



## Research Article

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### A COMPARATIVE ANTIMICROBIAL ACTIVITY OF APAMARGA (*ACHYRANTHES ASPERA* LINN.) PATRA AND BEEJA

Girigaon Yogesh H<sup>1\*</sup>, Kulkarni Yogini R<sup>2</sup>

Department of Dravyaguna, KLE University's Shri B. M. K. Ayurveda Mahavidyalaya, Belgaum, Karnataka, India

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#### \*Corresponding author

E-mail: yogesh10660@gmail.com

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#### ABSTRACT

Apamarga (*Achyranthes aspera* Linn.) is one such herb which is extensively used in the Indian systems of medicine. In *Rigveda* 190 medicinal plants were described, in that Apamarga is one of the plant. In *Yajurveda* this plant find its reference as having Rakshoghna property. *Atharvaveda* extensively quoted references regarding Apamarga. This plant removes the vitiated doshas from the body hence the name Apamarga. The properties attributed to this plant in *Atharvaveda* are Krimighna, Sahasravirya, Rakshoghna, Rasayana, Arshoghna, Kshudha, trishna, marana, vishaghna, Ashmarinashan and Ojovardhan. *Charka samhita* explained Apamarga under Krimighna varga. Keeping its immense qualities in mind screening of antimicrobial activity has been carried out on selected stains of organisms. Disc diffusion method has been adopted for antimicrobial activity. Study reveals significant result of trial drug when compared with the standard drug.

**Keywords:** Apamarga, Patra, Beeja, Disc diffusion

#### INTRODUCTION

Apamarga (*Achyranthes aspera* Linn.) is from the family of *Amaranthaceae*, found throughout tropical Asia, Africa, Australia and America. Apamarga is mentioned in various texts of *Ayurveda*. Viz. *Charaka samhita*, *sushruta samhita*, *Astanga samgraha*, and other treatises like *Bhavaprakasha*, *Dhanwantari nighantu*, *saligrama nighantu* etc. used to treat various ailments. Apamarga is herb, climbing sub shrub or shrub. Leaves- alternate or opposite, entire, stipulate. Flowers- small, bisexual or unisexual, or sterile and reduced, subtended by 1 membranous bract and 2 bracteoles, solitary or aggregated in cymes. Inflorescences- elongated or condensed spikes (heads), racemes, or thyroid structures of varying complexity<sup>1</sup>. Whole plant is useful for therapeutic purposes. For present study patra (leave) and beeja (seed) were selected to screen antimicrobial activity comparing with standard drug. For *in-vitro* study, *Staphylococcus aureus* (Gram +ve), *Escherichia coli* (Gram -ve), fungi *Candida albicans* were selected.

#### MATERIALS AND METHODS

Leaves and seeds of Apamarga were collected from M.K. Hubli; Belgaum, Karnataka, India and was authenticated from central research Laboratory B.M.K. Ayurvedic college Shahapur Belgaum, India (Apamarga patra dry; Sampale code: CRL/09/27 and beej Sample code: CRL/09/28). The collected drugs were dried under the shade and made into coarse powder and stored in moisture free air tight container. Thus powdered drug were subjected to pharmacognostic study as per pharmacopoeia standards. Aqueous and ethanolic extract were obtained and were subjected to phytochemical analysis, TLC and HPTLC study<sup>2</sup>.

#### Methodology

The agar dilution technique was used to measure qualitative *in vitro* activity of an antimicrobial agent

against the test bacterial. In this method, the petri dishes were filled with inoculated liquefied agar medium to uniform thickness. Then graded amount of test samples are incorporated in agar plates and inoculated in spots with the organisms under study. The Antimicrobial activity of a drug was generally expressed as its inhibiting effect towards the growth of bacterium in nutrient broth or on nutrient agar<sup>3</sup>.

For this study, following conditions were;

- The substance or extract must be in contact with the test organism.
- Conditions must be favorable for the growth of microorganisms in the absence of Antimicrobial substances.
- There must be means of estimating the amount of growth and thereby percentage of growth of inhibition.
- The activity of extract should be observed and determined by the growth response of microorganisms.

#### Experimental Procedure

In this present study, the Antimicrobial screening was done by cup plate method / cup diffusion method. In the method Muller Hinton, agar plates were prepared and inoculated with the microbial cultures and incubated at 37<sup>0</sup> C ± 1 for 24 hours. Then small wells (cups) were bored and the samples (stock solution of the drug) were taken and put in them and kept under observation for next 24 hours at 37<sup>0</sup> C ± 1. Then zone of inhibition were recorded.

This is one of the methods official in I.P. where the antimicrobial extract is diffused from the cup through an agar layer in a petri-dish or plate to an extent such that the growth of added microorganisms is restricted entirely in circular area or zone around the cavity containing the solutions of the test sample. The antimicrobial activity is

expressed as zone diameter in millimeters, which is measured<sup>4</sup>.

All the extracts of (ethanol and water) dried leaf powders were screened for antimicrobial activity against wide spectrum of microorganisms and the activity were compared with appropriate standards.

**Standards used in the study**

| Bacteria                     | Fungi                   | Standard drugs used |             |
|------------------------------|-------------------------|---------------------|-------------|
|                              |                         | Gram +ve            | Gram -ve    |
| <i>Staphylococcus aureus</i> | <i>Candida albicans</i> | Ofloxacin           | Ofloxacin   |
| <i>Escherichia coli</i>      | -----                   | Amikacin            | Norfloxacin |
| -----                        | -----                   | Roxithromycin       | Gentamycin  |

**Preparation of Antimicrobial agent stock solution**

- Remove the Antimicrobial agents from the freezer and warm to room temperature before opening to avoid condensation of water.
- Weigh appropriate amount of the powdered semi liquid antimicrobial Agent.
- Dissolve the Antimicrobial agent powdered / semi liquid in solvent to make 5 mg/ml and 10 mg/ml concentrations<sup>5</sup>.

