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Research Article

IN VITRO EVALUATION OF BILVADI AGADA (HERBO-MINERAL COMPOUND) FOR ANTI-MICROBIAL AND ANTI-FUNGAL ACTIVITY

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Abstract

'Bilvadi Agada' is one of the most frequently prescribed herbo-mineral compound preparations in variety of ailments ranging from mild fever to severe diarrhoea including various poisonous conditions. The present study was taken to investigate the *in vitro* antimicrobial and anti-fungal activity of this formulation. Agar-well method was used for *in vitro* antibacterial and antifungal screening. Zones of inhibition were observed in disc diffusion for antimicrobial investigation against selected standard bacterial and fungal strains. The formulation – 'Bilvadi Agada' showed average zone of inhibition ranging from 2-8 mm suggesting its activeness against the tested microorganisms.

Keywords: Bilwadi Agada, Agadatantra, *In-vitro*, Anti-microbial, Anti-fungal

INTRODUCTION

Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential, indeed, that they contained what we would currently characterize as antimicrobial principles, was well accepted. Since antiquity, man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies. Apart from this Many efforts have been made to ascertain novel antimicrobial compounds from diverse kinds of sources such as microorganisms, animals and minerals.[1] In India Ayurveda is one of the noteworthy systems of traditional medicine practice that uses mainly certain plants for the treatments of ailments in both man and other animals.[2-4] Plants are used directly as therapeutic agents, as well as starting material for the synthesis of drugs or as models for pharmacologically active compounds.[5] Now a day herbal medicines are gaining growing interest because of their cost-effective, eco-friendly attributes and true relief from disease condition. The increasing prevalence of multidrug resistant strains of bacteria and the recent emergence of strains with reduced susceptibility to antibiotics raises the spectre of untreatable bacterial infections and adds urgency to the search for new infection fighting strategies.[6] Consequently plants as single herb and compound drugs prepared from it should be probed to comprehend their properties, safety and efficacy for a search of new potent antimicrobial compounds.[7] Bilwadi Agada is mentioned in Ashtanga Hridaya chapter 36 / 84-85[8] comprises the effect in versatile infective conditions like Visuchika, Jwara, Garavisha and various poisonous conditions including venomous bites. So it is very much essential to evaluate the effects of this compound preparation against certain microbes. Present paper highlights the results of contents of Bilwadi Agada analyzed both as potential antimicrobial and anti-fungal in the form of compound drug as well as a source for natural compounds that act as new anti-infection agents.

Need and Significance

In last three decades, numbers of new antibiotics have produced, but clinical efficacy of these existing antibiotics is being threatened by the emergence of multi drug-resistant pathogens. In Ayurvedic literature various modalities as well as drugs are prescribed for the diseases which have infective focci according to modern medical science. Principal internal medicines administered in Ayurveda for such conditions are herbo-mineral in origin. Contrary to the synthetic drugs, antimicrobial substances of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious disease. Most of the higher plants produce a large number of diverse chemical compounds with different biological activities. Therefore such plants should be investigated to understand their properties, safety and efficacy and for a search of new potent antimicrobial compounds and fractions.

Aim and Objectives

The aim of this study was to evaluate the antimicrobial activity of compound herbal preparation, 'Bilwadi Agada'

- To evaluate anti-microbial effect of Bilwadi Agada on standard bacterial strains.

- To evaluate anti-fungal effect of Bilwadi Agada on certain standard fungal strains.

MATERIALS AND METHODS

Table 1: Ingredients of Bilwadi Agada: (A.H.U. 36 / 84-85)[8]

S. No	Name of the content	Latin Name
1	Bilva (moola)	*Aegle marmelos* corr
2	Surasa (pushpa)	*Ocimum sanctum* linn
3	Karanja (phala)	*Pongamia pinnata* Perri
4	Tagara	*Valeriana wallichii* DC
5	Devadaru	*Cedrus deodara* Roxb
6	Haritaki	*Terminalia chebula* Retz
7	Vibhitaki	*Terminalia bellirica* Roxb
8	Amalaki	*Emblica officinalis* Gaertn
9	Shunthi	*Zingiber officinale* Rosc
10	Maricha	*Piper nigrum* Linn
11	Pippali	*Piper longum* Linn
12	Haridra	*Curcuma longa* Linn
13	Daruharidra	*Berberis aristata* DC
14	Bhavana with Aja mutra (Goat's urine)	*Capra indica* - Urine

Photo Gallery of the Ingredients (Bilwadi Agada)

