ISSN: 2249- 5746
International Journal of Ayurvedic and Herbal Medicine 14:6 (2024) 4582-4590
Journal homepage: http://www.interscience.org.uk
10.47191/ijahm/v14i6.01
Impact Factor: 8.003

Phytochemistry and Therapeutic Potential of the Medicinal Plant: *Withania coagulans*

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ABSTRACT: Herbal plants have long been used in traditional medicine to address and heal a wide range of health-related issues. *Withania coagulans* (Family; Solanaceae), a valuable medicinal plant used to evaluates bioactives by using maceration. Phytochemical analysis evaluated by qualitatively for five different extract. Methanol extract has highest phytocostitutents so further used for analysis phenolic compounds (86.67 ± 3.19 mg GAE/g) and flavonoids (60.95 ± 2.70 mg QE/g). The methanol extract demonstrated significant antioxidant activity in the DPPH and FRAP assay with IC_{50} value 63.02±1.08 µg/ml and 70.2±1.11 µg/ml respectively and notable antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Additionally, the methanol extract exhibited substantial anti-diabetic potential, with IC_{50} value 602.6±2.98 µg/ml. This study demonstrated that the methanolic extract of *Withania coagulans* is a potent natural antioxidant, which could be beneficial in slowing the progression of various oxidative stress-related conditions.

KEYWORDS: *Withania coagulans*, antioxidant, anti-diabetic

INTRODUCTION

Plant based medicines have been used for centuries across different cultures to treat a variety of ailments. Many of these medicinal plants grow naturally in the wild, offering accessible and sustainable remedies for common health issues. The knowledge of using these plants often comes from traditional practices, passed down through generations [1]. Plant-based medicines are often considered more human- and environment-friendly because they tend to have fewer adverse effects compared to synthetic drugs [2]. Plants have useful phytochemicals and antioxidants which makes plants powerful therapeutic agents. One of the widely recognized medicinal plant within the genus *Withania* is *Withania coagulans* which holds significant importance in the ayurvedic medicinal system due to its potent nutraceutical and pharmaceutical properties. *Withania coagulans* extract has both hypolipedemic and antidiabetic activity; hence this can be used for effective treatment for diabetic [3]. The plant has wide benefits for diabetes and this is a rising epidemic throughout the world, has no signs of abatement and remains one of the most challenging health problems [4]. Medicinal plants play a vital role for the development of new drugs.

Solanaceae family is consisting about 3000–4000 herbs, bushes, tree species with 90 genera from all annuals, biennials to perennials. Different plants of this family have different kinds of secondary metabolites and widely used as ethno herbal. Presence of alkaloids, flavonoids and terpenes in Solanaceae species has broad significance in herbal industries world over [5]. *Withania coagulans* belonging to family Solanaceae is commonly known as Indian Cheese maker [6]. *Withania coagulans* has shown to have profound

hypoglycemic activity because it contains compounds which can utilize blood glucose and also repair the pancreatic β-cells, thus providing insulin to the body [7]. Various plant parts such as roots, fruits and leaves have different therapeutic effects. The phytochemical screening of extracts shows the presence of various primary and secondary metabolites such as, proteins, amino acids, alkaloids, phenols, tannins, steroids, saponins, etc. The ripe fruit of *Withania coagulans* is sweet and used for wound healing, asthma, dyspepsia, and as a sedative. It is also traditionally used in many countries to treat diabetes and as an antibacterial, antimicrobial, hepatoprotective, hypolipidemic, antioxidant, anti-tumor, antidepressant, immunosuppressive, and anti-inflammatory agent. The seeds reduce inflammation, act as a diuretic, and treat ophthalmia, while the flower buds exhibit anthelmintic activity [8]. In recent years, there has been resurgence in interest in plant-based medicines, driven by concerns about the side effects of synthetic drugs, a desire for more natural treatments, and the present study give new avenue for development of plant based medicines.

2. MATERIAL AND METHODS

2.1. Plant Sample Collection:

The fruits were procured from homestead gardens of Kolhapur district, Maharashtra, India. The samples were air dried and then pulverized into a fine powder (Figure1).

