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Screening Of *Withania Coagulans* Fruit For Antimicrobial And Antidiabetic Property

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Abstract

Various pharmaceutical preparations used to treat the microbial infections and diabetic problems may lead to hazardous side effects. Hence there is a need to find new ways for developing safe drugs to deal with the increasing complications caused by synthetic drugs. In the traditional health care system many plants are known to have therapeutic history. This property not only varies between species but also on different parts of the same plant. The fruit of the plant *Withania coagulans* used for the research work was collected from the market in Bengaluru. The crude extract of the sample was prepared by using methanol as a solvent. Antimicrobial analysis of the extract was studied by well diffusion method using the test organisms *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. α-Glucosidase inhibition assay was considered for the analysis of antidiabetic property of the sample. Results of the study reveal that *W. coagulans* fruit is an effective and alternative therapeutic with antimicrobial and antidiabetic property.

Key words:Withania coagulans, Fruit, Antimicrobial, Antidiabetic.

1. Introduction:

Enhanced resistance to antibiotics in microorganisms due to indiscriminate use of synthetic drugs has adversely increased the microbial infections all over the world [1]. This antimicrobial resistance is a serious threat for the effective treatment of microbial infections. Hence there is a need to find a suitable alternative means to prevent the infections. In traditional or folk medicine, various plants have been reported to cure many diseases and known to possess antimicrobial property [2].

Diabetes is one of the chronic, debilitating and rapidly increasing health conditions, which affect every organ of the body. Even though we have many drugs in the modern medicine and synthetic insulin hormone for the management of diabetes, long term use of these drugs are known to exhibit several dangerous side effects and complications to various organs of the body [3, 4]. Hence it is necessary to find suitable medications that help the patient to maintain the blood sugar level and to regulate the complications caused by diabetes [5].
Ever increasing interest to exploit the alternative therapies is to find more potent biomolecule than the existing

drug with reduced side effects [6]. Natural plants constitute a major source of medicines[7] and are found to be safe with least side effects compared to synthetic drugs[8]. In Ayurveda many plants and plant products have been used for treating various health related problems.

The sample medicinal plant selected for the study was *Withania coagulans* Dunal, commonly known as Rishyagandha, Paneer ke phool in Hindi and Indian cheese maker or Vegetable rennet in English [7]. Its usage for maintenance of health is mentioned in Charaka Samhita [9]. This plant is known to have many health benefits as a common natural remedy to cure various health related problems and diseases [10]. It is used for coagulation of milk, treating intestinal infection, toothache, diabetes [11] and it is also known for its antimicrobial activity [12].

Even though *W. coagulans* is a popular medicinal plant since ancient times, this medicinal plant with vast therapeutic potential can be utilized effectively only after the proper investigation and standardization. Hence the objective of the present research work was to evaluate the methanolic extract of *W. coagulans* fruit for its antimicrobial and antidiabetic property.

2. Materials and methods

Sample collection and preparation of extract

The fruit of the medicinal plant from Solanaceae family *Withania coagulans* Dunal used for the research work was collected from the local market in Bengaluru. Confirmation of the sample identification was done by botany taxonomists in Nrupathunga University. The sample extract was prepared by using methanol as a solvent (table 1).

Table 1: Details of test sample extraction

Sl. No.	Sample	Solubility	Stock solution
1	*Withenia coagulans* fruit	Methanol	100 mg/ml

Antimicrobial analysis of the extract by well diffusion method

The test organisms used for the experiment were bacteria *Escherichia coli* ATCC8739 (gram negative), *Staphylococcus aureus ATCC 6538* (gram positive) and fungus *Candida albicans ATCC 10231*. The details of the materials used for antimicrobial test are given in table 2.

Table 2: Details of materials used for antimicrobial test

Sl. No.	Particulars	Source	Catalogue No
1	Tryptone broth	Himedia	M463-500G
2	Soyabean Casein Digest Agar (SCDA)	Himedia	MH290-500G
3	Potato dextrose Agar (PDA)	Himedia	M096-500G
4	Petri plates	Genaxy	GEN-PTD-90

Test compounds

Sample: *Withenia coagulans* fruit (100 mg/ml).
S-Standard: Ciprofloxacin (0.1 mg/ml).
S-Standard: Itraconazole (100 mg/ml).
Control: Methanol (5%).

Inoculum

E. coli and *S. aureus* cell suspension were prepared, grown on tryptone broth and cultures were incubated for 24 hrs at 37°C. The cell suspensions of the cultures were adjusted to 1-2x10^6 cells/ml. *C. albicans* spore suspension were prepared and grown on potato dextrose broth and cultures were incubated for 5-7 days at room temperature.

