Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 4, 2012

Research Article

PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF *WITHANIA COAGULANS* (STOCKS) DUNAL (FRUIT)

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Received: 06 April 2012, Revised and Accepted: 13 June 2012

ABSTRACT

Phytochemical screening and antimicrobial activity of namely petroleum ether, chloroform, benzene, ethyl acetate, methanol and distilled water extracts of *Withania coagulans* (Stocks) Dunal (Fruit) were tested against various pathogens such as bacteria *(Shigella flexneri, Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus vulgaris, Enterobacter aerogenes)* and three fungi(*Aspergillus niger, Candida albicans, Trichophyton rubrum).* Phytochemical screening recorded that redusing sugar and terpenoids are present in all extracts, flavonoid are present methanol and distilled water extract tannin present only in methanol extract and saponins present in pet.ether, benzene, chloroform and ethyl acetate extract. Maximum antimicrobial activity was showed by ethyl acetate extract against *Enterobacter aerogenes* (28mm) on conc. 150mg/20disc, methanol extract showing the best activity against *Klebsiella pneumonia* (21mm) on conc. 250mg/20 discs.

Keywords: *Withania coagulans*, Antimicrobial activity, Pathogens, Medicinal plants.

INTRODUCTION

Infectious diseases are world's most important reason of untimely death, killing 50,000 people each day[1]. Resistance to antimicrobial agents is rising in a wide diversity of pathogens and numerous drug resistances are becoming common in diverse organisms[2]. The microbial fighting is mounting day by day and the viewpoint for the use of antimicrobial drugs in the prospect is still uncertain. Therefore, way to be taken to decrease this problem, for example, to control the use of antibiotic, build up research to enhance understand the genetic mechanisms of resistance, and to continue studies to develop new drugs either synthetic or natural. The final goal is to present suitable and well-organized antimicrobial drugs to the patient[3]. This has necessitated an investigation for new antimicrobial substances from other sources counting plants. Many higher plants mount up extractable organic approaches substances in quantities enough to be inexpensively management of disease. Plants have been a wealthy resource of medicines because they create wide array of bioactive molecules, most of which almost certainly evolved as chemical defence against predation or infection. There are numerous reports in the literature concerning the antimicrobial activity of crude extracts prepared from plants[4]. Therefore, it is sensible to wait for a selection of plant compounds with exact as well as general antimicrobial activity and antibiotic potential[5]. Over the past 20 years, there has been a lot of study on plants as source of new antimicrobial agents. But motionless there is an instant need to recognize novel substances lively in the direction of pathogens with high resistance[6].

Withania coagulans Dunal belongs to family Solanaceae.Withania is a little genus of vegetation, which is dispersed in the East of the Mediterranean region and extends to South Asia. The berries of the shrub are use for milk coagulation. It is generally recognized as Indian cheese maker. In Punjab, the fruits of *W. coagulans* are used as the foundation of coagulating enzyme for clotting the milk which is called paneer. The fruits are diuretic[7, 8, 9], hypoglycaemic and hypolipidemic[10]. Anti-inflammatory, antiumor, hepatoprotective, anti-hyperglycemic, cardiovascular, immuno-suppressive, free radical scavenging and central nervous system depressant activities of the plant have been reported. The use of medicinal plants by humans is as old as the origin of the human race.

MATERIALS AND METHODS

Collection

Plant sample *W. coagulans* (Fruit) was collected from various tribes living in tribal pockets of Mt. Abu, arid zone of Rajasthan, in the month of Feb, 2010. These plants were used by these tribes in their daily lives to cure various ailments.

Identification

These samples were authenticated and submitted in Ethnomedicinal Herbarium, Centre of Excellence (funded by DST), MGiaS, Jaipur (Rajasthan).

