RESEARCH ARTICLE

Pre-clinical appraisal of "*Dooshivishari Agada*" for antimicrobial, antifungal, and antioxidant activity: *In vitro* trial

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Abstract

Background: *"Dooshivishari Agada"* is one of the frequently prescribed herbomineral compound medications in a variety of ailments including venomous bites and stings in South India especially in Kerala. The compound preparation is a contribution of *Vagbhata's Ashtanga Hridayam*, *Uttaratantra*, and chapter 35/39–40 which indicates are benefits and versatile applicability in various poisonous infectious insect bites. **Materials and Methods:** The present study was planned to explore the *in vitro* antimicrobial, antifungal, and antioxidant activity of this formulation. Agar-well method was used for screening *in vitro* antibacterial and antifungal activity. Zones of inhibition were observed in disc diffusion for antimicrobial investigation against selected standard bacterial strains of *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhi* and *Shigella,* and fungal strains *Aspergillus niger,* and *Candida albicans*. Cefpodoxime and fluconazole were used as antibacterial and antifungal control with a concentration of 10 mg/ml, respectively, to compare the effects. **Results:** The formulation - "*Dooshivishari Agada*" showed an average zone of inhibition ranging from 17 to 33 mm suggesting its activeness against the tested microorganisms and confirmed its antimicrobial perspective. Antioxidant activity was calculated in terms of ascorbic acid which was observed as 10.91. **Conclusion:** *Dooshivishari Agada* undoubtedly exhibits its antimicrobial, antifungal, and antioxidant potentials. Precise and rational therapeutic application of this formulation will certainly prove beneficial.

Key words: *Agadatantra*, antifungal, antimicrobial, antioxidant, Ayurveda, *Dooshivishari Agada*

INTRODUCTION

Plants and many herbal formulations are being used since centuries as remedies in several traditional systems on medicine including Ayurveda. Adverse drug reactions and untoward expenses associated with the conventional system of medicines have motivated the need for research into herbal drugs. Holistic approach of Ayurveda medicines and procedures makes it effective as well as gentle without any complications. Apart from this in recent times, there is remarkable augmentation in antibiotic-resistant strains of various pathogens which are clinically important.[1-3] This embarrassing situation with the failure of antibiotics has led the entire world in search of alternative medication from herbs as it can play a significant role in as natural sources of life-saving drugs in designing newer formulations.[4,5] Non-availability and soaring cost of new generation antibiotics with limited effective span lead to increase in morbidity and mortality rate. Therefore, pharmaceutical industries are now being lured to work on several herbal drugs either in single or in combination form to appraise its quality and efficacy for diagnostic and or therapeutic purpose.

Another major issue related to human health is oxidative stress which leads to many chronic and degenerative diseases

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Received: 16-11-2017
Revised: 01-12-2017
Accepted: 15-12-2017

