International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 998-1005 www.ijprajournal.com ISSN: 2456-4494

Phytochemical Screening and Evaluation of Antimicrobial activity of Withania coagulans

Prashik Kamble , Pratiksha Kamble , Prachi Kamble , Manavi Kajave , Snehal Kamble .

Sarojini College of Pharmacy Rajendra Nagar, Kolhapur 416008 Maharashtra India.

Submitted: 02-10-2022 Accepted: 12-10-2022

ABSTRACT :
Withania coagulans belongs to family Solanaceae and mainly found in India, Pakistan, Afghanistan, South Asia. In the present study phytochemical screening of Withania coagulans ethanolic fruit extract was performed. Priliminary phytochemical study of ethanolic extract of Withania coagulans was found to be contain carbohydrates, steroids and alkaloids are present. Presence of steroids withanoloids in the ethanolic extract of Withania coagulans concluded that it was found to produced considerable antimicrobial effect. Different withanoloids having different therapeutic activity such as Antidiabetics, Anticancer, Antihyperglycemic, Anti inflammatory. The antimicrobial activity of ethanolic extract of Withania coagulans tested against pathogenic bacteria like Bacillus cereus, Staphylococcus Aureus, E.coli, Proteus vulgaris, S.mutant. It shows good antimicrobial avtivity against all pathogens but highest inhibitory activity was observed against Bacillus cereus .

KEYWORDS : Antimicrobial , Pathogens , Withania coagulans , Ethanolic extract , Phytochemical Screening.

I. INTRODUCTION:

From the ancient times the herbal plant extract is used as medicine to cure many diseases. And it is a only source on which they rely on. Herbal medicines are used for health promotion and therapy for chronic, as opposed to life threating conditions. Evidence exists for the use of medicinal plants up to 60,000 years ago but more recently, a 5000 years old Sumerian clay slab was discovered verifying the utilization of medicinal plants for the preparation of drugs.(1) Herbal medicines are natural drugs, so they have less side effect as compare to pharmaceutical medicines. Antimicrobial resistance, in today's world is becoming a threat to the effective prevention and treatment of infections caused by various bacterial strains. This is a result of the ever-increasing use of antibiotics in our daily lives. The phenomena of antibiotic resistance has raised alarm for the need of other alternatives for the cure and prevention of these infectious diseases.(2)According to a detailed research carried out by the World Health Organization (WHO), it was determined that about 80 percent of the world's population depends on conventional plants as healers.(3) Withania Coagluans, also popularly known as Paneer Doda in Pakistan and the surrounding regions, has primarily been derived from the Solanaceae family.(4) W. coagulans belongs to the Solanaceae, a family of common traditional therapeutic plants with wide range of pharmacological applications,(5) including antimicrobial, anti-inflammatory, (6) antitumor, (7) antihyperglycemic, (8) cardiovascular, and immunosuppressive properties. (9) The constituents of W. coagulans include free amino acids, essential oils, steroidal lactones and esterases, widely used for their pharmacological activities. (10) A few studies have also recommended the use of withanolide, withaferin and other biological entities found in W. coagulans for their bioreducing potential in the synthesis of nanoparticles, (11,12) and studies have reported the ecofriendly and less toxic preparation of nanoparticles and pharmacological studies using W. coagulans components.(13)

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 998-1005 www.ijprajournal.com ISSN: 2456-4494

Table No. 1.1 : Toxonomical Classification of Withania coagulans dunal[14,15]

Rank	**Scientific Name And Common Name**
Kingdom	Plantae-plants
Subkingdom	Tracheobionta-vascular plants
Superdivision	Spermatophyta-Seed plants
Division	Magnoliophyta-Flowering plants
Class	Magnoliopsida-Dicotyledons
Subclass	Asteridae
Order	Solanales
Family	Solanaceae
Genus	Withania
Species	Coagulans (L.) Dunal

Table No. 1.2 : Common names of Withania cogulans dunal[16]