Determination of antimicrobial activity

E. coli and S. aureus were inoculated on Soya bean casein digested agar plates (90 mm). Test compounds, sample (20, 10 µl), standard ciprofloxacin (20 µl) for *E. coli and S. aureus*, were added to the 5 mm well on

agar plates. The treated plates with *E. coli and S. aureus* were incubated in incubator at 37°C for 24 hrs.

C. albicans were inoculated on potato dextrose agar plates (90 mm). Test compounds, sample (20, 10µl), standard itraconazole (20 µl) for *C. albicans,* were added to the 5 mm well on agar plates. The treated plates with *C. albicans* were incubated at room temperature for 5-7 days. After incubation the treated plates were observed for zone of inhibition around the wells.

α-Glucosidase inhibition assay on antidiabetic property of *withania coagulans*

α-Glucosidase activity can be measured *in-vitro* by determination of the reducing sugar (glucose) arising from hydrolysis of sucrose by α-glucosidase enzyme, isolated from small intestine of rat. The purpose of this SOP is to provide clear and concise instructions for performing the inhibition studies on α- Glucosidase enzyme.

Materials and methods used for α-glucosidase inhibition assay

α-Glucosidase.
Sucrose-Reducing sugar free.
Acarbose.
Disodium hydrogen phosphate.
Sodium dihydrogen phosphate.
Sucrose.
Glucose reagent kit: Glucose oxidase method.

Preparation of working solution

Phosphate buffer (80 mM) ph 7.0

Sodium dihydrogen phosphate (NaH_2PO_4, $2H_2O$) - 12.48 g in 1000 ml of de-ionised water (solution A). Disodium hydrogen phosphate anhydrous (Na_2HPO_4) - 11.35 g in 1000 ml of de-ionised water (solution B). or Disodium hydrogen phosphate, dehydrate (Na_2HPO_4, $2H_2O$) -14.24 g in 1000 ml of de-ionised water. Mix 39 ml of solution A with 61 ml of solution B and make up to 200 ml with deionised water.

Preparation of enzyme

Rat intestine is removed and chilled with ice cold 80 mM phosphate buffer (pH 7.0). The intestine is then cut open; the mucosa is scraped off with a piece of glass rod and homogenized in homogenizer with four parts (v/w) of cold 80 mM buffer (pH 7.0). The tube is chilled with crushed ice during homogenization. Large cell debris are removed by centrifugation at 2000 to 4000 rpm for 10 minutes and supernatant was stored at -20°C.

Substrate (37 mm)

Dissolve 1.2665 gm of sucrose in 100 ml of 80 mM phosphate buffer pH 7.0.

Reference standard (inhibitor)

Dissolve 50 mg of acarbose in 50 ml of phosphate buffer and dilute appropriately to get concentration of 5 µg/ml using phosphate buffer pH 7.0.

Procedure: α- glucosidase inhibition bio-assay

To 50 µl of enzyme, add 250 µl of buffer or test sample and incubate at 37°C for 30 minutes. Add 500 µl of sucrose solution and incubate at 37°C for 20 minutes, heat on boiling water bath for 2 minutes to arrest the reaction and cool. Measure the glucose concentration by glucose oxidase method.

Glucose estimation by glucose oxidase method

Mix 100 µl of sample with 500 µl of glucose reagent (glucose reagent kit) then incubate at room temperature for 10 minutes. Measure the absorbance at 510 nm.

Calculation

The percentage inhibition of α-glucosidase is calculated as follows:

$$\% \text{ inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (test)}}{\text{Absorbance (control)}} \text{ X } 100$$

3. Results and discussion

Result of antimicrobial resistance of plant extract

The test sample *W. coagulans* fruit extract has shown antibacterial activity against *E. coli*, *S. aureus* and antifungal activity against *C. albicans*. As given in table 3 in standard the zone of inhibition for *E. coli*, *S. aureus*, *C. albicans* was 22, 22, 12 mm and that of sample *W. coagulans* at 2 mg per well concentration was 15, 17 and 10 mm respectively. But the zone of inhibition showed by well diffusion method for sample at 1 mg per well concentration against *E. coli*, *S. aureus*, *C. albicans* was 10, 12 and 6 mm respectively. The results of the present study clearly indicate that the zone of inhibition is directly proportional to the concentration of the sample. Zone of inhibition observed for different test samples against the test organisms are summarized in table 3 and figure 1, 2 and 3.

Table 3: Inhibitory activity of test compound against test organisms.