Figure 1: *Aegle marmelos*

Figure 2: *Ocimum sanctum*

Figure 3: *Pongamia pinnata*

Figure 4: *Valeriana wallichii*

Figure 5: *Cedrus deodara*

Figure 6: *Terminalia chebula*

Figure 7: *Terminalia bellirica*

Figure 8: *Emblica officinalis*

Figure 9: ***Zingiber officinale***

Figure 10: ***Piper nigrum***

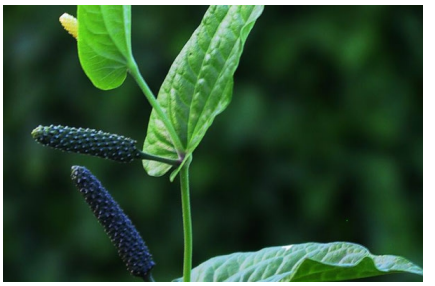

Figure 11: ***Piper longum***

Figure 12: ***Curcuma longa***

Figure 13: ***Berberis aristata***

Plan of Study

The antimicrobial activity was assessed qualitatively and quantitatively by the presence or absence of inhibition zones. Micro-organisms strains of *E. coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhi* were used for assessing the anti microbial activity with standard Cefpodoxime (10 mg / ml). Fungal strains *Asperigillus niger* and *Candida albicans* were used for anti-fungal activity with control Fluconazole in the concentration of (10 mg / ml).

Study Protocol

Antimicrobial activity will be determined by Agar well method. Muller Hinton and Saboured Dextrose Broth were used as medium for bacterial and fungal strains respectively. Positive control for bacterial culture was carried out under the similar condition by using Cefpodoxime dispersible tablets (10 mg / ml) and control for fungal culture was Fluconazole tablet (10 mg / ml). The petri-dishes with the bacterial and fungal cultures will be incubated at 37 ± 20 C for 24 h and 27 ± 20 C for 48 h respectively. The assessment of anti microbial activity was based on the measurement of diameter of inhibition zone formed. The experiment was repeated twice and the results were taken as mean of two readings.

Preparation of Extract

Bilwadi Agada – herbo-mineral formulation was subjected to simple percolation process. Distilled water was used for preparation of extracts required for the study.