Tamil	Panir poo
Hindi	Akri, punir
English	Indian cheese maker, Vegetable rennet
Punjabi	Spin bajja, panir
Marathi	Kaknaj
Sindhi	Punirjafota, punirband
Persian	Kaknajehindi, punirbad
Arabic	Javzulmizaja, Kaknajesindi
Telugu	Panneru-gadda
Urdu	Hab Kakmaj

II. LITERATURE REVIEW[17]

1) Chavan RT et. al., (2016) reported HPTLC fingerprint analysis and antimicrobial activity of leaf extracts of Cassia fistula L. The phytochemical screening showed the presence of alkaloids, carbohydrates, glycosides, saponins, triterpenes, tannins, flavonoids, photobatalin and anthraqunies

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 998-1005 www.ijprajournal.com ISSN: 2456-4494

in methanol and aqueous extracts of leaf. The analysis of methanolic extract of leaves by HPTLC confirmed the presence of flavonoids(Peak 4) and alkaloids(Peak 3).

2) Vandana Gupta et. al., (2013) suggested a review of Withania Coagulans Dunal. (Paneer Doda). Withanolides are steroidal lactones having significant pharmacological activities. In various studies it has been seen that the Withania coagulans possess several medicinal properties such as hepatoprotective, antiinflammatory, antihyperglycaemic, free radical scavenging, hypolipidaemic, antimicrobial, cardiovascular, central nervous system depressant, immunomodulating, antitumour and cytotoxic activities.

3) Prakash Chandra Gupta et. al., (2012) studied Withania Coagulans Dunal- An Overview. The fruits of the plant are reported to be sedative, emetic, alterative and diuretic. Further, they are used for liver complaints, asthma and biliousness. The active compounds, in particular, withanolides isolated from the plant are considered to have antimicrobial, anti-inflammatory, antitumor, hepatoprotective, antihyperglycemic, cardiovascular, immuno-suppressive, free radical scavenging and central nervous system depressant activities.

4) Sumathi P et. al., (2010) suggested Antimicrobial activity of some traditional medicinal plants. The results showed that the minimum inhibitory concentration (MIC) of P. niruri leaf extract was 50 µg/ml against Salmonella typhi and Staphylococcus aureus, whereas, the MICs of T. bellerica fruit extract against Escherichia coli and S. aureus were 50 and 200 µg/ml respectively. However, the leaf extracts of the Andrographis paniculata, T. Chebula and V. negundo have not shown any antimicrobial activity in the tested concentrations.

5) Islam MR et. al., (2010) obeserved Analgesic, anti-inflammatory and antimicrobial effects of ethanol extracts of mango leaves and analgesic bioassay, oral administration of the ethanol leaves extract significantly ($P<0.01$) reduced the writhing response. The degree of inhibition of leaves extract was 55.8% compared to the effect of standard analgesic drug, Diclofenac Sodium (75.28%). On the other hand, though leaves extract reduce paw edema but they did not show any significant effect.

6) Preethi MP et. al., (2014) performed work on Principal Component Analysis and HPTLC Fingerprint of In Vitro and Field Grown Root Extracts of Withania Coagulans. The HPTLC system was standardized and was found out that roots of Withania coagulans, AUF Wc 024 and AUF Wc 025 had maximum withanolide A accumulation (1.17mg/g). The extractive value was found to be high for AUF Wc 021 (392.4 mg/g). Toluene: Ethyl acetate: Formic acid (5:5:1) has been standardized as the best solvent system for HPTLC analysis of withanolides.

7) Deo SS et. al., (2011) studied Antimicrobial Activity and HPLC Fingerprinting of Crude Ocimum Extract. The crude aqueous extract of Ocimum sanctum showed strong antimicrobial activity against S.aureus and moderate against others. Whereas the crude aqueous extract of Ocimum kilimandsacharicum showed moderate activity against the gram positive and gram negative organisms and strong activity against C. albicans at higher concentration, same as that shown by the standard for C. albicans.