Sources of test organisms

Bacteria-Pure culture of all test organisms, namely *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi, Shigella flexneri, Proteus vulgaris, Enterobactor aerogenes* and fungi *Candida albicans, Aspergillus niger, Trichophyton rubrum* were obtained through the courtesy of Mahatma Gandhi Institute of applied Sciences (MGiaS), Jaipur, which were maintained on Nutrient broth media.Culture of test microbes

For the cultivation of bacteria, Nutrient Agar Medium (NAM) was prepared by using 20 g Agar, 5 g Peptone, 3 g beef extract and 3 g NaCl in 1 L distilled water and sterilized at 15 lbs pressure and 121°C temperature for 25-30 min. Agar test plates were prepared pouring approximately 15 ml of NAM into the Petri dishes (10 mm) under aseptic conditions. A saline solution was prepared (by mixing 0.8% NaCl) in distilled water, followed by autoclaving and the bacterial\ cultures were maintained on this medium by regular sub-culturing and incubation at 37°C for 24-48 hour. To prepare the test plates, in bacteria, 10-15 ml of the respective medium was poured into the Petri plates and used for screening. For assessing the bactericidal efficacy, a fresh suspension of the test bacteria was prepared in saline solution from a freshly grown Agar slant.

Preparation of test extracts

Crushed powders of species were successively soxhlet extracted. Later, each of the homogenates was filtered and the residue was re-extracted twice for complete exhaustion, the extracts were cooled individually. Each filtrate was concentrated to dryness in vitro and re dissolved in alcohol, until screened for antimicrobial activity.

Bactericidal assay

For both, bactericidal in vitro Disc diffusion method was adopted[11], because of reproducibility and precision. The different test organisms were proceeded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibition were measured around sterilized dried discs of Whattman No. 1 paper (6 mm in diameter), which were containing three different concentration, its control (of the respective solvent) and tetracycline as reference drugs (standard disk) separately. Such treated discs were air-dried at room temperature to remove any residual solvent, which might interfere with the determination, sterilized and inoculated. These plates were initially placed at low

temperature for 1 hour so as to allow the maximum diffusion of the compounds from the test disc into the agar plate and later, incubated at 37°C for 24 hour in case of bacteria, after which the zones of inhibition could be easily observed. Five replicates of each test extract were examined and the mean values were then referred.

The Inhibition Zone (IZ) in each case were recorded and the Activity Index (AI) was calculated as compared with those of their respective standard reference drugs (AI = Inhibition Zone of test sample/Inhibition zone of standard).

Phytochemical Screening

Phytochemical screening was performed using standard procedure:

Test for Reducing sugar (Fehlings Test) - The aqueous extract (0.5gm in 5 ml of water) was added to boiling fehling's solution (A and B) in a test tube. The solution was observed for a color reaction.

Test for Terpenoids (Salkowski Test) - To 0.5 gm each of the extract was added to 2ml of chloroform. Concentrated sulphuric acid (3ml) was carefully added to form a layer. Reddish brown coloration of the interface indicates the presence of terpenoids.

Test for Flavonoids- 4ml of extract solution was treated with 1.5ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated Hydrochloride acid was added and red colour was observed for flavonoids and orange colour for flavons.

Test for Tannins- About 0.5 g of the extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blueblack coloration.

Test for Saponins- To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

RESULT AND DISCUSSION

Phytochemical screening

Phytochemical analysis for the *W. coagulans* (Fruit) extract was performed and the phyto constituents reported in the Table no.1:

Table 1: Preliminary phytochemical screening of the plant *W. coagulans* (Fruit)

1. *Withania coagulans* (Paneerphal)-

Plants Extracts	Reducing sugar	Terpenoides	Flavonoids	Tannin	Saponin
Pet.ether	+ve	+ve	-ve	-ve	+ve
Benzene	+ve	+ve	-ve	-ve	+ve
Chloroform	+ve	+ve	-ve	-ve	+ve
Ethyl acetate	+ve	+ve	-ve	-ve	+ve
Methanol	+ve	+ve	+ve	+ve	-ve
Distilled water	+ve	+ve	+ve	-ve	-ve

