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### ANTI-MICROBIAL ACTIVITY OF *BRASSICA OLERACEA* LEAVES

**Shinde SA\*, Kadam SS, Suryawanshi JS, Tare HL**

\*Satara College of Pharmacy, Satara, Maharashtra, India.

#### ABSTRACT

On the planet there are numerous medicinal plant having antibacterial activity. So, *Brassica oleracea* is one of them. It is commonly known as Cauliflower & is an important medicinal plant reputed for its dilatory as well as therapeutic uses. The aim of present study is to do, an in vitro evaluation of antibacterial activity of, aqueous & organic extract of leaves of *Brassica oleracea* plant, on some vegetative microorganism like *Candida albicans*, *Staphylococcus spp.*, *Salmonella spp.*, *Klebsiella spp.*, *Bacillus spp.* and yeast, having different strain, with the help of agar well diffusion method. The result obtained by using extract of leaves having different concentration against different test bacteria, showed that the leaves of *Brassica oleracea* possess an antibacterial activity against above mentioned microorganism.

**Key words:** Antimicrobial Activity, *Brassica oleracea*.

#### INTRODUCTION

An infectious diseases produced by various microorganisms as well as their hazardous effects to human is not a recent problem but since ancient time people are suffering from the same problem. Infectious diseases are leading causes of death worldwide, especially in developing countries. When recent scientific study practically proved that, some bacterial strains has become resistant to strong antibiotic, as well as continuous use or overdose of synthetic drug against microbial infections causes adverse effect to human health, the total view of human population to see toward botanical studies or phytomedicines has changed. It is not the case that recently human has started to make use of herbal drugs since ancient time, the human herbalism has been supported, but the only difference is that now-a-days people have become more health conscious, their views regarding advantages, disadvantages of synthetic & herbal drugs have become very clear. By using various advanced techniques, isolation of various phytoconstituents are carried out for the benefit of human health. Bacteria are of both types that is beneficial & harmful but when person's immune system

become weak at that time useful bacteria like normal bacterial flora also start to act like harmful bacterias. There are so many infectious diseases which are water borne or food borne [1].

**1) Food borne diseases-** Gastroenteritis, creutzfeldt Jacob disease, mad cow disease etc.

**2) Water borne diseases-** Travellers diarrhoea, typhoid, cholera etc.

Out of these food resources is an important source for mode of transmission of bacterias. For example

**1) Cheese-** *Campylobacter spp.*, *E.coli.*, *Salmonella spp.*

**2)Egg-** *Salmonella spp.*,

**3)Meat(Beefpork)& poultry (chickenduck)-** *E.coli.*, *Salmonella spp.*, *Campylobacter spp.*,

**4) Milk or juice -** *Staphylococcus spp.*, *Salmonella spp.*, *E.coli.*,

**5) Rice-** *Bacillus spp.*,

**6) Vegetables-** *Aeromonas spp.*,

To treat the infectious diseases, it is necessary to know which resources are responsible for transmission of

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Corresponding Author :- **Shinde SA** Email:- seema\_kadam4@rediffmail.com

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various diseases & very important to know which are the possible sites present on human body where infection may occur [2]. The sites are as follows

So in such a way this information is very helpful to use herbal extracts because according to site of infection herbal extract can be used. Plant with possible antibacterial activity should be tested against an appropriate bacterial model to confirm the activity and to ascertain the parameter associated with it. The effect of plant extract on bacteria has been studied by a very large number of researchers in different part of the world [3].

### **Plant Profile**

**Scientific name-***Brassica oleracea*

**Synonym-** *Cauliflower*

**Biological source:** The drug consist of leaves of *Brassica oleracea* belonging to family *Brassicaceae*

### **Description plant**

**Distribution-** *Brassica oleracea* leaves is grown in tropical region & the relatively warmer temperature region. It is an annual plant.

**Propagation-**It is grown from seeds.

**Climate-** Warm & tropical climate are suitable for cauliflower.

**Growth rate-** It takes 45 -55 days to reach maturity.

**Height-** Height up to 4 inch.

**Leaves-** Hand shape leaves 10-20 cm long with 15-25 lobes.

### **Traditional uses**

- Cauliflower leaves are used as anti-helminthic.
- Leaves were considered as having anti-ulcerogenic activity.
- It is anti-parasitic.
- Fresh cauliflower juice has been shown to promote rapid healing of peptic ulcer.
- Cauliflower is used as excellent source of vitamin-c.
- It is also used in gastroprotective activity [4,5].

