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Evaluation of Anti-microbial activities of Kanduparangi Chooranam (*Pygmaeopremna herbacea*)

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Abstract

Introduction:

Kanduparangi chooranam, coded as KC is a classical Siddha monoherbal drug. Drugs which is made up of single herb have been using for the elimination of microorganisms that mentioned in ancient siddha literature. On the behalf of literary evidences, elimination and developing resistance against microorganisms that encourages us to find new drugs.

Aim and objectives:

The test formulation *Kanduparangi chooranam* (KC) is screened for anti – microbial activity in various extracts like aqueous & methanol. The studies were carried out in 50 µl and 100µl concentrations in each extracts against MTCC strains of 5 G+ve , 5 G-ve bacterium and 5 fungi.

Materials and methods:

The present research was carried out in the Inbiotics, a training unit, Institute of biology and clinical research, William hospital campus, Nagercoil, Tamil Nadu, India during December 2019. Antibiotic susceptibility tests were determined by agar disc diffusion (Kirby–Bauer) method. Anti-fungi assays were determined using disc diffusion method.

Results and discussion:

KC is highly sensitive in tested G +ve bacteria Staphylococcus *aureus* (11, 15 mm), *Enterococcus faecalis* (10,13mm), *Lactobacillus salivarius* (9,12 mm), *Streptococcus mutans* (8, 11mm) and *Bacillus subtilis* (15,19mm) and tested G - ve bacteria *E. coli* (16 mm , 19 mm), *Pseudomonas aeruginosa* (13,16 mm), both *Proteus vulgaris* & *Proteus mirabilis*(14,19 mm) respectively, *Klebsiella pneumoniae* (8 mm,10 mm) in methanol extract at 50 and 100 µl concentrations. The inhibitory actions for bacterium were compared with standard drug streptomycin.

Methanol extracts of KC 50 and 100 µl concentrations is highly sensitive in *Candida albicans* (14 mm, 18 mm) and *Aspergillus niger* (10 mm ,12 mm) then the aqueous extracts of KC 50 and 100 µl concentrations is sensitive in *Penicillium notatum* (11 mm,12 mm) and *Candida albicans* (9,12mm) and no activity was reported against *Aspergillus niger* , *Aspergillus flavus* and *Rhizopus stolonifer* Their actions was compared to standard anti-fungal drug Fluconazole. *Siddha* formulation KC can employed in the management of super opportunistic infections in asthmatic patients.

From the present research it was concluded that all the methanol and some aqueous extracts of KC have high antimicrobial activity against gram negative bacteria than gram positive bacteria as compared to positive control , streptomycin and high antifungal activity against *Candiada albicans* and *Aspergillus niger* as compared to positive control, fluconazole.

Keywords: Antibacterial, Anti fungal, *Kanduparangi chooranam*, Bronchial asthma, *Pygmaeopremna herbacea*

1. Introduction

According to the ancient philosophies, drugs from herbs have been using for the elimination of microorganisms. Many plants derivatives such as spices, fruit preparations, vegetable preparations or extracts have been used for centuries for the preservation and extension of the shelf life of foods (Chattopadhyay et al.2007).

Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicinal compound; have continued to play a dominant role in the maintenance of human health, since ancient times. Over 50% of all modern clinical drugs are of natural product origin and it also plays an important role in drug development programs in the pharmaceutical industry.

Herbal medicine holds an indispensable major position in *Siddha* pharmacology. The *Siddhars* had a systematic approach towards the selection of drugs starting with herbs and ending with higher order medicines of metals & minerals. Single-herbal / poly-herbal / herbo-mineral forms are practised in *Siddha*. *Eka mooligai prayogam* (single herbal therapy) is getting more popularity among scientific community and public extensively due to factors such as high efficacy, low cost, less side effects.

The present study is aimed at the evaluation of the antimicrobial properties of the methanol and aqueous extracts of *Kanduparangi chooranam*. It has shown good antimi- crobial activity when compared with standard drug.

1.1. *KANDUPARANGI CHOORANAM*– AN OVERVIEW

Kanduparangi Chooranam* (KC)** is a classical Siddha mono herbal formulation[14] mentioned in ***"Gunapadam - Mooligai Vagupu " siddha classical text book for the management of *Iya iraippu* which can be correlated in modern medicine as "Bronchial Asthma" as well as the drug is indicated for *Vatha*, *Pitha* and *Kabha* related disorders.