Table 2.1 : List of drug and materials

Sr. No.	Materials	Use
1	Withania coagulans fruits	Antimicrobial agent
2	Ethanol	Solvent for extraction
3	Pectin	For preparation of agar media
4	Sodium chloride	For preparation of agar media

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 998-1005 www.ijprajournal.com ISSN: 2456-4494

5	Yeast extract	For preparation of agar media
6	Beaf extract	For preparation of agar media
7	Mayer's Reagent	For phytochemical testing
8	Hager's Reagent	For phytochemical testing
9	Alpha Napthol	For phytochemical testing
10	Cons.H_2SO_4	For phytochemical testing
11	Chloroform	Solvent for extraction
12	B.cerus	Bacteria
13	S.aureus	Bacteria
14	E.coli	Bacteria
15	Proteus vulgaris	Bacteria
16	S.mutant	Bacteria

III. METHODS :

1) Selection and collection of herbal drug :

Discuss all types of herbal drugs in a group and on the basis of further study we select W.coagulans fruit.The dried fruit of W.coagulans plant were collected from herbal drug medicine shop in Kolhapur Maharashtra.

2) Preparation of Powder :

To form the powder of W.coagulans fruit we use grinder method and prepared the powder of W.coagulans fruit. To prepare sample for extraction the coarse powder is required for the experiment. To separate the coarse powder, sieving method is used. The powder of W.coagulans fruit is passed through 10 number sieve using sieving machine and collect the coarse powder from it.

3) Preparation of Extract :

i) Cold Maceration

For cold maceration 20gm of W.coagulans dried coarse powder was weigh. Take 250ml of conical flask, then transferred 20gm of coarse powder into the beaker and add 200ml of ethanol. And extraction was continued for 48 hours at room temperature.

ii)Filtration

After completion of cold maceration the extract was filtered by using simple filtration method . Then marc was obtained.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 998-1005 www.ijprajournal.com ISSN: 2456-4494

4) Distillation[14]:

Separate out ethanol and extract by using simple distillation method based on their different volatility boiling point. In distillation process the marc was put into distillation flask and heated at temperature 550 C. • After distillation the brown colour final extract was obtained in distillate and stored in closed container.

5) Identification of chemical constituents by preliminary phytochemical test[14,15]:

The extract was used to identify the chemical constituents present in W.coagulans.

Qualitative phytochemical analysis

The ethanolic extract of w.coagulance fruit were subjected to the following chemical test.

A) Test For Alkaloids :

1) Mayer's test : To 1 ml of the extract , added 2 ml of Mayer's reagent , a dull white precipitate revealed the presence of alkaloids.
2) Hager's test : To 1 ml of the extract , added 3 ml of Hager's reagent , the formation of yellow precipitate confirmed the presence of alkaloids.

B) Test for carbohydrates :

1) Molisch test : To 2 ml of the extract, added 1 ml of alpha naphthol solution, and conc.H2SO4 through the sides of test tube. Purple or reddish violet color at the junction of the 2 liquids revealed the presence of carbohydrates.
2) Fehling's test : To 1 ml of the extract, added equal quantities of Fehling's solution A and B, upon heating formation of a brick red precipitate indicated the presence of carbohydrates.

C) Test for Steroids :

1) Liebermann Burchard test : Dissolved the extract in 2 ml of CHCL3 in a dry test tube. Added 10 drops of conc.H2SO4. The solution become red, blue, and bluish green, indicated the presence of steroid.
2) Salkowski test : Dissolve the extract in chloroform and added equal volumes of conc.H2SO4. Formation of bluish red to cheery red colour in CHCL3 layer and green fluorescence in the acid layer represented the steroids components in the tested extract.