### **Chemical constituents**

The leaves of *Brassica oleracea* contains Norisoprenoids, alcohol, carbonyl compound, carbohydrate, lipid, terpenes, tannins, isoflavonoids, flavonoids, alkaloids, sulfuraphane, glucosinolates, carotenoids, etc..

### **Need of the Study**

On the earth not a single place is there, where microorganisms are absent. They may be present in the form of infectant , ingestants , injectant , contactants , and may produce harmful effect on human body. Within certain limit they are beneficial to human but beyond limit they are harmful. There are various sources of transmission of these microorganisms like through food, water, by simple contact with infectious person, soil, etc. Out of them food resources

is the commonest mode of transmission and which is unavoidable by human being [6,7].

e.g. 1) sea food (raw)- Astrovirus , *Vibrio* spp.

2) vegetable- *Aeromonas* spp.

These are the modes of transmission of harmful bacteria, they may cause diseases like cholera, typhoid, syphilis , and food borne diseases some of diseases may cause death of human also.

In order to counteract these infectious diseases synthetic medicines are preferred. Antibiotic like broad spectrum include erythromycin, sulpha drugs, or narrow spectrum including penicillin, cephalosporin, are used to reduce infectious diseases. But unfortunately, some bacterial strain has become very resistant & cannot be killed by antibiotic. So, in that case, herbal extract are preferred. Various researchers have also recently carried out work on efficiency of herbal extract for their antibacterial activities & antifungal activities. These researches will continue in future also because extract have-

1) Less side effect.

2) Cost benefit ratio is also good as compared to synthetic drug.

From above description the aim of present study is clear. In this, *Brassica oleracea* plant is selected, which is one of the commonest plant reputed for it's nutritional and therapeutic effect. Leaves of same plant are selected for evaluation of antibacterial activity, by using appropriate method and laboratory condition [8].

### **Objectives of Study**

To screen specific plant part for presence of phytoconstituent responsible for an antibacterial activity and to extract the active phytoconstituent from leaves of *Brassica oleracea* by maceration method. To evaluate sensitivity of vegetative bacterial species & yeast species by in vitro evaluation method against selected plant part which is considered to possess an antibacterial activity.

### **MATERIALS AND METHOD**

**Preparation of aqueous & organic extract of *Brassica oleracea* leaves**

**Collection of plant**

Fresh leaves of *Brassica oleracea* were collected from local area of satara

**Isolation of leaves of *Brassica oleracea***

In this stage the leaves were simply cut down by knife longitudinally.

**Drying**

Pieces of leaves were dried in open air in the shade to prevent the contact of ultraviolet rays in order to prevent the inactivation of chemical constituents present in it.

**Powdering**

Dried leaves were crushed so as to form fine powder with the help of grinder.

**Maceration**

Finely formed powder was macerated by adding water, chloroform and methanol in an appropriate amount. Maceration process was carried out for 6 days with occasional shaking. Added chloroform prohibited growth of fungus on the powder of *Brassica oleracea* which is in contact with water. This process is carried out at room temperature [9].

**Filtration**

After 6 days of extraction process, the macerated suspension was filtered and clear filtrate was collected in big porcelain dish.

**Evaporation**

Porcelain dish containing filtrate was kept on electric water bath for evaporation & temperature was maintained about 100°C. After evaporation of solvent the dark brown extract of leaves was obtained which was stored in refrigerator until its use in antimicrobial activity [10].

**Determination of an antibacterial activity of Brassica oleracea leaves**

For an evaluation of antibacterial activity of *Brassica oleracea* leaves cup and plate method or agar well diffusion method was used due to following advantages-

- It is very easy method.
- Less time consuming process
- Cheap method

**Preparation of dilution**

Dilutions were prepared in DMSO. The concentrations of dilutions were 400µg/ml, 600 µg/ml, 800 µg/ml, 1000 µg/ml.

**Preparation of nutrient agar medium**

pH of the medium was adjusted to 7.1 & medium was subjected for sterilization in autoclave at 15 psi pressure, 121°C temperature for 30 minutes.