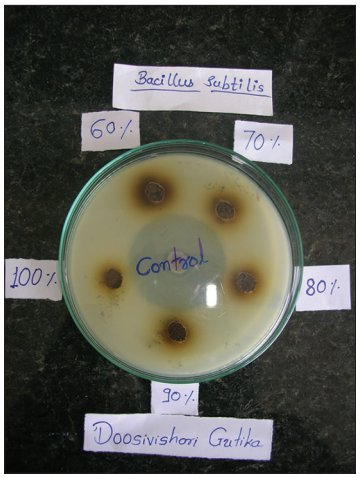

Figure 1: *Bacillus subtilis*

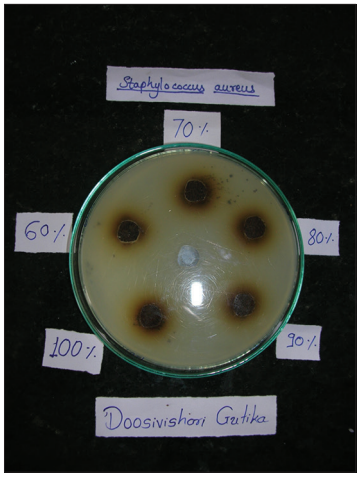

Figure 2: *Staphylococcus aureus*

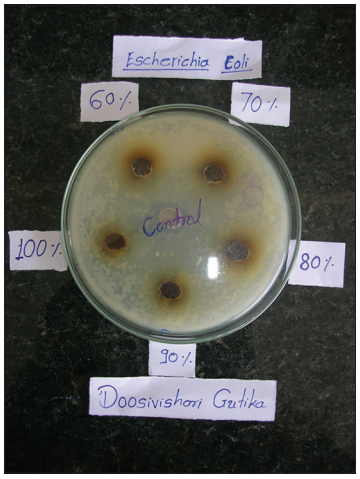

Figure 3: *Escherichia coli*

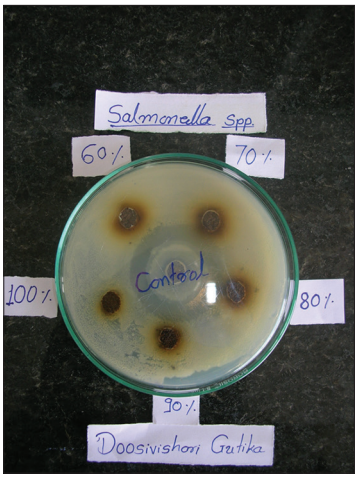

Figure 4*: Salmonella typhi*

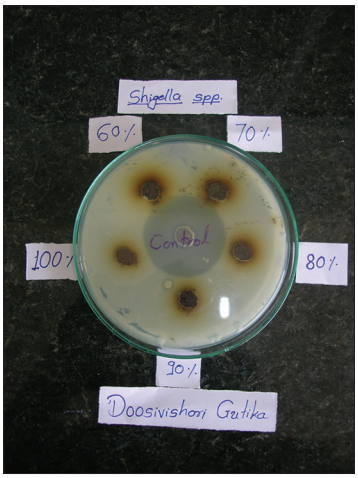

Figure 5: *Shigella flexneri*

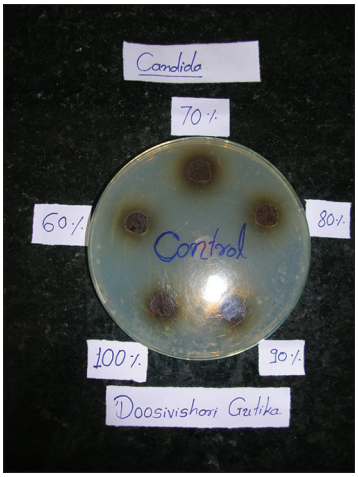

Figure 6: *Candida albicans*

of vital systems such as ischemic heart disease, diabetes mellitus, neurodegenerative diseases, and aging.[6] This oxidative progression produces free radicals in foods and human body.[7,8] The action of these free radicals can be reduced by the use of substances possessing antioxidative defense mechanism, popularly known as antioxidants. These substances help in breaking free radical chain reaction. Therefore, antioxidants have fascinated substantial attention of the medical field with regard health and longevity.[9,10]

Dooshivishari Agada is one of the Ayurvedic herbomineral formulations extremely useful in dormant and cumulative poisoning and its complications, insect poisoning and other associated signs and symptoms.[11] *Dooshivishari Agada*

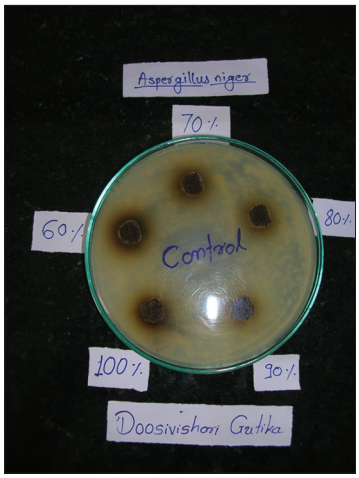

Figure 7: *Aspergillus niger*

is frequently prescribed by Ayurveda physicians as well as traditional Vaidyas of Kerala in day-to-day life in the management of various skin ailments and effects of polluted air and water. Keeping this in mind, the present study was premeditated to evaluate the *in vitro* effects of *Dooshivishari Agada* with special reference to its antimicrobial, antifungal, and antioxidant activities.

MATERIALS AND METHODS

Dooshivishari Agada was procured from market prepared by a reputed good manufacturing practice certified pharmacy in tablet form. It consists of following ingredients in equal quantity as shown in Table 1.

Preparation of Extract

Dooshivishari Agada was subjected to simple maceration, percolation, and infusion techniques process. Distilled water was used for the preparation of extracts required for the study.

Microorganisms

The microorganisms used in the present trial were procured from Nikhil Analytical and Research Pvt., Ltd., Sangli, Maharashtra. Standard cultures of different species of two Gram-positive, three Gram-negative bacteria, and two fungi strains including pathogenic and non-pathogenic were considered for this trial as shown in Table 2.