1.2. *PYGMAEOPPREMNA HERBACEA (KANDUPARANGI)* – AN OVERVIEW

1.2.1. Taxonomy [Integrated Taxonomic Information System (ITIS)]

Domain	: Eukaryota
Kingdom	: Plantae
Sub-kingdom	: Viridaeplantae
Phylum	: Tracheyophyta
Sub-phylum	: Euphyllophytina
Infraphylum	: Radiatopses
Division	: Angiospermae
Class	: Magnoliopsida
Subclass	: Lamiidae
Order	: Lamiales
Family	: Verbenaceae
Genus	: ***Pygmaeopremna***
Species	: ***herbacea***

Figure .1. *Pygmaeopremna herbacea*

PYGMAEOPREMNA HERBACEA (KANDU PARANGI) is perennial woody, flowering shrub of Verbenaceae[15][16] which ***herbacea*** is distributed throughout in the forest of India and Sri lanka. It is cultivated up to altitude 1400 ft. above sea level. It is also found in lower Himalaya too.

The root nodules of *Premna herbacea* Roxb. Syn., *Pygmaeopremna herbacea*, **(Verbenaceae)** is claimed to be useful in the ayurvedic system of medicine either alone or as an ingredient in the compound preparations for the treatment of several ailments, such as bronchitis, asthma, blood pressure, tumors, inflammation, epilepsy, helimenthiasis etc.

2. Materials and Methods

2.1. Collection and Identification of plant materials

The root of *Pygmaeopremna herbacea* was brought from country medical shop in Nagercoil, Tamil Nadu, India and it was authenticated by the Professors of department of Medicinal Botany at Government Siddha Medical College and Hospital, Palayamkottai Tirunelveli district, Tamil nadu.

2.2. Purification of raw drugs:

The raw drug was purified by removing unnecessary parts as per the methods mentioned in the Siddha literature.

2.3. Preparation of the drug *Kanduparangi chooranam* (KC)

The purified drug was dried well in shadow and made into micronized powder. Finally the powder was sieved using pure white cloth which is mentioned as *Vasthirakayam* in Siddha. Finally stored in a clean and air tight container.

Table 1: Ingredient of drug KC

S. no	Ingredient	Botanical Name	Family Name	Part used	Quantity
1	***Kanduparangi***	***Pygmaeopremna herbacea* .Linn**	**Verbenacae**	**Root**	**100 gms**

2.4. Antimicrobial activity procedure

2.4.1. Antibacterial Activity Procedure:

Dilution: 1mg in 1ml

Standard control: Streptomycin (S25)

Test Organism:

Gram positive: *Staphylococcus aureus* (MTCC 916), *Enterococcus faecalis* (MTCC 439), *Lactobacillus salivarius* (MTCC 1026), *Bacillus subtilis* (MTCC 1134), *Streptococcus mutans* (MTCC 916)

Gram negative: *Klebseilla pneumoniae* (MTCC 530), *E.coli* (MTCC 1671), *Pseudomonas aeruginosa* (MTCC 741), *Proteus vulgaris* (MTCC 426), *Proteus mirabilis* (MTCC 1429)

The test microorganisms used for antimicrobial analysis Microbial strains were purchased from Microbial Type Culture Collection and Gene Bank (**MTCC**) Chandigarh.

Nutrient Broth Preparation:

Pure culture from the plate were inoculated into Nutrient Agar plate and sub cultured at 37ºC for 24 h. Inoculum was prepared by aseptically adding the fresh culture into 2 ml of sterile 0.145 mol/L saline tube and the cell density was adjusted to 0.5 McFarland turbidity standard to yield a bacterial suspension of 1.5×108 cfu/ml. Standardized inoculums was used for Antimicrobial test.

Cleaning and sterilization:

The glass-wares used were cleaned with cleaning solution and sterilized in hot air oven to 180℃ for 3 hours. All nutrient media were sterilized by autoclave (121℃, 15psi for15-20 mins).