**Preparation of agar plates**

In each petriplate approximately 20 ml of agar medium was poured in an aseptic condition and kept at room temperature for solidification at least for 20 minutes [11].

**Preparation of saline**

In 100 ml distilled water 0.9 gm of sodium chloride was dissolved & is sterilized by autoclaving. Then different strains of bacteria were inoculated in saline for preparation of bacterial suspension.

**Selection of bacterias**

To prepare bacterial suspension in order to spread on an agar plate to see an antibacterial activity of aq. & organic extract on various strains of bacterias following spp. have been selected.

- A) Gram positive bacterias- 1) *Bacillus subtilis* 2) *Staphylococcus aureus*
- B) Gram negative bacterias-1) *Klebsiella pneumonia* 2) *Salmonella typhi*
- C) Yeast- *Candida albicans*

**Spreading of bacterial suspension**

After pouring 0.1 ml standard bacterial suspension with the help of sterile pipettes on respective agar plate the suspension was spread uniformly with the help of glass spreader in an aseptic condition [12].

**Table 1. List of infectious bacteria & their site of infection**

| Site for infection on human body | Infectious bacteria                             |
|----------------------------------|---|
| Eye                              | <i>Streptococcus spp., Staphylococcus spp.</i>  |
| Heart                            | <i>Staphylococcus spp., Verodans</i>            |
| Urinogenital tract               | <i>E. coli, Neisseria spp.</i>                  |
| Brain                            | <i>Neisseria spp., Streptococcus spp.</i>       |
| Gastrointestinal tract           | <i>Shigella spp., E. Coli., Salmonella spp.</i> |

**Table 2. Scientific classification**

|                    |                 |
|--------------------|-----------------|
| <b>Kingdom</b>     | Plantae         |
| <b>Subkingdom</b>  | Tracheobionta   |
| <b>Division</b>    | Magnoliopida    |
| <b>Subdivision</b> | Spermatophyte   |
| <b>Subclass</b>    | Billeniidae     |
| <b>Order</b>       | Capparales      |
| <b>Family</b>      | Brassicaceae    |
| <b>Genus</b>       | <i>Brassica</i> |
| <b>Species</b>     | <i>Oleracea</i> |

**Table 3. Composition of agar medium**

| Sr.no. | Ingredient              | Quantity (gm) |
|--------|-------------------------|---------------|
| 1.     | Beef extract            | 04 gm         |
| 2.     | Peptone                 | 04 gm         |
| 3.     | Sodium chloride         | 02 gm         |
| 4.     | Distilled water to make | 400 ml        |
| 5.     | Agar-agar               | 10 gm         |

**Table 4. Concentration of standard antibiotic as well as extract**

| Concentration of standard antibiotic ( $\mu\text{g/ml}$ ) | Concentration of Extract ( $\mu\text{g/ml}$ ) |
|---|---|
| 600   | 400   |
|   | 600   |
|   | 800   |
|   | 1000  |

**Table 5. Results for aqueous extract**

| Sl. No | Bacterial spp.               | Concentration ( $\mu\text{g/ml}$ ) | Zone of inhibition (mm) |
|--------|------------------------------|------------------------------------|-------------------------|
| 1      | <i>Bacillus subtilis</i>     | 400                                | 12                      |
|        |                              | 600                                | 15                      |
|        |                              | 800                                | 16                      |
|        |                              | 1000                               | 18                      |
|        |                              | (Ciprofloxacin) Std-600            | 34                      |
|        |                              | DMSO                               | 11                      |
| 2      | <i>Staphylococcus aureus</i> | 400                                | -                       |
|        |                              | 600                                | -                       |
|        |                              | 800                                | -                       |
|        |                              | 1000                               | -                       |
|        |                              | (Ciprofloxacin) Std-600            | 12                      |
|        |                              | DMSO                               | -                       |
| 3      | <i>Klebsiella pneumoniae</i> | 400                                | 20                      |
|        |                              | 600                                | 25                      |
|        |                              | 800                                | 26                      |
|        |                              | 1000                               | 28                      |
|        |                              | (Ciprofloxacin)Std-600             | 40                      |
|        |                              | DMSO                               | -                       |
| 4      | <i>Salmonella typhi</i>      | 400                                | 11                      |
|        |                              | 600                                | 12                      |
|        |                              | 800                                | 13                      |
|        |                              | 1000                               | 15                      |
|        |                              | (Ciprofloxacin)Std-600             | 26                      |
|        |                              | DMSO                               | -                       |
| 5      | <i>Candida albicans</i>      | 400                                | -                       |
|        |                              | 600                                | -                       |
|        |                              | 800                                | -                       |
|        |                              | 1000                               | -                       |
|        |                              | Std-600                            | 45                      |
|        |                              | DMSO                               | -                       |