2.2: Maceration:

In maceration clumsily powdered material with appropriate solvents closely packed kept at least three days with frailly shaking illustrated in **Figure 2**. At the end of extraction, the micelle is separated by filtration. Solvents for extraction used during maceration were distilled water, methanol, ethanol, acetone, chloroform [9].

2.3. Phytochemical analysis:

Various qualitative tests were performed to find the profile of extracts for their chemical composition. *Withania coagulans* including aqueous, methanol, ethanol, acetone, chloroform extract were analyzed [10].

2.3.1. Phenols:

1 ml of extract with 3 ml of D/W; few drops of neutral 5% FeCl3 formation of dark green color indicates the presence of phenols.

2.3.2. Alkaloids:

2 ml extract was taken followed with 2 ml of concentrated HCL then few drops of Mayer’s reagent also added. Green shade signifies the alkaloids.

2.3.3: Flavonoids:

5 ml of seed extract was taken; 5 ml of dilute ammonia. Afterwards concentrated H_2SO_4 was added into solution. Reaction gives yellow coloration which indicates the existence of flavonoid.

2.3.4. Anthocyanin and Betacyanin:

Aliquots of 2 ml of extract were taken with 1ml of 2N NaoH was added and heated for 5min.at 100 ^{0}C. Yellow color indicated the presence of Betacyanin.

2.3.5. Carbohydrate:

2 ml of extracts was taken then 1ml of Molish’s reagent added thereafter some drops of concentrated H_2SO_4 integrated. Presence of carbohydrates was indicated by purple color formation.

2.3.6. Amino acids:

1ml extract; 1ml of (0.25%) Ninhydrin reagent and boiled for few min. formation of blue color indicates presence of amino acids.

2.3.7. Cardiac glycosides:

1ml extract with glacial acetic acid containing trace of $FeCl_3$ 1 ml of conc. H_2SO_4 along the side of tube formation of purple ring at interface indicates presence of cardiac glycosides

2.3.8. Saponins:

2 ml seed extracts were taken with 2 ml of D/W then 15min wait with shaking. Foam formation gives response of the presence of saponins.

2.3.9. Acids:

1ml of extract; add 1 ml sodium bicarbonate solution formation of effervescence indicates presence of acids.

2.3.10. Quinones:

1 ml extract and 1ml of strong H_2So_4 was taken. Red color formation shows the existence of Quinones.

2.4. Quantitative phytochemical analysis:

Withania coagulans methanolic extract (WCME) qualitatively analyzed and has major phytoconstituents so this extract has used for further analysis.

2.4.1. Phenolic content determination:

The amount of phenolic constituents was quantified using the Folin-Ciocalteu colorimetric method [11], After incubating for 15 min at room temperature, the absorbance was measured at 765 nm to determine the phenolic amount expressed as mg gallic acid /g of dry extract, following the addition of mixture comprising 5 ml of Folin-Ciocalteu reagent and 4 ml of Na_2Co_3 to 0.5 ml of the extract.

2.4.2. Flavonoid content determination:

The flavonoids were quantified by the method of Peri and Pompei with some changes. In test tube 1ml of sample extracts with concentrations (1mg/ml) was taken. The volume was adjusted with 1ml D/W. To this 0.5ml of folins phenol reagent (1:2) was integrated and then followed with 5ml of 35% Na_2Co_3 and keep it for 5 min at room temperature. Blue color was observed. OD was taken at 640 nm. Same procedure followed for quercetin which act as standard. The flavonoid content is expressed in mg QE /g dry extract [12].