6) Antimicrobial activity :

To carry out antimicrobial activity of given sample Nutrient agar plates were prepared in sterile condition. Then all plates were spreaded with 24hrs culture of fresh culture of all the test organisms. Well known method for antimicrobial assay compound i.-e. well diffusion method [1] was used to carry out antimicrobial activity of given sample against Gram -positive and gram-negative bacterial Pathogens. To carry out this activity small well with diameter 1cm prepared on each plate aseptically by using cork borer. Then each well is filled with 25µl, 50µl, 100µl of synthesized compound. After that all plates were kept inside refrigerator for proper diffusion of the sample inside the agar plate. Then all plates were incubated at 37 °C for 24 h. after 24 Hrs incubation plates were observed for antimicrobial activity.

IV. RESULT AND DISCUSSION :

Preliminary Qualitative Phytochemical analysis of W.coagulans :

The aqueous extract of W.coagulans to prepared to conduct preliminary phytochemical analysis. Below table shows the presence of phytochemical analysis of W.coagulans.

Table no 7.1: Preliminary Qualitative Phytochemical Test :

Sr. No.	Test	Aqueous Extract
1	Alkaloids a) Mayer's test b) Hager's test	 Present Absent
2	Carbohydrates a) Molisch test b) Fehling's test	 Present Absent
3	Steroids a) Liebermann Burchard test b) Salkowski test	 Present Present

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 998-1005 www.ijprajournal.com ISSN: 2456-4494

The phytochemical analysis of aqueous extract of w.coagulans shows that bioactive compounds such as alkaloids, carbohydrates and steroids were detected to be present in the w.coagulans . since this fruit had been used in the treatment of different ailments such as insomnia, emetic, disability, impotence ,etc. to presence of this biologically active compounds in the extract has made the plant to be known for its medicinal use especially for antimicrobial activity against pathogenic organisms.

Table No 7.2 . Confirmatory test : For the confirmation of withanoloids.

Test	**Inference**	**Confirmation of chemical constituents**
Salkowaski	Steroid Present	Withanoloids

This test proved that withaonoloids present in W.coagulans ,which can shows antimicrobial activity.

V. MATERIALS AND METHODS :

Materials: To carry out antimicrobial activity of given sample we have used Gram-positive bacterial pathogen Staphylococcus aureus, and Bacillus cereus also we have used Gram-negative pathogens such as Escherichia coli, and Proteus Vulgaris and S. mutant. All the above strains were collected from Department of Microbiology, Shivaji University Kolhapur, Maharashtra, India.

Result: The antibacterial study of given sample against gram positive and gram-negative organisms showed that the given compound is having good antimicrobial activity against all the pathogens that were used for study. Zone of inhibition in millimeter were shown in table 1 and figures were shown in Figure 1 to 5.

Table No 7.3 : Antimicrobial activity of given sample against tested pathogens.

Name of bacteria	**Zone of Inhibition in mm**		
	25µl	50µl	100µl
Bacillus cereus	21	23	26
Staphylococcus Aureus	17	19	21
Escherichia coli	15	19	24
Proteus vulgaris	14	23	26
S. mutant	-	-	15

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 998-1005 www.ijprajournal.com ISSN: 2456-4494

Figure: 1
Showing the results of the antimicrobial activity of given sample against Gram-positive bacterial pathogen Bacillus cereus.

Figure: 2
Showing the results of the antimicrobial activity of given sample against Gram-positive bacterial pathogen Staphylococcus Aureus.

Figure: 3
Showing the results of the antimicrobial activity of given sample against Gram-negative pathogens Escherichia coli.

Figure: 4
Showing the results of the antimicrobial activity of given sample against Gram-negative pathogens Proteus vulgar is.

Figure: 5
Showing the results of the antimicrobial activity of given sample against Gram-negative pathogens S. mutant.

VI. CONCLUSION :

From the entitled " Extraction And Evaluation of Antimicrobial Activity of W.coagulans ", the conclusion could be , the study has to supported the traditional used of W.coagulans have scientifically proved the antimicrobial activity. preliminary phytochemical study of ethanolic extract of W.coagulans was fond to be contain carbohydrates ,steroids and alkaloids are present. Presence of steroids in the ethanolic extract of W.coagulans concluded that , it was found to produce considerable antimicrobial effect .The ethanolic extract of W.coagulans was subjected to the antimicrobial test showed significant inhibitory effect on both gram positive and gram negative bacteria.

