

Antibacterial activity of crude extract from *Kadukkai choornam* (*T. chebula* Retz): A Siddha medicine

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Abstract

Among the single herbs widely used in Traditional Siddha System of Medicine, *Kadukkai* (*Terminalia chebula*, Combretaceae). It finds a very special place, because it is been considered superior to the nourishing mother. Apart from its efficacy in treating tastelessness, fever, cough, asthma, urinary diseases, worms, rheumatism, etc. It is the only plant having five tastes among the six tastes mentioned in the Siddha literatures. This research paper deals with the anti bacterial activity of crude extract from *Kadukkai Choornam* using Nutrient Agar and Muller Hinton Agar. The extract was found to be effective against micro-organisms such as *Protease*, *Vibrio cholera*, *Staphylococcus*, *Pseudomonas* and *Helicobacter pylori*. The specialty lies in its efficacy of inhibiting the micro-organisms even at minimum concentration of 100 µl. This is only a preliminary study and more studies are to be carried out to explore the wonderful therapeutic properties of this herbal medicine.

Keywords: Traditional system; Siddha medicine; *Kadukkai chooranam*; Anti-microbial

1. Introduction

The concept of Single herbs as Therapeutic agents is widely recognized nowadays. In Traditional *Siddha* system of Medicine, there is a concept known as, 'Yegha Mooligai Prayogam' (Yega – single, Mooligai – herb and Prayogam – application) which deals with single herbs and their clinical application^[1]. Unlike poly herbal formulas consisting of herbs, metals and minerals, single herb therapy is safe, convenient, economical and time tested. Thereby, the single herb *Kadukkai* (*Terminalia chebula*, Combretaceae) finds a special place, which has been compared superior to the nourishing mother. According to *Siddha* literature, *Kadukkai* is one of the major constituent of commonly used *triphala chooranam* in the household. It has high efficacy in treating fever, cough, asthma, urinary diseases, intestinal worms, etc. The special character of the herb is that it is the only plant having five tastes among the six tastes^[2]. Anti bacterial activity of crude extract from *Kadukkai Chooranam* is studied using Nutrient Agar and Muller Hinton Agar.

2. Material and methods

The raw drug was dried in direct sun light, powdered as per Standard Operative Procedure (SOP); this end product was filtered and stored in an air tight container. This product was used for analysis.

2.1. Media used

- Nutrient Agar
- Muller Hinton agar

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2.2. Media Preparation

2.2.1. Nutrient Agar per litre/gram (NA)

- Peptone -5.0g
- Yeast extracts -2.0g
- Na Cl -5.0g
- Agar -18.0g
- Distilled water-1000ml pH -7.0

2.2.2. Muller Hinton agar

- Approximate Formula* Per Litre
- Beef Extract Powder -2.0 g
- Acid Digest of Casein-17.5 g
- Starch -1.5 g
- Agar -17.0 g
- pH -7.0

2.3. Crude extraction Procedure

2.3.1. Preparation of plant extract

The dried powdered fruit of *Kadukkai* (10g), was extracted by mechanical maceration with aqueous at 35 °C for 24 h. The extracted liquids were filtered and filtrates concentrated under vacuum, followed by drying (40 °C).

2.3.2. Culture collection and maintenance:

The studies of organisms *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Pseudomonas* and a clinical isolates of *H. pylori* were obtained from CAS in Botany and Department of Biochemistry, University of Madras, Guindy Campus, Chennai- 600 025.

2.4. Principles and Procedure ^[3]

Preparation of culture for antibacterial studies: Twelve hour old bacterial suspension was adjusted to 0.5 OD and 1.0ml from the above was inoculated into 50 ml of nutrient broth and incubated at 37 °C in an orbital shaker at 150 rpm. To determine the growth rate, culture was removed at 4hr interval and the growth was monitored by measuring the optical density at 540nm in spectrophotometer (Deep vision model 1371 VU/Vis Spectrophotometer, India). The growth curve was drawn by plotting OD value against the incubation time.

2.4.1. Antibacterial susceptibility test

The disk diffusion (Kirby–Bauer) technique, which conforms to the recommended standards of the National Committee for Clinical Laboratory Standards (NCCLS) (now known as Clinical and Laboratory Standards Institute (CLSI)), was used as previously described (Ndip et al., 2005). Brain heart infusion (BHI) agar (Conda Pronadisa, Spain) was used for susceptibility testing.