2.5. *In vitro* antioxidant activity:

2.5.1 DPPH assay:

DPPH radical scavenging assay was extensively employed to assess the scavenging capability of organic constituents. Assay relies on ability of antioxidants to scavenge stable radicals. Free radical scavenging activity of the seed extracts was evaluated *in vitro* using the DPPH radical, following the method characterized by Shimada et al. [13] with some changes. Various concentrations of WCME ranging from 20-100 µg/ml were mixed with 1 ml of 0.8 mM DPPH. After incubating 30 minutes, at 517 nm, absorbance was measured against a blank. Gallic acid served as the standard. The inhibition percentage was calculated using the formula:

$$\%\ \text{inhibition} = \frac{AC - AT}{\text{AC}} \times 100$$

Where, AT denotes the sample absorbance, AC represents absorbance of the control

2.5.2. FRAP Assay:

FRAP assay was executed by using Iris and Strain method with some modifications [14]. This assay evaluates the antioxidant capacity of WCME by converting ferric ions to ferrous ions. The complex formed with TPTZ (2,4,6-tripyndyl-s-triazine) can be quantified at 593 nm using spectrophotometer. The assay was conducted in 96- well plate. FRAP reagent formulated by mixing 10 mM TPTZ (0.25 ml) in 40 mM HCL, 20 mM $FeCl_3$ (0.25 ml) and acetate buffer in the ratio of (1:1:10). Later on in each well 170 µl FRAP reagent with 30 µl extract were added. Addition was followed in dark and plates were allowed to incubate

for 30 min. Ascorbic acid work as a standard. Finally absorbance was recorded at 593 nm. Relative % of reducing power calculated by following formula:

Relative % of reducing power = $[(AT - AC)/(Amax - Ac)] \times 100$

Where, AT denotes the sample absorbance, AC represents absorbance of the control, and Amax shows highest absorbance of the standard.

2.6. Antibacterial Activity:

The antibacterial activity of WCME against the selected *E.coli & Staphylococcus aureus* was carried out using well diffusion method. The bacteria strains were spread on the nutrient agar using sterile a spreading rod. 6mm diameter wells were bored using a sterile cork borer. 100µl of prepared extract and standard was added to the wells. The petri plates incubated at 37°C for 24 h. Streptomycin (1mg/ml) was prepared as a positive control and respective solvents were taken as negative control. The zone of inhibition was observed after 24 h of incubation.

2.7. *In vitro* α amylase inhibition activity:

In vitro amylase inhibition was studied by the method of Bernfeld with some medication [15]. 100 µl of different concentrations of extract (200-1000 µg/ml) was all owed to react with 500 µl of 0.1 M phosphate buffer pH 6.9 containing α-amylase enzyme. After 10 min incubation at 25°C, 500 µl of 1% starch solution in 0.1 M phosphate buffer pH 6.8 was added then incubated at 25°C for 10 min. The same was performed for the controls where 500 µl of the enzyme was replaced by buffer. After incubation, 1000 µL of dinitro salicylic acid reagent was added to both control and test. They were kept in boiling water bath for 10 min and cooled. The absorbance was recorded at 540 nm using spectrophotometer and the percentage inhibition of α-amylase enzyme was calculated using following formula.

$$\% \ \alpha \text{ amylase inhibition} = \frac{absorbance\ of\ control - absorbance\ of\ test}{absorbance\ of\ control} \times 100$$

2.8. Statistical Analysis:

To ensure replicability all the experiment done in triplicate, numerical results are expressed as $mean \pm standard\ error$. Statistical analysis included one-way ANOVA for the group comparison and Mann-Kendall test for trend detection, with significance set at $p<0.05$

3. RESULT & DISCUSSION

3.1: Qualitative Analysis:

The phytochemical screening for methanol extract of *Withania coagulans* contains alkaloids, glycosides, flavonoids, carbohydrate, flavonoid, cardiac glycoside, phenol, quinones and terpenoids. Likewise ethanol and acetone showed positive results for alkaloids, carbohydrate, cardiac glycoside, quinones and terpenoids. Distilled water extract only give positive response for amino acid, cardiac glycoside, quinones and terpenoids. And lastly chloroform extract showed results same like distilled water expect amino acid.

Table 1: Qualitative phytochemical analysis of *Withina coagulans*.