REFERENCES :

[1]. Sumner J. The natural history of medicinal plants. London:Timber Press;2000. pp. 16.
[2]. J.C.Smith,"The true cost of antimicrobial resistance,"BMJ, 2013
[3]. R.D. Smith, and J.C. Coast,"Antimicrobial resistance; response," NCMH,vol. 80, no. 2, pp. 8, 2002

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 5 Sep-Oct 2022, pp: 998-1005 www.ijprajournal.com ISSN: 2456-4494

[4]. M.K.Khodaei, M.J.Jafari, and M.N.Noori, "Remedial Use of Withanolides from Withania Coagulans (Stocks) Dunal,"sapub, 6-9, 2012
[5]. Prasad S., Singh P., Wahi A., Hemalatha S. Pharmacognostical Standardization of Withania coagulans Dunal. Pharmacogn. J. 2010
[6]. Ismail H., Rasheed A., Haq I.-U., Jafri L., Ullah N., Dilshad E., Sajid M., Mirza B. Five Indigenous Plants of Pakistan with Antinociceptive, Anti-Inflammatory, Antidepressant, and Anticoagulant Properties in Sprague Dawley Rats. Evid.-Based Complement Altern. Med. 2017
[7]. Ahmad R., Fatima A., Srivastava A., Khan M.A. Evaluation of apoptotic activity of Withania coagulans methanolic extract against human breast cancer and Vero cell lines. J. Ayurveda Integr. Med. 2017
[8]. Maurya R., Akanksha, Jayendra, Singh A.B., Srivastava A.K. Coagulanolide, a withanolide from Withania coagulans fruits and antihyperglycemic activity. Bioorganic Med. Chem. Lett. 2008
[9]. Gupta V., Keshari B.B. Withania Coagulans Dunal. (Paneer Doda): A Review. Int. J. Ayurvedic Herb. Med. 2013
[10]. Khodaei M., Jafari M., Noori M. Remedial Use of Withanolides from Withania Coagolans (Stocks) Dunal. Adv. Life Sci. 2012
[11]. Qasim S., Zafar A., Saif M.S., Ali Z., Nazar M., Waqas M., Haq A.U., Tariq T., Hassan S.G., Iqbal F., et al. Green synthesis of iron oxide nanorods using Withania coagulans extract improved photocatalytic degradation and antimicrobial activity. J. Photochem. Photobiol. B Biol. 2020
[12]. Tripathi D., Modi A., Narayan G., Rai S.P. Green and cost effective synthesis of silver nanoparticles from endangered medicinal plant Withania coagulans and their potential biomedical properties. Mater. Sci. Eng. C. 2019;100:152–164. doi: 10.1016/j.msec.2019
[13]. Maurya R., And A. Jayendra Chemistry and pharmacology of Withania coagulans: An Ayurvedic remedy. J. Pharm. Pharmacol. 2010
[14]. Chetan Salwaan, Amrinder Singh, Anuj Mittal, Prabhsimran Singh. Investigation of the Pharmacognostical, Phytochemical and Antioxidant Studies of Plant Withania coagulan Dunal. Journal of Pharmacognosy and Phytochemistry 2012.
[15]. .Prakash Chandra Gupta. Withania coagulans Dunal- an Overview. International Journal of Pharmaceutical Sciences Review and Research 2012.
[16]. Sangwan RS, Chaurasia ND, Mishra LN, Lal P, Uniyal GC, Sharma R. Phytochemical variability in commercial 83 herbal products and preparation of Withania somnifera (Ashwagandha). Curr Sci. 200.
[17]. R.Saratha;(Reg.no.261525152).Pharmacol ogical evalution of extraction of ethanolic extract of w. coagulans dunal (Flower buds).2017.