**Table 6. Results for organic extract**

| Sl. No | Bacterial spp.               | Concentration (µg/ml)  | Zone of inhibition (mm) |
|--------|------------------------------|------------------------|-------------------------|
| 1      | <i>Bacillus subtilis</i>     | 400                    | 12                      |
|        |                              | 600                    | 13                      |
|        |                              | 800                    | 15                      |
|        |                              | 1000                   | 19                      |
|        |                              | (Ciprofloxacin)Std-600 | 33                      |
|        |                              | DMSO                   | -                       |
| 2      | <i>Staphylococcus aureus</i> | 400                    | -                       |
|        |                              | 600                    | -                       |
|        |                              | 800                    | -                       |
|        |                              | 1000                   | 13                      |
|        |                              | (Ciprofloxacin)Std-600 | 41                      |
|        |                              | DMSO                   | -                       |
| 3      | <i>Klebsiella pneumonia</i>  | 400                    | 21                      |
|        |                              | 600                    | 24                      |
|        |                              | 800                    | 27                      |
|        |                              | 1000                   | 30                      |
|        |                              | (Ciprofloxacin)Std-600 | 40                      |
|        |                              | DMSO                   | -                       |
| 4      | <i>Salmonella typhi</i>      | 400                    | 14                      |
|        |                              | 600                    | 16                      |
|        |                              | 800                    | 19                      |
|        |                              | 1000                   | 22                      |
|        |                              | (Ciprofloxacin)Std-600 | 34                      |
|        |                              | DMSO                   | -                       |
| 5      | <i>Candida albicans</i>      | 400                    | -                       |
|        |                              | 600                    | -                       |
|        |                              | 800                    | -                       |
|        |                              | 1000                   | -                       |
|        |                              | (ciprofloxacin)Std-600 | 47                      |
|        |                              | DMSO                   | -                       |

**Preparation of cups**

With the help of sterile borer of 8 mm diameter total 6 cups were prepared on two agar plates for each bacterium that is 3 cup on one plate. Accordingly they were labelled. Different concentrations of extract (0.1 ml) poured in it along with standard antibiotic as mentioned below [13].

**Refrigeration & incubation**

After addition of extract in respective cups the plates were kept in refrigerator for at least two hours in order to facilitate diffusion process. Then plates were transferred for incubation in an incubator at 37°C temperature for 24 hrs [14].

**DISCUSSION**

An antimicrobial activity of leaves of *Brassica oleracea* was observed by agar well diffusion method & by measuring the diameter of zone of inhibition, the above mentioned results were obtained. Among the tested, aqueous extract of cauliflower leaves has shown high

degree of inhibition against most of the selected bacteria but not against yeast *Candida albicans*. The zone of inhibition increases with increase in concentration of extract indicating concentration dependant effect. The observation suggests that certain bioactive compounds are responsible for an antimicrobial activity of cauliflower leaves [15-20]. The effectiveness of cauliflower leaves is not due to the one of its constituent, but the combined action of other constituents too; those compounds might be certain lipid, tannins, or phenolic compounds. The present study proves that *Brassica oleracea* leaf possesses an antibacterial activity which gives an indication regarding presence of biological principle & hence the same can be utilized to develop an antibacterial agent in future. Following images of petriplates indicates an antimicrobial activity of cauliflower leaves against *Bacillus subtilis*, *Salmonella typhi*, *Klebsiella pneumoniae*.

**CONCLUSION**

From the obtained results of an antimicrobial activity of *Brassica oleracea* leaves, it can be concluded

that the extract exhibits an antibacterial activity, against used bacterial strain & no activity against yeast *Candida albicans*. It also can be confirmed that the extract of leaves can be used in case of infection of test bacterial strain. As we know, thin layer chromatography provides the good

evidence for the presence of different phytochemicals in extract it further need the investigation to determine phytochemically active compound from the leaves of *Brassica oleracea*.

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