**Preparation of Standard Solutions**

- Required amount of standard ofloxacin – 2 µg / disc and norfloxacin – 10 µg/disc solutions are prepared.
- The concentration of standard equivalent to 100 µg/disc of Gresiofulvin was prepared as standard for antifungal activity.

**Preparation of Test solution**

Each test compound (5mg) was dissolved in dimethyl formamide (5ml) to give a 1000µg/ml. Total of 2 test samples of two different concentrations 0.5 ml (500µg) and 0.25 ml (250µg) were prepared which are as follows.

- Sample 1-- Aqueous Extract --500µg
- Sample 2-- Ethanol Extract--500µg
- Sample 3-- Aqueous Extract --250µg
- Sample 4- Ethanol Extract--250µg

**Culture medium**

Medium types of media have been used according to the types of organism. In the present investigation, test sample medium (Muller – Hinton Agar – Himedia) were employed.

**Experimental Procedure**

The sterile borer was used to prepare 10 cups of 8 mm diameter in the medium of each petridish. An accurately measured 0.1 ml solution of each concentration of solutions of extracts and standard samples were added to the cups with the help of micropipette. All the plates were kept at room temperature for effecting diffusion of drug extracts and standards. Later, they were incubated at 37 ± 1<sup>0</sup>C of 24 hours. The presence of definite zones around the cup of any size indicates antimicrobial activity. The controls were run simultaneously to assess the activity of ethanol and water, which were used as a vehicle for extracts. The diameter of the zone of inhibition was measured and recorded<sup>6</sup>.

The zones inhibitions for the antimicrobial and antifungal activities of extracts were calculated by measuring the inhibitory effect towards the growth of bacterial and fungus around nutrient agar cup.

**RESULT AND DISCUSSION**

Antimicrobial study was carried out on Gram + ve bacterial (*Staphylococcus aureus*) Gram –ve bacterial (*E. coli*) and fungi (*Candida albicans*).

In the study, for the test drug 500µg extract, the zone of inhibition was observed comparing to the modern different standard drugs. The zone of inhibition of standard drugs for Gram-positive *S.aureus* were Ofloxacin-20. Roxithromycin.—18 and Amlkacin.—18.

The Ethanol extract showed good activity when compared to other samples.

The results for aqueous extract were somehow nearer to the standard drugs. The zone of inhibition of a standard drugs for Gram negative *E-coli* were Ofloxacin-20mm, Norfloxacin.—16mm and Gatifloxacin.—18mm.

**Table 1: HPTLC Result**

| Plant name   | Amount of extract on plate (µg) | @ UV 254 nm             | @ UV 366 nm                               |
|--|---------------------------------|-------------------------|---|
| <b>Apamarga: Patra; (<i>Achyranthes aspera</i> L., Leaves)</b> |                                 |                         |   |
| a) Ethanol extract   | 500µg                           | <b>0.27, 0.87</b>       | <b>0.27, 0.34, 0.81, 0.89</b>             |
| b) Aqueous extract   | 500µg                           | <b>0.27, 0.87</b>       | <b>0.18, 0.27, 0.34, 0.36, 0.47. 0.87</b> |
| <b>Apamarga: Beej; (<i>Achyranthes aspera</i> L., Seeds)</b>   |                                 |                         |   |
| a) Ethanol extract   | 500µg                           | <b>0.39, 0.58</b>       | <b>0.18, 0.69, 0.87</b>                   |
| b) Aqueous extract   | 500µg                           | <b>0.41, 0.58, 0.87</b> | <b>0.75, 0.87</b>                         |

**Table 2: Antimicrobial study**

| Drug                       | Zone of Inhibition in mm    |                               |                                |
|----------------------------|-----------------------------|-------------------------------|--------------------------------|
|                            | Gram Negative <i>E.coli</i> | Gram positive <i>S.aureus</i> | Fungus <i>Candida albicans</i> |
| <b>1) Apamarga Patra</b>   |                             |                               |                                |
| a) Aqueous Extract (500µg) | 17                          | 4                             | 0                              |
| b) Ethanol Extract (500µg) | 18                          | 18                            | 17                             |
| <b>2) Apamarga Beej</b>    |                             |                               |                                |
| a) Aqueous Extract (250µg) | 2                           | 2                             | 0                              |
| b) Ethanol Extract (250µg) | 20                          | 18                            | 17                             |
| Ofloxacin                  | 20                          | 20                            | -                              |
| Norfloxacin                | 16                          | -                             | -                              |
| Gatifloxacin               | 18                          | -                             | -                              |
| Roxithromycin              | -                           | 18                            | -                              |
| Amlkacin                   | -                           | 18                            | -                              |
| Gresiofulvin               | -                           | -                             | 20                             |

In Anti fungal activity aqueous extract did not show zone of inhibition therefore it was found to be resistant whereas ethanol extract of both leaf and seed showed the zone of inhibition is nearer to standard drug<sup>7-8</sup>.

## CONCLUSION

In study Anti-fungal *Candida albican* showed resistance in agar cup diffusion method. Ethanolic extract showed antimicrobial activity and sensitivity against *E.coli* and *S.aureus*.

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