Sr.No.	Test	Distilled water	Methanol	Ethanol	Acetone	Chloroform
1.	Alkaloid	-	+	+	+	-
2.	Acid	-	+	-	-	-
3.	Aminoacid	+	-	+	-	-
4.	Betacyanin	-	-	-	-	-
5.	Carbohydrate	-	+	+	+	+

6.	Flavonoid	-	+	-	-	-
7.	Cardiacglycoside	+	+	+	+	+
8.	Phenol	-	+	-	-	-
9.	Quinone	+	+	+	+	+
10.	Steroid	-	-	-	-	-
11.	Tannins	-	-	-	-	-
12.	Terpenoids	+	+	+	+	+
13.	Saponin	-	-	-	-	-

(+ positive/present, - negative/absent)

3.2 Quantitative phytochemical estimation:

The phenolic content and flavonoids content of WCME by various extraction methods are illustrated in **Table 2**. Quantitative evaluation of phytochemicals has roles in providing antioxidant properties to plants. The quantitative analysis of the extract in order to estimate the concentration of flavonoids and phenols was performed and following observations were made. The estimated amount total phenols expressed as 86.67 ± 3.19 mg GAE/g and flavonoids was expressed 60.95 ± 2.70 mg QE/g and. Nazneen et al. studied the phytochemical evolution of *Withina coagulans* and according to study plant have 5.5 mg /g flavonoid, 9.5 mg/g alkaloids and 5.2 mg/g phenols [16]. Muhammad Azhar et al estimated phytochemical analysis of leaves, stem and root extract of *Withina coagulans* and from that study it is found that leaves has higher phytochemicals than stem and roots [5].

Table 2: Analysis of total content of Phenolic and Flavonoid for *Withina coagulans* methanolic extract (WCME) (Results are presented as Mean ± SD)

Sample	Total Phenolic content (Gallic acid equivalent in mg/g)	Total Flavonoid content (Quercetin equivalent in mg/g)
WCME	86.67 ± 3.19	60.95 ± 2.70

3.3 Antioxidant activity:

Two different assays were studied in which antioxidants act as free radical scavengers (DPPH assay), as reducing agents (FRAP assay) and that, determine the antioxidant activities of extracts of selected plant material with IC_{50} value and graphical representation showed in **Figure 3 & 4**. Antioxidant activity of WMCE could probably be attributed to various mechanisms like peroxides decomposition, scavenging or radicals, reducing capacity, chain initiation prevention are some of these mechanisms. Upon reduction of DPPH by an antioxidant intense violet color of DPPH changes to pale yellow. The change in the color is directly proportional to the antioxidants potency and a significant radical scavenging activity of the test material is due to significant reduce in the absorbance of the reaction mixture. Antioxidant activity of extracts increases in concentration dependent manner for both DPPH and FRAP assay having IC_{50} value 63.02±1.08 μg/ml and 70.2±1.11 μg/ml respectively. In comparison with standard 51.11±2.71 μg/ml and 45.72±1.83 μg/ml for DPPH and FRAP extract showed significant antioxidant activity. The anti-oxidant activity of *Withania coagulans* Dunal was studied by Chetan Salwaan et al using DPPH and Nitric oxide method and it was observed that it has antioxidant activity [17]. Additionally, the Mann-Kendall test was applied, confirming a monotonic increase in standard %RSA inhibition with concentration, yielding a p-value of 0.027 ($p < 0.05$) at a significance level of 0.05. This result indicates that the trends is statistically significant in the both DPPH and FRAP assays.

3.4. Antibacterial Assay:

Antibacterial sensitivity test of extract against various microorganisms like *Staphylococcus aureus*, *Escherichia coli* revealed. Zone of inhibition of all these extracts is shown in **Figure 5**. MCME give maximum zone of inhibition for *Staphylococcus aureus,* whereas *Escherichia coli* give moderate activity. Sudhanshu et al studied antimicrobial activity of petroleum ether, benzene, chloroform, ethyl acetate, methanol and distilled water extract of *Withania coagulans* (Fruit) for various 8 bacteria's and 3 fungus [18].