Plan of Study

For evaluation of antifungal activity

For the evaluation of antimicrobial activity agar diffusion assay method was employed.[12]

Sterile Mueller-Hinton agar well media was prepared in Petri dish, and bacteria were inoculated in the media. Each dish

Table 1: Ingredients of *Dooshivishari Agada*

Sanskrit Name	Latin/English name
Pippali	*P. longum* Linn.
Dhyamaka	*C. martini* Roxb.
Jatamansi	*N. jatamamsi* DC.
Lodhra	*S. racemosa* Roxb.
Ela	*E. cardamomum* Maton.
Suvarchika	Salt petre
Kutannatam	*O. indicum* Benth.
Natam	*V. wallichii*
Kushta	*S. lappa* DC.
Yashtimadhu	*G. glabra* L.
Chandana	*S. album* L.
Gairika	Red ochre

P. longum: Piper longum, C. martini: Cymbopogon martini, N. jatamansi: Nardostachys jatamansi, S. racemosa: Symplocos racemosa, E. cardamomum: Elettaria cardamomum, O. indicum: Oroxylum indicum, V. wallichii: Valeriana wallichii, S. lappa: Saussurea lappa, G. glabra: Glycyrrhiza glabra, S. album: Santalum album

Table 2: Bacterial and fungal strains

Organism	Strain
Gram-positive Bacteria	*B. subtilis* (MTCC 441)
	S. aureus (MTCC 96)
Gram-negative Bacteria	*E. coli* (MTCC 443)
	S. typhi (MTCC 733)
	S. flexneri (MTCC 1457)
Unicellular fungi	*C. albicans* (MTCC 227)
Filamentous fungi	*A. niger* (MTCC 282)

B. subtilis: Bacillus subtilis, S. aureus: Staphylococcus aureus, E. coli: Escherichia coli, S. typhi: Salmonella typhi, S. flexneri: Shigella flexneri, C. albicans: Candida albicans, A. niger: Aspergillus niger

comprised 6 wells prepared under stern aseptic measures. In these, various concentrations of the extracts in the ratio of 60–100% were introduced. The antimicrobial activity of *Dooshivishari Agada* was assessed qualitatively and quantitatively by the presence or absence of inhibition zones in comparison with standard Cefpodoxime dispersible tablets in the concentration of 10 mg/ml.

For evaluation of antifungal activity

Saboured Dextrose agar was used as a medium for the cultivation of fungal strains. 6 wells were prepared in similar fashion as mentioned in each Petri dish under aseptic conditions and fungal spores were inoculated. Extracts of *Dooshivishari Agada* were introduced in it with different concentration ranging from 60 to 100%. For the evaluation of antifungal activity of *Dooshivishari Agada*, fluconazole was used as a control in the concentration of 10 mg/ml.

Both these cultures (bacteria and fungal) were incubated at 37 ± 2°C and 27 ± 2°C for 6 days, respectively. At the end of the incubation period, the media were examined for the zone of inhibition. The assessment of antimicrobial and antifungal activity was based on the measurement of the diameter of inhibition zone formed. The zones of inhibition were measured with the help of Vernier caliper in millimeter. The experiment was repeated twice, and the results were taken as the mean of two readings.

For evaluation of total antioxidant capacity

Dooshivishari Agada was coarsely converted powder and extracted with 95% methanol by a Soxhlet apparatus at 45°C. The solvent used was completely separated by rotary evaporator. The extract was concentrated and dried with reduced pressure. Thus obtained crude extract of *Dooshivishari Agada* was used further to evaluate its antioxidant properties. The total antioxidant capacity of methanol extracts is evaluated by the method of Prieto *et al*.[13] using ammonium molybdate reagent and a spectrophotometer. It is compared to that of ascorbic acid. Quantitative analysis of antioxidant activity of *Dooshivishari Agada* was attributed as the number of gram equivalents of ascorbic acid.

For evaluation total polyphenols

Total polyphenols were evaluated using a method using Folin–Ciocalteu reaction, with tannic acid as a reference.[14,15]

OBSERVATIONS AND RESULTS

Antibacterial and antifungal activity of *Dooshivishari Agada* were studied in different concentrations ranging from 60 to 100%. Table 3 summarizes that zone of inhibition exhibited by *Dooshivishari Agada* for *Staphylococcus aureus* (MTCC 96) 25 mm at 60% concentration. It was gradually increasing, and at 100% concentration, it was observed as 33 mm in comparison to 25 mm of Cefpodoxime. Similar results were seen in other organisms. For *Bacillus subtilis* (MTCC 441) the zone of inhibition was ranging from 17 to 20 mm, for Gram-negative bacteria such as *Escherichia coli* (MTCC 443) it was least, i.e., 13 mm at 100% concentration, whereas for *Salmonella typhi* (MTCC 733) it was 14 mm in comparison to 18 and 28 mm for control, respectively. However, promising results were observed against *Shigella flexneri* (MTCC 1457) wherein the zones of inhibition were ranging from 15 to 22 mm as compared to 34 mm of control, i.e., Cefpodoxime [Figures 1-5].

