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# Preliminary Antimicrobial Screening of Compounds (*Emblica Officinalis* Gaertn., *Terminalia chebula* Retz., *Piper longum* Linn., *Plumbago zeylanica* Linn.)

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## Abstract

Evaluation of antimicrobial effect of hydroalcholic extract of polyherbal drug *Amalakyadi gana* of sushruta samhita in sutra sthana 38th chapter containing *Amalaki,* (Emblica officinalis Gaertn.), *Haritaki* (Terminalia chebula), *Pippali* (Piper longum Linn.,) and *Citraka* (Plumbago zeylanica Linn.) mentioned as *Sarvajvarahara* (to alleviate all kind of fever). It is *Caksusya* (Beneficial to eye), *Dipana* (enhances the agni), *Vrsya* (Aphrodisiac) and *Kapharocakan* (Eversion of food due to Kapha). The antimicrobial activity of newly synthesized compound was first screened by disc diffusion method against Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC(American Type Culture Collection) 27893, Staphylococcus aureus ATCC 25323 (Gram-positive) and four fungal strains namely Candida albicans ATCC 90028, Candida krusei ATCC 6258, Candida tropicalis ATCC 750, Candida parapsilosis ATCC 22019 according to the guidelines of National Committee for Clinical Laboratory Standards (NCCLS, 1997). The Zone of inhibition (in mm) was formed maximum in Staphylococcus aureus (25±0.35) in comparison to Standard drugs (10µg/disc) - 24(Ampicilin). In other microorganism Pseudomonas aeruginosa, Candida albicans, Candida tropicalis, Escherichia coli, Candida krusei, C. parapsilosis and Plesiomonas shigelloides , the zone of inhibition was observed as follows; 16±0.57, 12±0.47, 12±0.39, 11±0.68, 11±0.67, 11±0.29 and 10±0.41, respectively.

**Keywords** *Amalakyadi gana, Amalaki* (Emblica officinalis Gaertn), *Haritaki* (Terminalia chebula), *Pippali* (Piper longum Linn.,), *Citraka*

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## INTRODUCTION

Infectious diseases are the world's leading cause of Fever, killing almost 50 thousand people every day. Moreover, the increasing emergence of resistant pathogenic strains to the existing drugs and new infectious diseases has necessitated the need for searching novel molecules with better antimicrobial properties than the existing ones[2] (Bhagat et al., 2012). Plant extracts and essential oils have been used as alternatives to antibiotic due to their antimicrobial activities and favorable effect on the animal intestinal favourable effect on the animal intestinal system[3] (Al-Kassien, 2009). Spices and herbs and their constituents are generally recognized to be safe, either because of their traditional use without any documented detrimental impact or because of dedicated toxicological studies[4] (Frankic, 2009). Being a rich source of secondary biomolecules which exhibit significant pharmacological effects, spices and herbs appeal to many consumers who question the safety of synthetic food additives[5] (Craig, 1999). Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper cost than modern medicine.

## SOURCE OF DRUG

The fruit of *Amalaki* (Emblica officinalis Gaertn.), *Haritaki* (Terminalia chebula Retz.), *Pippali* (Piper longum Linn) and root of *Citraka* ( Plumbago zeylanica Linn) was taken. The mature fruit of *Amalaki* and *Haritaki* was collected from the Ayurvedic Dravyaguna garden, B.H.U., *Citraka* root was collected from the Rajiva Gandhi south Campus Barkacha, Mirzapur. The fruit of *Pippali* was purchased from the local crude market goladinanath Varanasi. After ensuring that the drug is more than 1year old . Drug identified by the teacher of *Dravyaguna* department in faculty of Ayurveda B.H.U.

**Table - 1 Literature Review of Drugs in *Samhita* and *Chikitsa grantha***

| S. No | Samhita and Chikitsa grantha | Name | Prayoga | Reference |
|---|---|---|---|---|
| 1 | Caraka samhita | Amalaka, Abhaya Amalaka,Pippali,Haritaki Dhatriphal, Pippali,Citraka | Jvarahara MahakaÒaya Visamajvarahara Granthijvarahara | Su.4/39 Ci.16/93,94 Ka.1/16 |
| 2 | Sushruta | Amalaka,Haritaki,Pippali, | Sarvajvaraha | Su.38/60 |

| | | | | |
|---|---|---|---|---|
| | samhita | Citraka | ra | |
| 3 | Astanga samgraha | Abhaya, Amalaka, Pippali | Visamajvarahara | Ci.10/17 |
| 4 | Astanga Hridaya | Dhatri, Pippali, Haritaki | Jvarahara | Ci.1/100,101 |
| 5 | Siddhayoga | Amalaka,Abhaya,Krisna, Citraka | Sarvajvaraharа | Ci. 1/177 |
| 6 | Chikitsa Kalika | Krisna,Agni,Pathya, Amalaka | Sarvajvaraharа | Ci. 1/108 |
| 7 | Cakradatta | Amalaka,Abhaya,Krisna, Citraka | Sarvajvaraharа | Ci. 1/106 |
| 8 | Vangasena | Amalaka,Abhaya,Krisna, Citraka | Sarvajvaraharа | Ci. 1/136 |
| 9 | Sharngadhara | Amalaka,Citraka,Pathya, Pippali | Sarvajvaraharа | Madhya Khanda.6/7 |
| 10 | Bhavaprakasha | Amalaka,Citraka,Pathya, Pippali | Sarvajvaraharа | Madhya Khanda.1/821 |
| 11 | Yogaratnakara | Amalaka, Abhaya,Krisna, Citraka | Sarvajvaraharа | Ci.2/201 |
| 12 | Bhaisajyaratnavali | Amalaka, Abhaya,Krisna, Citraka | Sarvajvaraharа | Ci.5/146 |

## Media used

Muller-Hinton agar and broth (Hi-media, Mumbai, India), Sabouraud dextrose agar pH 7.3±0.2 (Hi-media), were used for antibacterial and antifungal activity respectively.