(+: present) (- : absent)

The result of the phytochemical screening of *Withania coagulans* (Fruit) is presented in Table 1. This reveals moderate concentration of reducing sugar, flavonoids, saponins, terpenoids and tannin in different extraction solvents, some of which chemical compounds have been associated to antimicrobial activities and thus have curative properties against selected bacteria and fungi. Standard method were used for preliminary phytochemical screening of the extract was performed to know the phyto-constituents in the extract and it was found that petroleum extract contains reducing sugar, terpenoids and saponins, benzene extract contains reducing sugar, terpenoids and saponins, chloroform extract contain reducing sugar, terpenoids and saponins, ethyl acetate extract contains reducing sugar, terpenoids and saponins, methanol extract contains reducing sugar, terpenoids, flavonoids and tannin and distilled water extract contains redusing sugar, terpenoids and flavonoids.

Antimicrobial activity

The traditional healers use primarily water as the solvent, but plant extracts prepared with different solvents provided more consistent antimicrobial activity as also reported earlier[12]. To support this view, the six extracts of fruit of *Withania coagulans* (Fruit) showed good inhibitory activity against all the pathogens tested (Table 2).

Table 2: Antimicrobial activity of petroleum ether, benzene, chloroform, ethyl acetate, methanol and distilled water extract of *Withania coagulans* (Fruit).

I.Z. - Inhibition Concentration of zone diameter (mm). A.I. - Activity index, Extract (mg/ml)

				Sf	*Sa*	*St*	*Pv*	*Kp*	*Pa*	*Ea*	*An*	*Ca*	*Tr*
1	Pet. Ether	A1	I.Z.	11	7	0	8	0	6	6	0	0	9
			A.I.	.31	.35	0	.18	0	.37	.14	0	0	.32
		A2	I.Z.	10	8	0	9	8	7	7	0	0	0
			A.I.	.28	.40	0	.20	.20	.43	.17	0	0	0
		A3	I.Z.	13	9	0	11	0	7	7	0	0	11
			A.I	.37	.45	0	.25	0	.43	.17	0	0	.39
		A4	I.Z.	11	8	0	9	7	7	7	0	0	7
			A.I.	.31	.40	0	.20	.17	.43	.17	0	0	.25
2	Benzene	A1	I.Z.	9	8	9	8	0	8	8	8	9	12
			A.I.	.25	.40	.30	.18	.0	.50	.19	.33	.24	.42
		A2	I.Z.	8	9	8	8	7	7	7	8	8	11
			A.I.	.22	.45	.26	.18	.17	.43	.17	.33	.21	.39
3	Chloroform	A1	I.Z.	10	11	0	16	12	8	9	9	0	11
			A.I.	.28	.55	0	.36	.30	.50	.21	.37	0	.39
		A2	I.Z.	12	10	17	18	19	13	10	11	17	12
			A.I.	.34	.50	.56	.40	.47	.81	.24	.45	.45	.42
4	Ethyl acetate	A1	I.Z.	14	11	9	11	11	12	11	11	11	11
			A.I.	.40	.55	.30	.25	.27	.75	.26	.45	.29	.39
		A2	I.Z.	15	13	8	20	16	15	16	11	14	11
			A.I.	.42	.65	.30	.45	.40	.93	.39	.45	.37	.39
		A3	I.Z.	11	11	9	13	19	20	28	11	16	14
			A.I.	.31	.55	.30	.29	.47	1.25	.68	.45	.43	.50