Preparation of test drug samples:

1gram of test drug was diluted in 1ml of distilled water and methanol respectively. The percolation time was 5- 7 days. The sample thus prepared in methanol was stored in room temperature and aqueous extract in 4℃ to avoid the fermentation of the sample. Then the extracts were subjected to anti-microbial assay.

Anti-bacterial assay:

The medium was prepared by dissolving 38 g of Muller Hinton Agar Medium (Hi Media) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 Lbs pressure at 121^0C for 15 min (pH 7.3). The autoclaved medium was cooled, mixed well and poured Petri plates (25 ml/plate) the plates were swabbed with Pathogenic Bacteria culture. Finally, the Sample or Sample loaded Disc was then placed on the surface of Mullar-Hinton medium and the plates were kept for incubation at 37°C for 24 hours. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in milli meters. The size of the zone of inhibition (including disc) was measured in millimeters. The absence of zone inhibition was interpreted as the absence of activity **(Kohner *et al*., 1994; Mathabe *et al*., 2006).** The activities are expressed as resistant, if the zone of inhibition was less than 7 mm, intermediate (8-10 mm) and sensitive if more than 11 mm (Assam *et al*., 2010).

2.4.2.Anti-fungi assay by disc diffusion method (Bauer *et al*., 1966)

Dilution **:** 1mg in 1ml

Standard control **:** Fluconazole

Test Organism: *Aspergillus flavus* (MTCC 535), *Aspergillus niger* (MTCC 281), *Penicillium notatum* (MTCC 2647), *Rhizopus stolonifer* (MTCC 162), *Candida albicans* (MTCC 183).

Cleaning and sterilization: Glass-wares used were cleaned with cleaning solution and sterilized in hot air oven to 180℃ for 3 hours. All nutrient media were sterilized by autoclave (121℃, 15psi for15-20 mins).

Preparation of test drug samples:

1gram of test drug was diluted in 1ml of distilled water and methanol respectively. The percolation time was 5- 7 days. The sample thus prepared in methanol was stored in room temperature and aqueous extract in 4℃ to avoid the fermentation of the sample. Then the extracts were subjected to anti-microbial assay.

Anti-fungal assay: Antibiotic susceptibility tests were determined by agar disc diffusion (Kirby–Bauer) method. Fungi strains were swabbed using sterile cotton swabs in SDA agar plate. Up to 40 µl of each concentration of the extract were respectively introduced in the sterile discs using sterile pipettes. The disc was then placed on the surface of SDA medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 22°C for 48 hours. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in millimetre.

3. Results

Siddha drug KC was evaluated for their antimicrobial potential against five gram-positive and five gram-negative bacteria strains and five fungi strains in this study.

Tables 2, 3 & 4 summarizes the results obtained.

3.1. Anti - bacterial activity of Kanduparangi Chooranam on gram positive bacterial strains

Table. 2. Anti - bacterial activity of methanol and aqueous extracts of KC (Gram positive bacterial strains)

Sample Code	Gram Positive Bacteria Strains Name				
	Staphylococcus aureus (G+) MTCC 916	*Enterococcus faecalis* (G+) MTCC 439	*Lactobacillus salivarius* (G+) MTCC 1026	*Bacillus subtilis* (G+) MTCC 1134	*Streptococcus mutans* (G+) MTCC 916
KC. M. 50	11 mm	10 mm	9 mm	15 mm	8 mm
KC. M. 100	15 mm	13 mm	12 mm	19 mm	11 mm
KC. Aq. 50	8 mm	-	8 mm	9 mm	-
KC. Aq. 100	11 mm	-	11 mm	11 mm	8 mm
PC	15 mm	19 mm	15 mm	18 mm	13 mm
NC	-	-	-	-	-

PC - Positive control (Streptomycin), **NC** - Negative control(plain disc), **-** - No Zone of inhibition, **mm** - millimetre , **G+** - Gram Positive , **G -** - Gram Negative

Figure -2. Antibacterial activity (Gram positive bacterial strains)