Dooshivishari Agada also showed antifungal activity against *Aspergillus niger* (MTCC 282) and *Candida albicans* (MTCC 227) ranged from 10 to 20 mm of growth inhibition zones.

Table 4 summarizes that total ash value of *Dooshivishari Agada* was 13.47% w/w, total phenols 258.32 mg/100 g, and antioxidant activity was observed as 10.91 mg/100 g in terms of ascorbic acid. Total microbial count was 12×10^4 cfu/g and 04×10^2 cfu/g for bacterial and fungal strains [Figures 6 and 7].

DISCUSSION

Standardization and preclinical appraisal of Ayurveda drugs have become very essential nowadays as there is increased demand of these medicines from entire world. *Dooshivishari Agada*, one of the herbomineral Ayurvedic formulations is popular for its versatile therapeutic applications among Ayurveda physicians. Present work was a small step to assess and establish the *in vitro* findings of this compound preparation.

There are several similar studies suggesting the antimicrobial, antifungal, and antioxidant activities of single herbs as well as compound preparations from Ayurveda.[16-20] Previous studies also show that some of the individual ingredients of *Dooshivishari Agada* also have potential antimicrobial as well as antifungal activities.[21-23] One of the remarkable finding observed in the present study is that *"Dooshivishari Agada"* does not exhibit strong antimicrobial activity against *E. coli* which is one or the other way beneficial to the gut without affecting intestinal flora and intestinal functioning. Total

Table 3: Antibacterial and antifungal activities of *Dooshivishari Agada*

Parameter	Control	*Dooshivishari Agada* concentration				
Antibacterial activity	Cefpodoxime	60%	70%	80%	90%	100%
S. aureus (MTCC 96)	24 mm	25	26	27	31	33
B. subtilis (MTCC 441)	32 mm	17	17	18	19	20
E. coli (MTCC 443)	18 mm	-	-	-	10	13
S. typhi (MTCC 733)	28 mm	-	-	09	12	14
S. flexneri (MTCC 1457)	34 mm	15	17	18	18	22
Antifungal activity	Fluconazole	60%	70%	80%	90%	100%
A. niger (MTCC 282)	22 mm	-	-	10	12	14
C. albicans (MTCC 227)	31 mm	13	16	18	18	20

B. subtilis: Bacillus subtilis, S. aureus: Staphylococcus aureus, E. coli: Escherichia coli, S. typhi: Salmonella typhi, S. flexneri: Shigella flexneri, C. albicans: Candida albicans, , A. niger: Aspergillus niger

Table 4: Antioxidant activity and total phenols in *Dooshivishari Agada*

Parameter	Value	Unit
Total ash	13.47%	w/w
Total phenols	258.32	mg/100 g
Antioxidant activity in terms of ascorbic acid	10.91	mg/100 g
Total plate count	12×10^4	cfu/g
Total fungal count	04×10^2	cfu/g

microbial load count was within the range of safe consumption.

The total polyphenol content directly affects the effectiveness of drug and also the antioxidant activity. These phenolic components are phenolic acids and phenolic diterpenes.[24] Redox properties of these phenolic compounds play an imperative role in absorbing and neutralizing free radicals, extinguishing singlet and triplet oxygen and or decomposing peroxides.[25] As per results of the present study, the total phenols are 258.32 mg/100 g, showing the antioxidant capacity of *Dooshivishari Agada* is 10% of the ascorbic acid, therefore, can be considered as good.

The ash values are beneficial in determining the quality and purity of the drug as well as to assess the existence of inorganic substances in the formulation. Here it was evaluated as 13.47% w/w which might be due to the presence of *Gairika* in *Dooshivishari Agada*. Earlier studies conducted on *Dooshivishari Agada* for its standardization shows that water soluble extract of *Dooshivishari Agada* was more in comparison to alcoholic extract indicating its bioavailability in water media.[26]

CONCLUSION

The antimicrobial, antifungal, and antioxidant activity of *Dooshivishari Agada,* when compared at a higher concentration with the established conventional drugs, confirmed its potentials. Results of this study support and indicate its application as a preventive remedy for various microbial diseases. However, authentic use of *Dooshivishari Agada* may be required for further distinguished work with other pathogens.

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Source of Support: Nil. **Conflict of Interest:** None declared.