OBSERVATION AND ANALYSIS

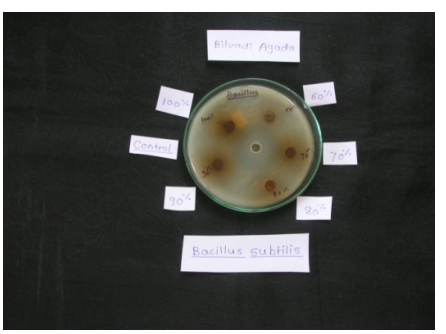

Figure 14: ***Bacillus subtilis***

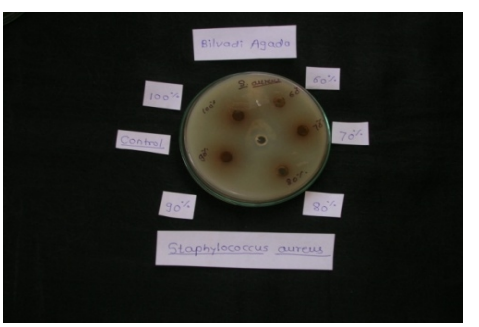

Figure 15: ***Staphylococcus aureus***

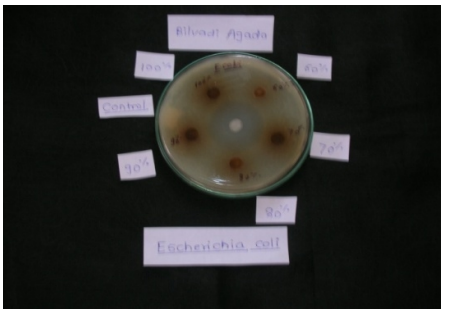

Figure 16: ***Escherichia coli***

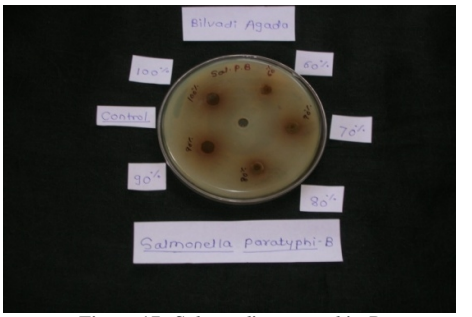

Figure 17: ***Salmonelia paratyphi - B***

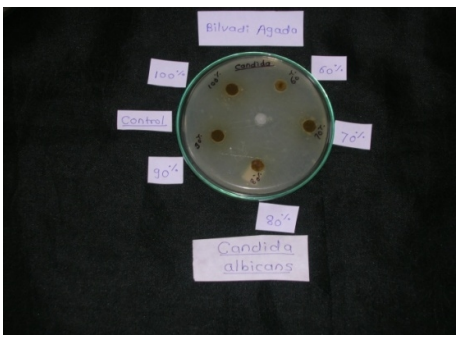

Figure 18: ***Candidia albicans***

Figure 19: ***Aspergillus niger***

Table 2: Antimicrobial Activity of Bilwadi Agada

S. No.	Organism	Bilwadi Agada Concentrations					Control
		60 %	70 %	80 %	90 %	100 %	
1	*Bacillus subtilis*	--	--	--	--	--	25 mm
2	*Staphylococcus aureus*	--	--	--	1 mm	2 mm	05 mm
3	*Escherichia coli*	--	--	--	--	--	27 mm
4	*Salmonelia paratyphi - B*	--	2 mm	4 mm	6 mm	8 mm	33 mm

Table 3: Anti-fungal Activity of Bilwadi Agada

Organism	Bilwadi Agada Concentrations					Control
	60 %	70 %	80 %	90 %	100 %	
Candidia albicans	--	--	--	2 mm	4 mm	6 mm
Aspergillus niger	--	--	--	2 mm	4 mm	--

Table 4: Total Microbial Load Count

Parameter	Value	Unit
Total Plate count	14×10^3	Cuf / g
Total fungal count	3×10^3	Cuf / g

RESULTS

The potential sensitivity of Bilwadi Agada was obtained against all the microorganisms tested as zone of inhibition. The final results are based on the measurement of mean diameter of inhibition zone formed on the completion of study.

In Table 2 the drug displayed various activities against microbes inhibiting it at various concentrations ranging from 60 % to 100 %. The inhibition zones against *Staphyloccocus aureus* were 1 mm and 2 mm at 90 % and 100 % concentration respectively comparable to the standard antibiotic Cefpodoxime with inhibition zone of 5 mm. Bilwadi Agada also showed activity against *Salmonelia paratyphi – B* with inhibition zone of 2 mm, 4 mm, 6 mm and 8 mm in concentration of 70, 80, 90 and 100 % respectively. However it has no activity against *E. coli* and *Bacillus subtilis*.

In Table 3 Bilwadi Agada showed activity against *Candidia albicans* and *Aspergillus niger* with 2 mm and 4 mm zone of inhibition at 90 % and 100 % concentration respectively in comparison to the control drug Fluconazole with 6 mm zone of inhibition to *Candidia albicans.*

Table 4 clearly indicates that the microbial count is within the range of safe consumption i.e. TPC $\leq$ 50,000 and TFC $\leq$ 5000

DISCUSSION

Reviews of activities of individual ingredients of Bilwadi Agada showed potent anti-microbial as well as antifungal activities. Crude extracts of *Aegle marmelos* (hexane, cold methanol and hot methanol extracts at a concentration of 100 mg / ml) showed positive results especially against *E. coli*.[9] another study showed that ethanolic leaf extracts of *Aegle marmelos* exhibited significant activity towards bacterial strains like *B. subtilis* and *E. coli* were more sensitive towards the treatment when compared to *S. aureus*.[10]

Similarly *O. sanctum* displayed marked antibacterial efficacy against *E. coli*, *S. aureus* and *B. subtilis.*[11,12] Likewise *Pongamia pinnata*[13-16] *Valeriana wallichii*[17-20] *Cedrus deodara*[21,22] *Terminalia chebula* as anti-microbial[23-33] and anti-fungal[34-37] *Terminalia bellirica*[38-40] *Emblica officinalis*[41-45] *Zingiber officinale*[46-49] *Piper nigrum*[50-52] *Piper longum*[53-55] *Curcuma longa*[56-60] *Berberis aristata*[61-65] also have exhibited remarkable activity against various gram +ve and gram –ve bacteria in number of studies conducted by scholars earlier. One of the noteworthy finding observed in present study is that, 'Bilwadi Agada' does not demonstrate any activity against *E. coli* which are one or the other way beneficial to the gut for its normal functioning comparable to the control drug which may affect intestinal flora and disturb G.I. functioning.

CONCLUSION

The antimicrobial and anti-fungal activity of Bilwadi Agada at higher concentration is comparable with Cefpodoxime and Fluconazole with concentration of 10 mg / ml each. Present study has confirmed the antimicrobial potentials of compound herbo-mineral preparation supporting its application may be as a preventive remedy for various microbial diseases. It also exhibited moderate to mild antimicrobial activity against most of the tested bacteria and fungi. Nevertheless authentic use of Bilwadi Agada may require further fundamental study vis-à-vis higher concentrations and samples.

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