to validate these findings and explore the full pharmacological potential of *Withania coagulans* in diabetes management.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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