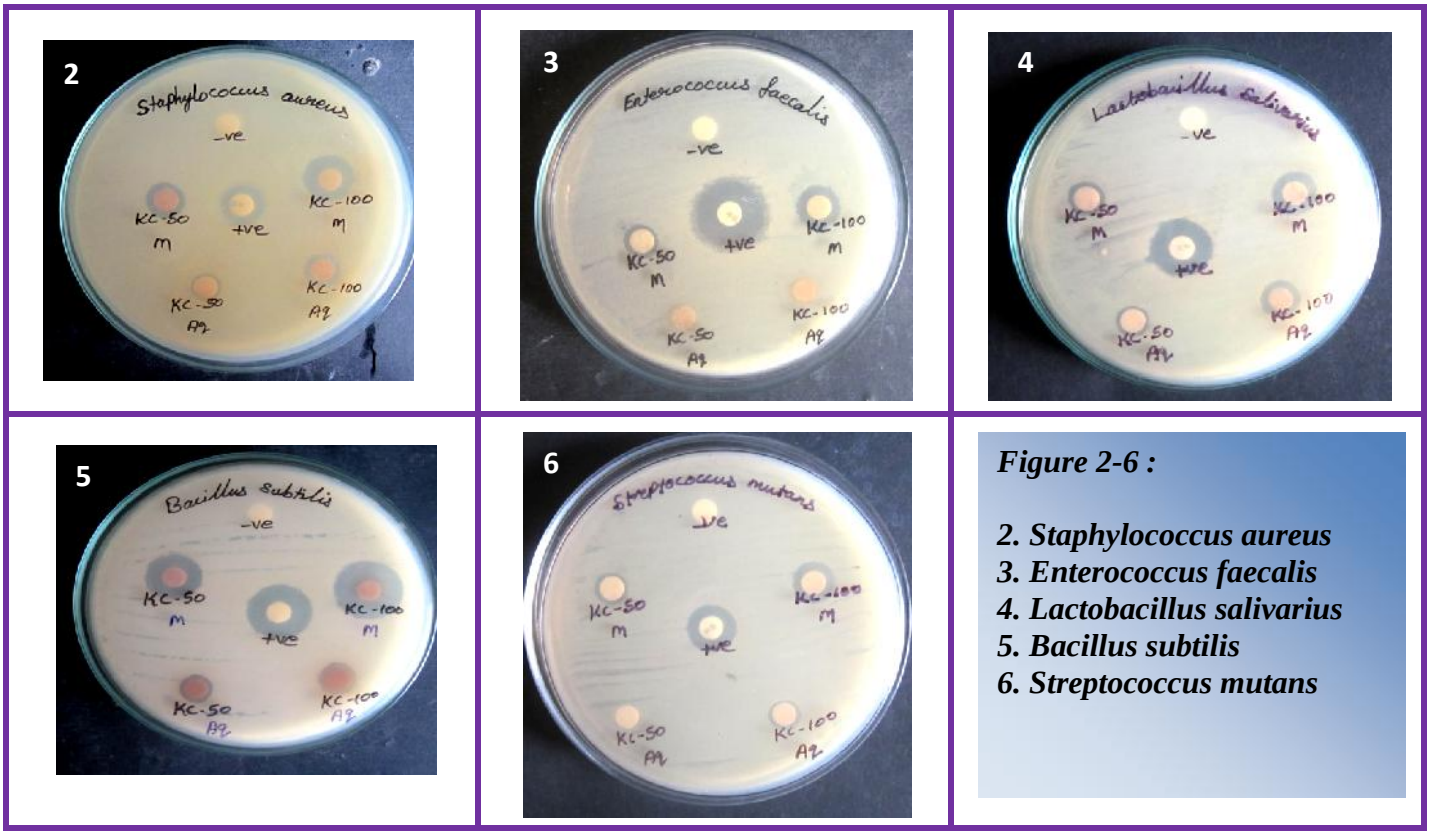

Figure 2-6 :

2. Staphylococcus aureus
3. Enterococcus faecalis
4. Lactobacillus salivarius
5. Bacillus subtilis
6. Streptococcus mutans

In methanol extract

As per table no: 2 & Fig; 2- 6, the methanol extract of KC 100 µl in G +ve strains shows more inhibition in the order of *Bacillus subtilis* (19 mm) followed by *Staphylococcus aureus* (15 mm) , *Enterococcus faecalis* (13 mm) respectively. Comparatively *Lactobacillus salivarus* (12 mm) showed lesser inhibition and *Streptococcus mutans* (11mm) possess least activity.

