Antibacterial Activity of Jwarahara Dashemani an Experimental Evaluation

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Abstract

Antibacterial activity of Syrup and Tablet form of the Jwarahara Dashemani of Charaka was tested against both Gram-positive and Gram negative organisms by using bioassay method with concentrations of 1000, 500 and 250 mcg/ml. Both the formulation i.e. JHD Syrup and JHD Tablet showed antibacterial activity against Pseudomonas aeruginosa and Staphylococcus aureus. But minimal activity showed against Escherichia coli and Streptococcus pyogenes. The above results were supported by phytochemical analysis. Both formulations exhibited concentration dependent activity.

Keywords

Antibacterial, JHD Syrup, JHD Tablet, JHD: Jwarahara Dashemani

Introducation

It is common folk knowledge that pyrexia is an extremely common symptom in approx all diseases of our society. Pyrexia or fever gets manifested as a symptom due to impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states. It is the body's natural defense mechanism to create an environment where infectious organism or damaged tissue cannot survive[1].

The contemporary system of medicine has ample of potent antipyrexial remedies but they also have a high risk of developing side effects. Amid of all these, it is the high time to search remedies from the traditional treasure houses, which may be proven as safe and effective anti-infectious, and antipyretic agents. Jwarahara Dashemani one of the combination explained by Acharya Charaka at Sutrasthana 4th chapter[2] has been selected for the present work and its antibacterial activity has been evaluated in two different forms.

The root of Hemidesmus indicus (Sariva) belongs to family Asclepiadaceae and ethanolic extracts posses antibacterial activity[3,4]. The Candy sugar (Sharkara) prepared from sugar cane (Saccharum spp.) which is one type of carbohydrate. The root of Cissampelos pareira (Patha) belongs to family Menispermaceae used in urinary infections, intestinal worms, etc[5]. The root of Rubia cordifolia (Manjistha) belongs to family Rubiaceae and it is credited with tonic, antiseptic, and deobstruent properties[6]. The fruit of Vitis vinifera (Draksha) belongs to family Vitaceae said to be an antioxidant, antibacterial etc. activity[6]. The fruit of Salvadora persica (Pilu) belongs to family Salvadoraceae, are carminative & diuretic and given in enlargement of spleen, rheumatism and tumors[7]. The fruit of Grewia asiatica (Parooshaka) belongs to family Tiliaceae, is astringent and stomachic. It is reported that when unripe, Phalsa fruit alleviates inflammation and is administered in respiratory, cardiac, and blood disorders, as well as in fever reduction[8]. The fruit of Terminalia chebula (Haritaki) belongs to family Combretaceae and is reported that a crude extract of it have potent and broad spectrum antibacterial activity against human pathogenic Gram positive and Gram negative bacteria[9]. The fruit of Emblica officinalis (Amalaki) belongs to family Euphorbiaceae and showed that, aqueous and ethanol extracts of P. emblica have been found to be both antifungal and antimicrobial in vitro, without any indication of cellular toxicity[10]. Medical studies conducted on Amla fruit suggest that it has antiviral properties[11] and also functions as an antibacterial and anti-fungal agent[12]. The fruit of Terminalia bellirica (Bibhitaki) belongs to family Combretaceae having four lignans (termilignan, thannilignan, hydroxy-3',4'-(methylenedioxy) flavan, and anolignan B) possessed demonstrable antifungal

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activity in vitro[13].

Materials and Methods

Plant material

The ingredients of Jwarahara Dashemani were collected during the month of July 2007 from the Gujarat Ayurved University Pharmacy, Jamnagar, Gujarat and authenticated by pharmacognosy department of IPGT & RA, Gujarat Ayurved University, Jamnagar.

Preparation of formulation

JHD Syrup

Individual drug was powdered (passed through mesh no.8) using a grinding mill. The powder of each (125 g) material (total 1125 g) was suspended in 18000 ml of water for 12 h at room temperature, and then heat was given (between 70-85°C) till 2200 ml liquid remains. The filtrate was collected and sugar candy powder (1450 g) was added and again heated (range between 70-85°C) it till the volume becomes 2200 ml, filtered and stored at room temperature in amber colored glass bottle.

JHD Tablet

Individual drug was powdered (passed through mesh no.8) using a grinding mill. The powder of each (125 g) material (total 1125 g) was suspended in 18000 ml of water for 12 h at room temperature, and heat was given (between 70-85°C) till 2200 ml liquid remained and filtered. The filtrate was collected and again heated (range between 70-85°C) it to evaporate the watery part. When material gets concentrate collected and placed in oven at 45°C for drying. After complete drying, sugar candy powder was added and converted in to granules and compressed in to tablets of 500 mg, stored in stopper glass bottle for further compliance.

Bacteria

Gram-negative bacteria such as Escherichia coli (MTCC-442), Pseudomonas aeruginosa (MTCC-441); Gram-positive Staphylococcus aureus (MTCC-96), Streptococcus pyogenes (MTCC-443) were chosen based on their clinical and pharmacological importance[14]. The bacterial strains obtained from Institute of Microbial Technology, Chandigarh and sub cultured on selective medium at 37°C for 24 h incubation.