Disks of 6mm in diameter were punched from a sheet of Whatman filter paper, sterilized, and impregnated with 25 µl of 0.2 g/mL extract or solvent alone and dried at 30–35 °C for 12–24 hr (Nariman et al., 2004). The bacterial inoculum was prepared from subcultures of bacteria as follows: four to five colonies of the isolates were emulsified in sterile distilled water and the turbidity adjusted to 1.5×10⁸ CFU/mL (corresponding to 0.5 McFarland standards). A sterile cotton swab was dipped into the standardized bacterial suspension and used to evenly inoculate the BHI agar plates. The plates were allowed to dry for 3-5 min.

Thereafter, all disks were placed on the plates and pressed gently to ensure complete contact with agar. A distance of at least 15mm was maintained from the edges of the plates to prevent overlapping of inhibition zones. A clarithromycin disk (15µg) was used as the positive control. DMSO impregnated disks were used as negative control. Fifteen minutes following placement of the disks, the plates were incubated at 37 °C for 2–5 days. They were then examined and the diameter of the zone of inhibition measured. *Helicobacter pylori* control strain NCTC 11638 was included in all the experiments.

3. Results

3.1. Effect of crude extract on the growth of Pathogenic bacteria by disc diffusion method

The *Kadukkai* (*T. chebula*) fruit aqueous crude at different concentration (100, 200, 300 and 400µl) was tested against *Protease mirabilis*, *Vibrio cholerae*, *Staphylococcus aureus*, *Pseudomonas spp.*, and *H. pylori* at 37°C for 24 hr. The fruit aqueous crude extract exhibited more bactericidal action (Table-1) with higher inhibition zone was found at 400µl concentration. *H. pylori* showed higher bacterial growth inhibition than the other bacteria.

Although Sato et al., (1997) reported gallic acid and ethyl gallate in *Kadukkai* (*T. chebula* Retz) and have shown antibacterial activity of ethanol extracts of this plant against both methicillin resistant and sensitive *Staphylococcus aureus* and other bacteria, the components of *Kadukkai*(*T. chebula* Retz) aqueous extracts responsible for the observed bactericidal activity remain unknown. The antibacterial activity of aqueous extracts of *Kadukkai choornam* against bacteria and particularly *H. pylori* was also demonstrated. It is concluded that the traditional *Siddha* medicinal use of *Kadukkai choornam* powder to treat gastric infections is substantiated by the antibacterial activity of its extracts against *H. pylori*.^[4]

Table 1 The antibacterial activity of crude extract from *Kadukkai* (*T. chebula* Retz) was tested against bacterial pathogen and effective inhibition was determined by disc diffusion method

Test Micro-organism	Zone of Inhibition (mm ²)			
	Different concentrations crude extract (µl)			
	100 µl	200 µl	300 µl	400 µl
<i>Protease mirabilis</i>	09	14	16	18
<i>Vibrio cholera</i>	07	10	13	16
<i>Staphylococcus aureus</i>	10	13	14.5	17
<i>Pseudomonas spp.</i>	07	11	13	16
<i>H. pylori</i>	08	12	16	19

The inhibitory diameter was measured by means of calipers. All the assays were duplicated, and the mean values were recorded.

4. Discussion

The anti-bacterial activity of *Kadukkai Chooranam* was test by in vitro studies which explicate that it is effective against micro organisms such as *Protease*, *Vibrio cholera*, *Staphylococcus* and *Pseudomonas*. And it shows higher zone of inhibition to *Helicobacter pylori* in 400 µl. This study, reveals that, a single herb *Kadukkai Chooranam* is effective in treating gastric ulcers, stomach irritations in both therapeutic and prophylactic purposes.

5. Conclusion

It is inferred that, the Siddhars (usually referred as the olden day scientist) had the inner knowledge of the science of medicinal plants without the use modern technologies for medicinal activity of the plant. *Kadukkai* is the important constituent of *triphala* which been widely used for treating gastric ulcers, constipation, used as Anti-oxidants by *Siddha* practitioners and traditional healers.

Compliance with ethical standards

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Disclosure of conflict of interest

There are no conflicts of interest.

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