As per table no: 2 & Fig; 2- 6, the methanol extract of KC 50 µl in G +ve strains shows more inhibition in the order of *Bacillus subtilis* (15 mm) followed by *Staphylococcus aureus* (11mm), *Enterococcus faecalis* (10 mm) respectively. Comparatively *Lactobacillus salivarus* (9 mm) showed lesser inhibition and *Streptococcus mutans* (8 mm) possess least activity.

In aqueous extract

As per table no: 2 & Fig; 2- 6, the aqueous extract of KC 100 µl in G +ve strains shows more inhibition in the equal order by *Staphylococcus aureus, Lactobacillus salivarus* & *Bacillus subtilis* (11 mm) respectively.Then *Streptococcus mutans* (8 mm) showed lesser inhibition. *Enterococcus faecalis* did not show any inhibition.

As per table no: 2 & Fig; 2- 6, the aqueous extract KC of 50 µl in G +ve strains shows lesser inhibition in the order of *Bacillus subtilis* (9 mm) followed by both *Staphylococcus aureus* & *Lactobacillus salivarus* (8 mm) respectively. *Streptococcus mutans* and *Enterococcus faecalis* did not show any inhibition.

When tested, the methanol extracts of the KC showed significant activity against all the tested gram-positive microorganisms when compared to aqueous extract.

The highest gram-positive antibacterial activity recorded KC M 100 µl in *Bacillus subtilis* (19 mm) which was compared with positive control, Streptomycin (18mm).

3.2. Antibacterial activity of Kanduparangi chooranam on gram negative bacterial strains

In methanol extract

As per table no: 3 & Fig; 7- 11, the methanol extract of KC 100 µl in G -ve strains shows more inhibition in the equal order of *Proteus vulgaris, Proteus mirabilis* & *E. coli* (19mm) respectively followed by *Pseudomonas aeuroginosa* (16 mm) and *Klebsiella pneumoniae* (10mm) possess least activity comparatively.

As per table no: 3 & Fig; 7- 11, the methanol extract of KC 50µl in G -ve strains shows more inhibition in the order of *E. coli* (16 mm) followed by both *Proteus vulgaris* & *Proteus mirabilis* (14mm) respectively. Comparatively *Klebsiella pneumoniae* (8mm) possess least activity.

In aqueous extract

As per table no: 3 & Fig; 7 -11, the aqueous extract of KC 100 µl in G -ve strains shows more inhibition in the order of *E.coli* (15mm) followed by *Proteus mirabilis* (14 mm) both *Proteus vulgaris & Pseudomonas aeuroginosa* (12 mm) comparatively possess least activity. *Klebsiella pneumoniae* did not show any inhibition.

As per table no: 3 & Fig; 7-11, the aqueous extract of KC 50 µl in G - ve strains shows lesser inhibition in the equal order of *E.coli* & *Proteus mirabilis* (11 mm) followed by *Proteus vulgaris* (10 mm) & *Pseudomonas aeuroginosa* (9mm) showed lesser activity. *Klebsiella pneumoniae* did not show any inhibition.

Table. 3. Antibacterial activity of methanol and aqueous extracts of KC (Gram negative bacterial strains)

Sample Code	Gram Negative Bacteria Strains Name				
	Klebsiella pneumonia (G-) MTCC 530	*E.coli* (G-) MTCC 1671	*Pseudomonas aeruginosa* (G-) MTCC 741	*Proteus vulgaris* (G-) (MTCC 426	*Proteus mirabilis* (G-) (G-) MTCC 1429
KC.M. 50	8 mm	16 mm	13 mm	14 mm	14 mm
KC.M. 100	10 mm	19 mm	16 mm	19 mm	19 mm
KC. Aq. 50	-	11 mm	9 mm	10 mm	11 mm
KC. Aq. 100	-	15 mm	12 mm	12 mm	14 mm
PC	13 mm	16 mm	14 mm	18 mm	18 mm
NC	-	-	-	-	-