Antibacterial activity

Study was carried out using the modified bioassay method:

Susceptibility of bacteria to both formulation and standard antibiotics

Susceptibility of bacterial strains to both formulations i.e. JHD Syrup and JHD Tablet, and standard antibiotics was determined by modified bioassay methods of Berova et al. (1994)[15] and Chen et al. (1996)[16]. The reconstitute JHD Syrup and Tablet were diluted to give 1000 mcg / ml, 500 mcg / ml, 250 mcg / ml concentration in buffer solution. Muller Hinton sterile agar plates were seeded with indicator bacterial strains (10^8cfu) and allowed to stay at 37°C for 3 h. Using a sterile cork borer, wells were made on the seeded plates and these were filled with JHD Syrup and JHD Tablet of various dilutions with standard drug concentrations, and to the second set of seeded plates antibiotic impregnated discs were added. The two sets of plates were incubated at 37°C for 18 h and the zone of inhibition measured by zone reader scale.

Phytochemical screening

Alkaloids[17]: A portion of the methanolic extract was taken in a watch glass and was evaporated; to the residue few drops of dilute Hydrochloric acid and few drops of Mayer's reagent (potassium mercuric iodide solution) were added. The creamy or white precipitate indicates the presence of Alkaloids.

Tannins[18]: To a portion of aqueous solution of sample, a few drops of very dilute solution of ferric chloride was added, Greenish to bluish black color was formed, which indicates presence of tannin.

Flavonoids[18]: Small quantity of methanolic extract was treated with sulphuric acid (20 %). Appearance of yellow coloration indicates the presence of flavanoids.

Saponins[19]: The extract was diluted with distilled water and made up to 20 ml. The suspension was shaken vigorously for 15 min. 2 cm layer of foam indicates the presence of saponins.

Triterpenes and sterols[18]: 1 ml of the extract

was evaporated to dryness. 1 ml of chloroform and 1 ml of acetic anhydride were added to the residue followed by gentle addition of few drops of conc. Sulphuric acid from the side. Formation of purple colored ring at the junction of two layers indicates the presence of triterpenes and sterols.

The present investigation of preliminary phytochemical studies, JHD Syrup and JHD Tablet revealed the presence of saponins, flavonoids, triterpenoids, sterols and tannins.

Results

Antibacterial activity of JHD Syrup and JHD Tablet was tested using the broth dilution method. Both the formulations showed greater inhibition against Pseudomonas aeruginosa and Staphylococcus aureus than other species of bacteria. However, the JHD Syrup and JHD Tablet showed minimal antibacterial activity against Escherichia coli and Streptococcus pyogenes bacteria. The results of the antibacterial activity of the compounds tested against selected organisms are presented in Table 1.

Table 1. The effect of JHD Syrup and JHD Tablet against pathogenic bacteria:

Test Sample	Concentration mcg/ml	Size of inhibition zone (mm)			
		Ec	*Pa*	*Sa*	*Sp*
JHD Syrup	1000	10	30	21	13
	500	09	25	21	11
	250	09	24	20	10
Gentamycin	100	22	22	20	22
JHD Tablet	1000	10	29	22	12
	500	08	28	21	10
	250	07	27	20	09
Gentamycin	100	22	22	20	22

Bacteria: *Ec - Escherichia coli*, *Pa - Pseudomonas aeruginosa*, *Sa - Staphylococcus aureus*, *Sp - Streptococcus pyogenes*

Table 2. The Phytochemical screening of JHD Syrup and JHD Tablet:

Sr. No.	Components	JHD Syrup	JHD Tablet
1	Tannin	Present	Present
2	Terpenoid/Sterols	Present	Present
3	Alkaloid	Absent	Absent
4	Saponin	Present	Present
5	Flavonoid	Present	Present
6	Phenols	Absent	Absent

Discussion

The present work clearly indicates the JHD Syrup & Tablet in the highest concentrations of 1000 mcg/ml, effectively control the growth of specific bacteria. Both the formulations were less effective against Escherichia coli. As the combination is known to be colon friendly nature, it may prove a great boon to the antibacterial community.

The ingredients of JHD Syrup & Tablet were reported to contain flavonoids, tannins, terpenoids and saponins. The JHD Syrup & Tablet contains flavonoids, tannins, terpenoids and saponins by qualitative results were confirmed (table 2).

The secondary metabolites of various chemical types present in the plant species are known to possess antimicrobial activities. Flavonoids are found to be effective antimicrobial substances against a wide range of microorganisms, probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall; more lipophilic flavonoids may also disrupt microbial membrane[20]. Phenolics and polyphenol compounds present in the plants are known to be toxic to microorganisms[21]. The tannin components are responsible in inactivates microbial adhesions, enzymes and cell envelope transport proteins[22]. Many plant genetic resources have been analyzed for their active constituents possessing antibacterial activities. For example, broad spectrum antibacterial activity of leaf extract of Bolusanthus speciosus is due to flavonoids[23]. Landolphia owrrience is known to possess glycosides, flavonoids, tannins, saponins, which either individually or in combination, exert antibacterial activity[24]. The antibacterial activity exhibited by JHD Syrup and JHD Tablet may be attributed to the various active constituents present in it, which exhibit antibacterial activity either due to their individual or combined action. The present findings provide a scientific base for some of the medicinal claims of JHD Syrup and JHD Tablet. The above mentioned compounds, which are present in the JHD Syrup and JHD Tablet, may influence activity in an effective manner and may also have multiple actions against bacteria. Elucidation of these processes is necessary to define the exact mechanisms of active principles present in JHD Syrup and JHD Tablet.

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