3.5. Alpha amylase inhibition:

Alpha-amylase, a key enzyme in digestion process, plays a crucial role in breaking down polysaccharides and is primarily found in saliva and pancreatic juice [19]. One potential approach to reducing high postprandial blood glucose levels is by targeting and inhibiting this enzyme [20]. **Figure 6** illustrates the alpha amylase inhibition potential of WCME, showing a dose-dependent relationship where increased extract concentration (200-1000μg/ml) leads to greater enzyme inhibition. The IC_{50} values indicate that WCME has moderate inhibition potential with IC_{50} of 602.6±2.98 μg/ml and 483.71±1.96 μg/ml for extract and standard respectively. Navjot Kaur estimated phytoconstituents, antioxidant and antidiabetic activity of polyherbal extracts in which ethanolic extract of *Withania coagulans* gives antidiabetic activity [21]. The Mann-Kendall test yielded a p-value of 0.027, which indicates that the monotonic increase in alpha amylase inhibition with increasing concentration is statistically significant.

CONCLUSION

The phytochemical analysis, both qualitative and quantitative, has revealed a significant abundance of key phytochemicals in fruit *Withania coagulans.* Biologically active compounds offering a wide range of therapeutic and antioxidant effects make them promising candidates for diabetes treatment. The results demonstrated that methanol extract exhibited the highest phenolic and flavonoid content, contributing significantly to antioxidant potential as confirmed by the DPPH and FRAP assay. Extract significantly reduced the alpha amylase levels. Further research on the isolation and characterization of specific constituents is necessary to confirm our findings. This study could serve as a foundation for developing a natural antioxidant, which may be formulated as a dietary supplement to combat various diseases.

DECLARATION OF CONFLICT OF INTEREST

Authors declare no any conflict of interest.

ACKNOWLEDGMENT: We gratefully acknowledged Shivaji University for providing financial support Golden Jubilee fellowship to carry out the work associated with this article

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Figures:

Figure 1: A. Dried fruits of *Withania coagulans* B. *Withania coagulans* fruit powder