PC - Positive control (Streptomycin), **NC** -Negative control(plain disc) , - - No Zone of inhibition
mm - millimetre, **G+** - Gram Positive, **G -** - Gram Negative

Figure 7- 11: Antibacterial activity (Gram negative bacterial strains)

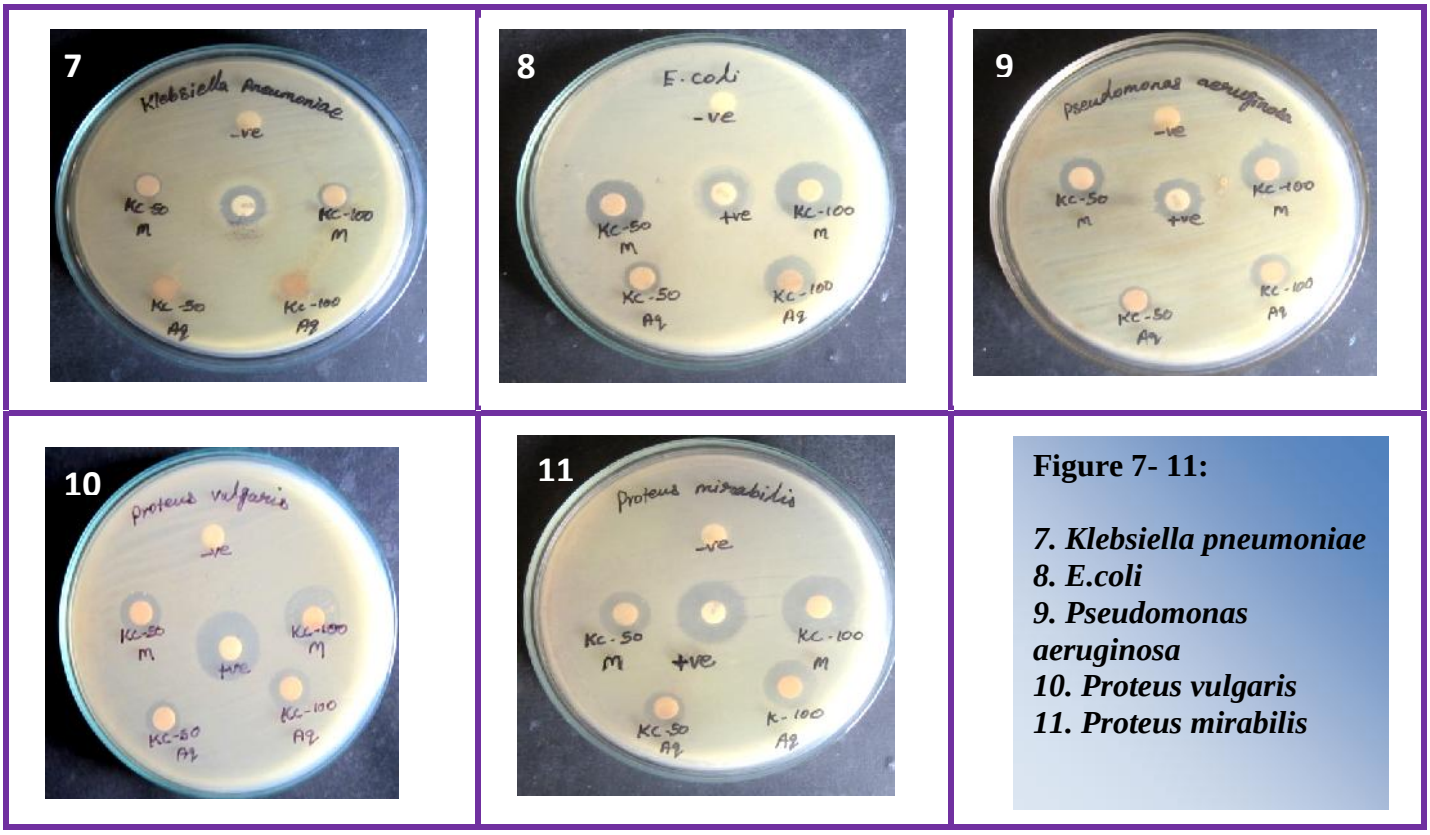

Figure 7- 11:

7. Klebsiella pneumoniae
8. E.coli
9. Pseudomonas aeruginosa
10. Proteus vulgaris
11. Proteus mirabilis

According to the result, the methanol extracts of the KC showed significant antimicrobial activity against all the tested gram-negative microorganisms when compared to aqueous extract.