Sample	Test Organisms	Test Compounds	Conc. per well	Zone of inhibition (mm)
W. coagulans	*E. coli*	Control	5%	-
		Ciprofloxacin (20 µl)	2 µg	22
		Sample (20 µl)	2 mg	15
		Sample (10 µl)	1 mg	10
	S. aureus	Control	5%	-
		Ciprofloxacin (20 µl)	2 µg	22
		Sample (20 µl)	2 mg	17
		Sample (10 µl)	1 mg	12
	C. albicans	Control	5%	-
		Itraconazole (20 µl)	2 mg	12
		Sample (20 µl)	2 mg	10
		Sample (10 µl)	1 mg	6

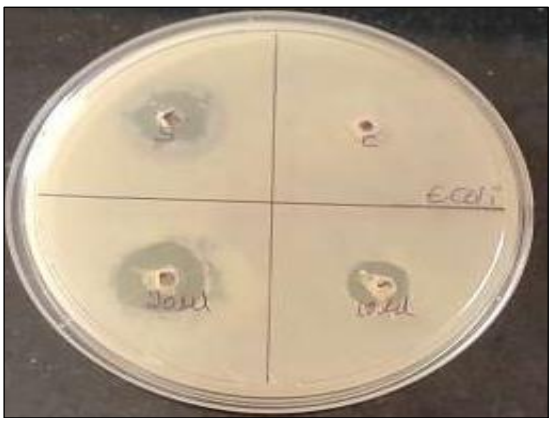

Fig. 1: Inhibitory activity of test sample *W. coagulans* against *E.coli*, S-Standard (Ciprofloxacin); C-Control (Methanol)

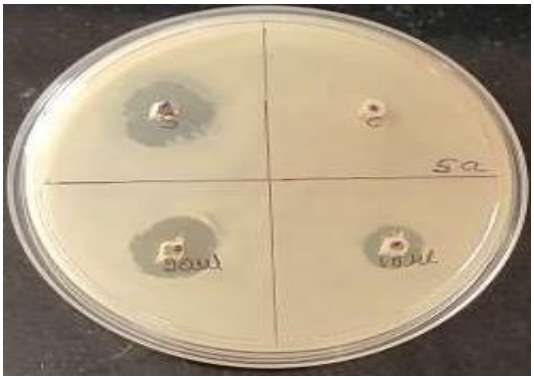

Fig. 2: Inhibitory activity of test sample *W. coagulans* against *S. aureus*, S-Standard (Ciprofloxacin); C-Control (5% Methanol)

Fig. 3: Inhibitory activity of test sample *W. coagulans* against *C. albicans* S. Standard (itraconazole); C-Control (5% Methanol)

Result of α-glucosidase inhibition assay for antidiabetic test

*W. coagulans*plant fruit crude extract sample tested has showed very good percentage of alpha glucosidase inhibition activity like that of standard acarbose. Percentage of inhibition is directly proportional to the concentration of the tested sample. The details of α-glucosidase inhibitionassay for standard acarbose and sample *W. coagulans*plant fruit crude extract are given in table 4, figure 4 and table 5, figure 5 respectively.

Table 4: Alpha glucosidase activity of standard (Acarbose)

Sample	Conc. µg/ml	OD at 510 nm	% Inhibition
Control	0	0.895	0.00
Acarbose	0.3125	0.831	7.19
	0.625	0.728	18.63
	1.25	0.644	28.02
	2.5	0.480	46.38
	5	0.356	60.22
	10	0.178	80.16