5	Methanol	A1	I.Z.	8	10	10	0	15	14	14	8	14	6
			A.I.	.22	.50	.33	0	.37	.87	.34	.33	.37	.21
		A2	I.Z.	14	9	8	0	0	14	14	7	10	0
			A.I.	.40	.45	.26	0	0	.87	.34	.29	.27	0
		A3	I.Z.	11	11	19	16	18	14	15.33	9	17	0
			A.I	.31	.55	.63	.36	.45	.87	.37	.37	.45	0
		A4	I.Z.	10	9	10	0	10	13	15	0	11	0
			A.I.	.28	.45	.33	0	.25	.81	.36	0	.29	0
		A5	I.Z.	13	9	14	16	21	20	15	11	19	0
			A.I.	.37	.45	.46	.36	.52	1.25	.36	.45	.51	0
6	D. Water	A1	I.Z.	9	7	0	0	0	0	0	0	0	0
			A.I.	.25	.35	0	0	0	0	0	0	0	0
		A2	I.Z.	9	8	0	8	0	0	9	12	0	10
			A.I.	.25	.40	0	.18	0	0	.21	.50	0	.35
		A3	I.Z.	13	8	9	0	8	9	9	9	7	10
			A.I	.37	.40	.30	0	.20	.56	.21	.37	.18	.35
		A4	I.Z.	9	9	9	0	0	8	10	9	8	10
			A.I.	.25	.45	.30	0	0	.50	.24	.37	.21	.35
		A5	I.Z.	10	9	9	0	7	10	9	10	10	14
			A.I.	.28	.45	.30	0	.43	.62	.21	.41	.27	.50

Sf - *Shigella flexneri*, Sa - *Staphylococcus aureus*, St - *Salmonella typhi*, Pv - *Proteus vulgaris*, Kp - *Klebsiella pneumoniae*, Pa - *Pseudomonas aeruginosa*, Ee - *Enterobacter aerogenes*, An - *Aspergillus niger*, Ca - *Candida albicans*, Tr - *Trichophyton rubrum* ; 0 - no inhibition zone.

A1-50 mg/20 disc, A2-100 mg/20 disc, A3-150mg/20 disc, A4-200mg/20 disc, A5-250mg/20 disc. I.Z. - Inhibition zone, I.A. – Activity index.

Among the extracts studied, the petroleum extract showed inhibition at all concentration and showing the best activity against *Shigella flexneri* (13mm) concentration 150mg/20 discs but not showing the activity against *Salmonella Typhi, Aspergillus niger, Candida albicans*. The benzene showing the best activity against *Trichophyton rubrum* (12mm) conc. 50mg/20 disc, chloroform showing the best activity against *Klebsiella pneumonia* (19mm) on conc. 100mg/20 disc and ethyl acetate extract showing the best activity against *Enterobacter aerogenes* (28mm) on conc. 150mg/20disc, methanol extract showing the best activity against *Klebsiella pneumonia* (21mm) on conc. 250mg/20 disc and distilled water extract showing the best activity against *Trichophyton rubrum* (14mm) conc. 250mg/20 disc exhibited moderate inhibition against all the organisms. It is evident from the above results that the fruit extracts exhibit potential activity.

Withania coagulans (Stocks) Dunal is used to treat nervous exhaustion, debility, insomnia, wasting diseases, failure to thrive in children, impotence. Its fruits are used for liver complaints, asthma and biliousness Flowers of *W. coagulans* (Stocks) Dunal are used in the treatment of diabetes[13]. The root is harvested in autumn and dried for later use[14]. Some caution is advised in the use of these plants since it is toxic[15]. The phytoconstituents quantified in the one study exhibits great deal of medicinal importance like Phenolic compound as a good anti-oxidants[16], tannins having protein precipitating property[17], whereas flavonoids and flavones possessed good anti-inflammatory[18,19] and anti-oxidants activity [20]. The alcoholic extract and total alkaloids of *W. coagulans* also showed significant anti-inflammatory effect in acute inflammation induced with egg albumin induced with formalin and granulation tissue formation by cotton-pellet method [21].

ACKNOWLEDGEMENT

Author acknowledge with thanks the financial support from Department of Science and Technology, Government of Rajasthan, in the form of Centre with Potentials for Excellence in Biotechnology, sanction no F 7(17) (9) Wipro/Gaprio/2006/7358-46(31/10/2008).

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