The highest gram-negative antibacterial activity recorded KC M 100 µl on *E. coli* (19 mm) which was compared with positive control, Streptomycin (16 mm).

3.3. Anti fungal activity of kanduparangi chooranam

In methanol extract

As per table no: 4 & Fig; 12 -16, the methanol extracts of KC 100 µl in fungal strains possess more inhibition in the order of *Candida albicans* (18 mm) followed by *Aspergillus niger* (12 mm) respectively. *Aspergillus flavus* (8 mm) showed least activity. *Penicillium notatum* & *Rhizopus stolonifer* did not show any inhibition

As per table no: 4 & Fig; 12 -16, the methanol extracts of KC 50 µl in fungal strains possess more inbition in *Candida albicans* (14 mm). *Aspergillus niger* (10 mm) showed lesser inhibition. *Aspergillus flavus* (7 mm) showed least activity. *Penicillium notatum* & *Rhizopus stolonifer* did not show any inhibition.

In aqueous extract

As per table no: 4 & Fig; 12 -16, the aqueous extracts of KC 100 µl in fungal strains possess more inhibition in the equal order of *Penicillium notatum and Candida albicans* (12mm) respectively. *Aspergillus niger*, *Aspergillus flavus* & *Rhizopus stolonifer* did not show any inhibition.

As per table no: 4 & Fig; 12 -16, the aqueous extracts of KC 50 µl in fungal strains possess more inhibition in the order of *Penicillium notatum* (11 mm) *followed by Candida albicans* (9mm) respectively. *Aspergillus niger*, *Aspergillus flavus* & *Rhizopus stolonifer* did not show any inhibition.

The highest antifungal activity was recorded at KC M 100 µl in fungal strains against *Candida albicans* (18 mm) which compared with positive control , fluconazole (14mm).

Table.4. Antifungal activity of methanol and aqueous extracts of KC (Fungal strains)

Sample code	Fungi strains name				
	***Aspergillus flavus* (F) 535**	***Aspergillus niger* (F) 281**	***Penicillium notatum* (F) 2647**	***Rhizopus stolonifer* (F) MTCC 162**	***Candida albicans* (F) MTCC 183**
KC.M. 50	**7 mm**	**10 mm**	**-**	**-**	**14 mm**
KC.M. 100	**8 mm**	**12 mm**	**-**	**-**	**18 mm**
KC. Aq. 50	**-**	**-**	**11 mm**	**-**	**9 mm**
KC. Aq. 100	**-**	**-**	**12 mm**	**-**	**12 mm**
PC	**26 mm**	**-**	**29 mm**	**12 mm**	**16 mm**
NC	**-**	**-**	**-**	**-**	**-**

PC - Positive control (fluconazole) **NC** - Negative control(plain disc)
- - No Zone of inhibition **mm** - millimetre

Figure. 12-16: Antifungal activity of methanol and aqueous extracts of KC (Fungal strains)