**Tested microorganism**

A total of 3 bacterial strains viz. Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27893, Staphylococcus aureus ATCC 25323 (Gram-positive) and four fungal strains namely Candida albicans ATCC 90028, Candida krusei ATCC 6258, Candida tropicalis ATCC 750, Candida parapsilosis ATCC 22019 were used in the investigation. All cultures were obtained from American Type Culture Collection (ATCC), MTCC (Microbial Type Culture Collection), clinical strain preserved at Department of Microbiology, Institute of Medical Sciences, BHU, and Varanasi, India. The fresh bacterial broth cultures were prepared before the screening procedure.

**Preparation of sample extract for microbiological assay**

About 1 g of extract was dissolved in 10 ml (100 mg/ml) of peptone water to obtain a stock solution and the working solution was prepared. The extract was diluted as 1:10 equivalent to 100 mg/ml and 1:5 dilution equivalent to 50 mg/ml, from which 5 µl was dispensed on a sterile disc of whatman's filter paper No.1 of 6 mm diameter for susceptibility testing.

**Antimicrobial susceptibility test**

The disc diffusion method was used to screen the antibacterial activity and antifungal activity[17]. Muller Hinton agar (MHA) plates were prepared by pouring 15 ml of molten media into sterile petridisc. The fresh grown bacteria were suspended in sterile saline to achieve concentration of $10^{6}$ cfu/ml. This suspension was spread on the surface of MHA agar plates. The plates were allowed to dry for 5 min. The different

concentrations of extract (50 mg/ml) were put on 6 mm sterile disc of Whatman filter paper No.1. The disc was then placed on the surface of medium and the compound was allowed to diffuse for 5 min and the plates were kept for incubation at 37°C for 24 h for bacteria and 48 h at 35°C for fungal agents. At the end of incubation, inhibitions zones were examined around the disc which if present were measured with transparent ruler in millimeters.

| Microorganism | Zone of inhibition (in mm) | Standard drugs (10µg/disc) |
|---|---|---|
| Pseudomonas aeruginosa ATCC 27893 | 16±0.57 | 30 Tobramycin |
| Escherichia coli ATCC 25922 | 11±0.68 | 26 (Norfloxacin) |
| Staphylococcus aureus ATCC 25323 | 25±0.35 | 24 (Ampicilin) |
| Candida albicans ATCC 90028 | 12±0.47 | 25µg/disc (Fluconazole) |
| Candida krusei ATCC 6258 | 11±0.67 | 25µg/disc (Fluconazole |
| Candida tropicalis ATCC 750 | 12±0.39 | 25µg/disc (Fluconazole) |
| C. parapsilosis ATCC 22019 | 11±0.29 | 25µg/disc (Fluconazole |

## RESULTS AND DISCUSSION

The Hydro-alcoholic extract obtained through hot-perculation of *Amalaki* (Emblica officinalis Gaertn.), *Haritaki* (Terminalia chebula Retz.), *Pippali* (Piper longum Linn), *Citraka* (Plumbago zeylanica Linn) shows antimicrobial activity. The zone of inhibition (in mm) was formed maximum in Staphylococcus aureus (25±0.35) in comparision to Standard drugs (10µg/disc) - 24 (Ampicilin)(concentration of Ampicilin per disc). In other microorganism Pseudomonas aeruginosa, Candida albicans, Candida tropicalis, Escherichia coli, Candida krusei, C. parapsilosis and Plesiomonas shigelloides , the zone of inhibition was observed as follows; 16±0.57, 12±0.47, 12±0.39, 11±0.68, 11±0.67, 11±0.29, 10±0.41, respectively.

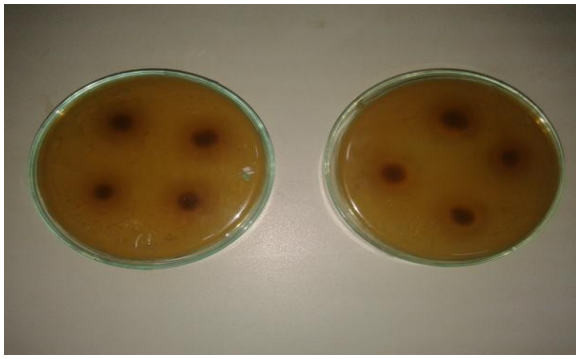

**Figure (1) showing antimicrobial activity and Zone of inhibition around hydro-alcohalic extract**

## CONCLUSION

The Hydro-alcoholic extract of Amlakyadi gana studied for anti-microbial activity. The

Zone of inhibition (in mm) was formed maximum in Staphylococcus aureus.Hence this formulation seems to be effective in Jvara (Fever).

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