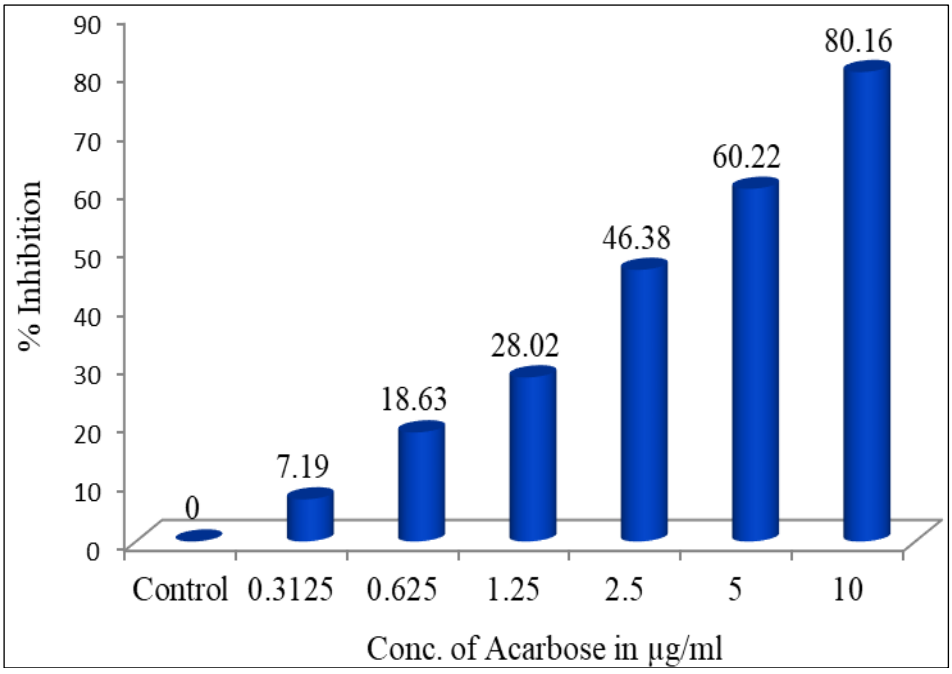

Fig. 4: Alpha glucosidase inhibition assay for standard (Acarbose)

Table 5: Alpha glucosidase activity of sample *W. coagulans*

Sample	Conc. µg/ml	OD at 510 nm	% Inhibition
Control	0	0.895	0.00
W. coagulans	50	0.819	8.50
	100	0.784	12.42
	200	0.649	27.56
	400	0.594	33.62
	800	0.491	45.21
	1200	0.339	62.11

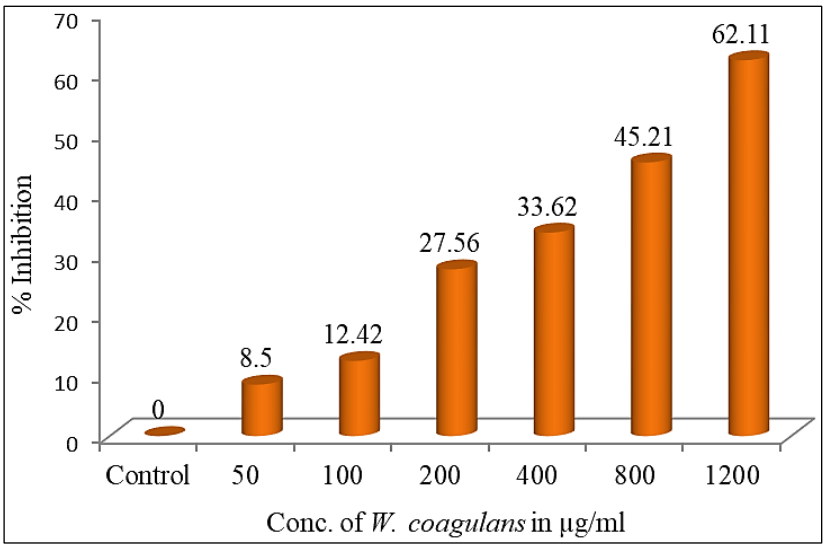

Fig 5: Aalpha glucosidase inhibition assay for *W. coagulans*

It was tried to establish the antimicrobial property of *W. coagulans* against gram positive, gram negative bacteria and fungus. The sample showed positive results against all the three selected test organisms and the percentage of inhibition is also directly proportional to the concentration of the sample. Study conducted by Murtaza *et al*.,[1] also reported the cost effective, ecofriendly and antimicrobial property of *W. coagulans* extract using different solvents.

Drastic decline in the polyphagia (82.5%), polyurea (56.07%), weakness (66.67%), joint pain (82.5%) and burning and tingling sensation, the characteristics of diabetes have been reported in the *W. coagulans* study conducted by Upadhyay *et al*., in 2011[3]. Results of the work carried out by Shukla *et al*.,[10] using *W. coagulans* successfully reduced the glucose level after 30 days, similar to that of the results of treatment with standard drug glib enclamide that induces the pancreatic cells to secrete the hormone insulin[13]. Sampathkumar *et al*.,[6] reported nearly 2 fold increased secretion of insulin from the β cells of pancreas treated with *W. coagulans* in rat.

The results of the study are also in line with the earlier reports. This clearly reveals the antimicrobial and antidiabetic property of *W. coagulans*. Hence with increase in diabetes all over the world, *W. coagulans*, fruit can be a good source of active compound with potential pharmacological activity for medical applications. Further *invivo* studies should be conducted to confirm the safe and efficient antidiabetic property of this plant.

4. Conclusion

Based on the findings of the present study it can be concluded that the *W. coagulans*, fruit crude extract has bioactive compounds with antimicrobial and antidiabetic property. So this natural resource can be used as an effective source to develop a potent antimicrobial and antidiabetic therapeutic.

Conflict of interest

The authors declare no competing financial interest.

Author contributions

Mohan Kumar H. M., Swechchha K. M., Rajeev Ramachandra Kolgi, and Shankara S conceived the study, performed the experiment and wrote the article.

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