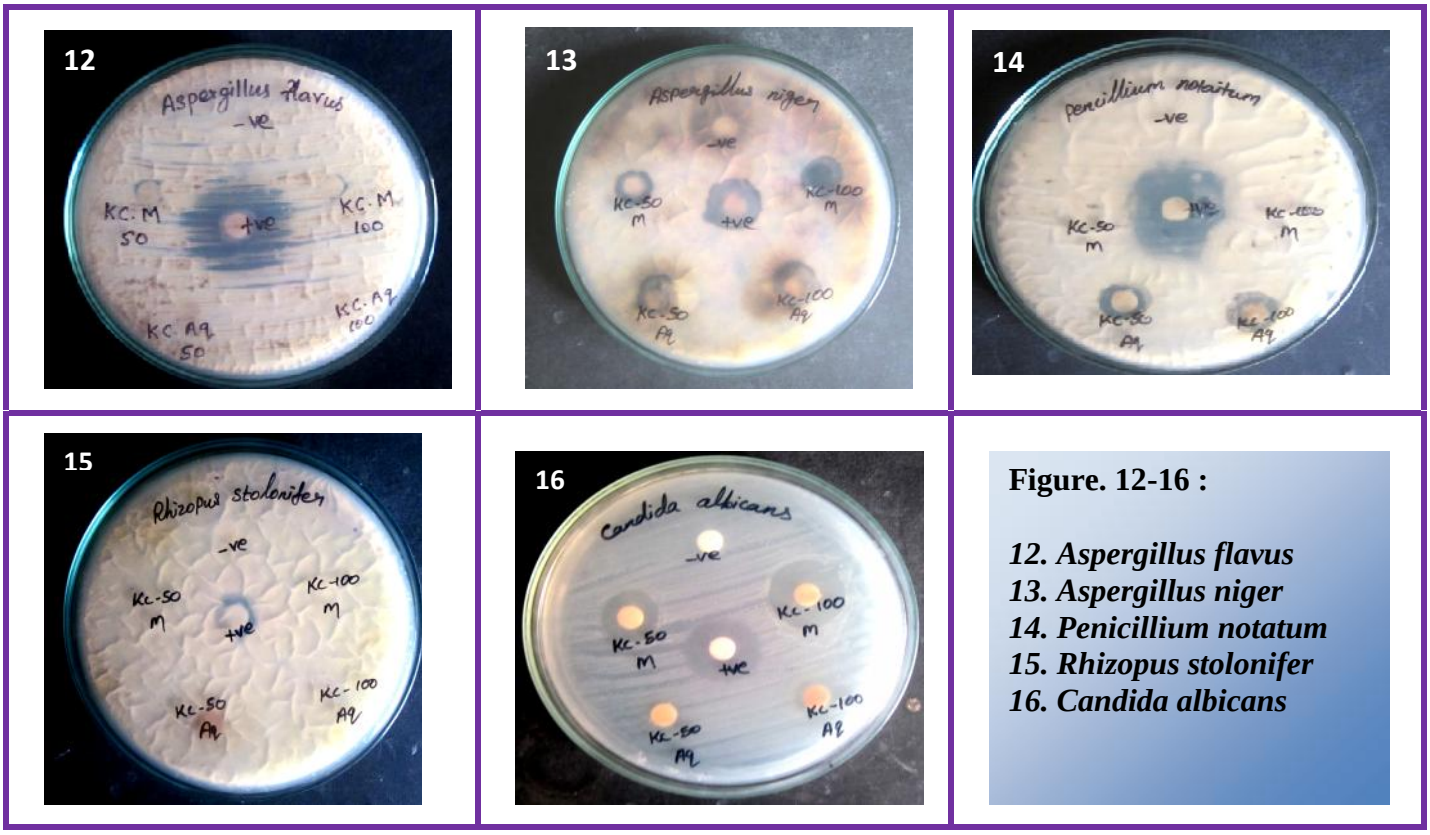

Figure. 12-16 :

12. Aspergillus flavus
13. Aspergillus niger
14. Penicillium notatum
15. Rhizopus stolonifer
16. Candida albicans

4. Discussion

In the alternative methods, the uses of plant materials to control pathogenic microorganisms have been considerable interest in the recently **(Aqil et. al., 2005)** and plants products have been shown resistant against pathogenic bacteria **(Nostro et. al., 2006).** The emergence of multidrug resistant strain of many pathogens is a serious threat and makes more difficult to cure diseases. The development of effective natural and non-toxic drug for treatment must be directed towards. (Chandra 2013). The present study was to explain the antimicrobial property of siddha mono herbal formulation ***Kanduparangi chooranam***.

5. Conclusion

The Siddha formulation *Kanduparangi chooranam* (KC) has promising action in the management of super opportunistic infections caused by both Gram positive as well as Gram negative organisms in asthmatic patients. KC showed highly sensitive inhibitory actions against both classes of bacteria and fungus especially *Candida albicans*.

It is concluded that this study would exhibit some valuable compound that has to be used to more potential antimicrobial drugs of natural origin. Further studies are needed to identify the biologically active compounds and to evaluate the efficiency of the compound against pathogenic microorganisms associated with various human diseases.

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