Figure 2: Bioactives extraction from *Withania coagulans*

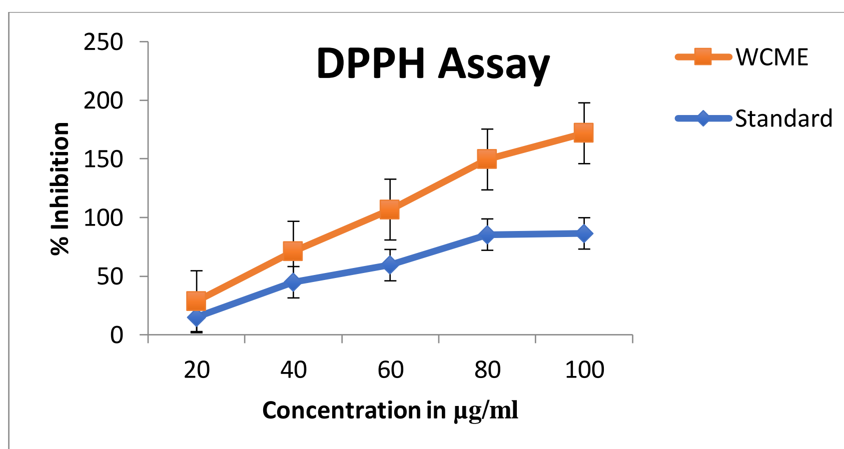

Figure 3: DPPH free radical scavenging activity of WCME

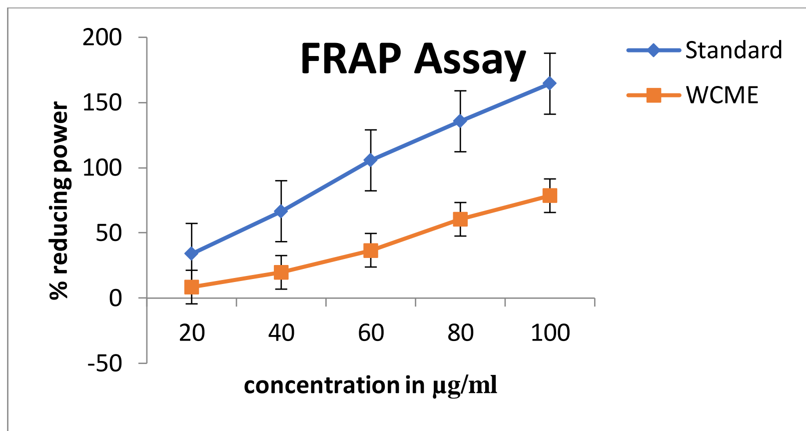

Figure 4: Ferric reducing activity of selected WCME

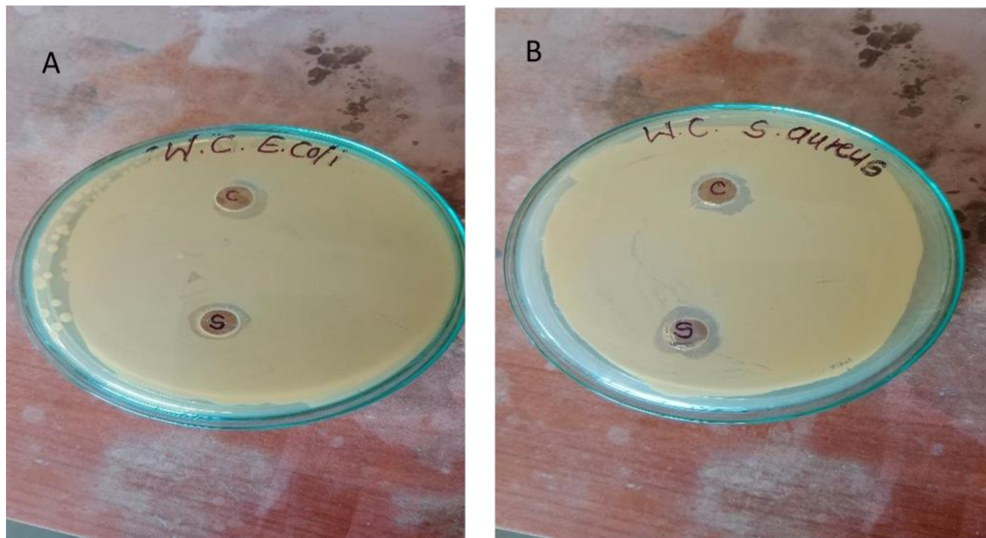

Figure 5: Antibacterial activity of WCME against A. *Escherichia coli* and B. *Staphylococcus aureus*

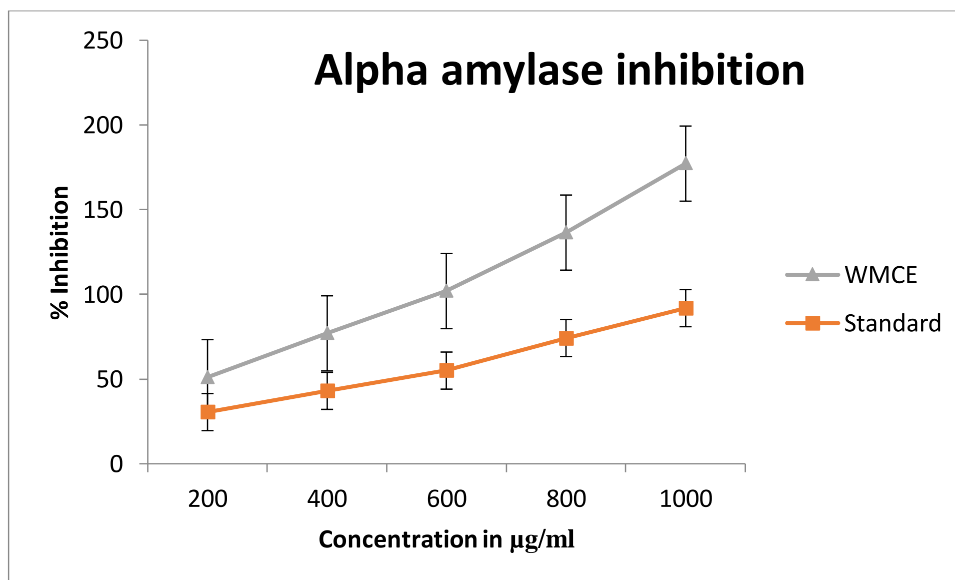

Figure 6: % inhibition of α-amylase for WMCE