Comparative analysis of antibacterial activities observered in some particular medicinally important plants

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Abstract: The present investigation is to evaluate antibacterial activity of aqueous extract of *Ocimum sanctum* and *Centella asiatica* were screened against three pathogenic bacteria *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus. Ocimum sanctum* was also showed antibacterial activity against *Staphylococcus aureus.* The antibacterial activity was tested by disc diffusion method.

Key words: *Ocimum sanctum L, Centella asiatica L, Antibacterial activity.*

1. INTRODUCTION

Plants are one of the most important sources of medicines. Today the large number of drugs in use are derived from plants, like morphine from Papaver somniferum, Aswagandha from Withania somnifera, Ephedrine from Ephedra vulgaris, Atropine from Atropa belladonna, Reserpine from Roulphia serpentina etc. The medicinal plants are rich in secondary metabolites (which are potential sources of drugs) and essential oils of therapeutic importance. According to a survey (1993) of World Health Organization (WHO), the practitioners of traditional system of medicine treat about 80% of patients in India, 85% in Burma and 90% in Bangladesh.

The medicinal use of plants is very old. The writings indicate that therapeutic use of plants is as old as 5000 – 45000 B.C. and Chinese used first the natural herbal preparations as medicines. In India, however, earliest references of use of plants as medicine appear in Rigveda which is said to be written between 10000 – 5000 B.C. Later the properties and therapeutic uses of medicinal plants were studied in detail and recorded empirically by the ancient physicians in Ayurveda (an indigenous system of medicine) which is a basic foundation of ancient medical science in India.

The herbal medicines used in ayurveda are claimed to cure the patient permanently rather than suppressing the disease without causing any side effects. The medicinal and aromatic plants are abundantly found in India, due to varied agro climatic conditions.

Ocimum sanctum (L.) named in Hindi as Tulsi or Holy basil is a member of the family Lamiaceae/Labiatae.[2] It is an aromatic plant that is native to the tropics of Asia and Africa and is widespread as a cultivated plant. It is an erect softy hairy small shrub with many branches and strongly scented green leaves. The leaves are ovate and slightly toothed. The flowers are purplish to white. It contains some biological active chemical compounds like ursolic acid, luteolin, alkaloids and apigenin extract from leaves. In Ayurvedic medicine, *O. sanctum* is used to treat common cold, headaches, stomach disorders, inflammation, heart disease & malaria. Recent studies have also shown that *O. sanctum* contains high levels of eugenol & there is effective as a painkiller.

Centella asiatica (L.) Urban, syn-Hydrocotyle asiatica (L.) named in Hindi as Brahmi–manduki is a member of the Apiaceae. The plant is prostrate, perennial, slender and faintly aromatic herb found throughout India and Sri Lanka in marshy places up to an altitude of 1828m.[1] The plant is reported to possess antileprotic, antitumor, antistress, antifeedant and antifilarial properties and is used as a tonic in Ayurvedic formulations. Main constituents of *C.asiatica* are saponin, triterpenic acids, polyacetylene, flavonoids, steroids, lipids constituents and Nitrogen containing constituents isolated from this plant.

2. MATERIALS AND MATHODS

2.1. Collection of plant material

Fresh leaves of *O. sanctum* were collected during October and November from the home cultivar as a religious plant. The plant *C. asiatica* was collected from the Botanical garden of Junagadh Agriculture University, Junagadh. The plant material was identified at Botany Department, Bahauddin Science College, Junagadh (India). A voucher specimen has been kept in the laboratory for future references. The leaves were washed, shade dried and pulverized by a grinder, passed through mesh sieve and stored in sealed container.

2.2. Collection and maintenance of the Bacterial strains for inoculums

Pure microbial cultures of *Bacillus subtilis*, *Escherichia coli, and Staphylococcus*

aureus were obtained from the pathological laboratory. All the strains were stored at 4^0 C in a freezer and periodically sub-culturing were made. Sample for inoculation were selected from the exponential growth phase of bacteria.

2.3 Preparation of extract

The powdered leaves (100 grams) of *O. sanctum* and *C. asiatica* were extracted separately with distilled water in soxlet apparatus up to 4 cycles (Paech et al., 1956). The extracted material was filtered through sterile muslin cloth and the filtrate obtained was evaporated to dryness in vacuo below 40^0 C. The yield was 9.25% w/w with respect to dry powdered material for *O*. sanctum and *C. asiatica.* The dried powder was weighed and reconstituted in sterile phosphate buffer saline (PBS, pH 7.4, 0.01 M) at the rate of 20 mg of extract per ml of phosphate buffer saline. The dose of O. sanctum and *C. asiatica* extract was standardized in the pilot study, by taking 3 Bacterial samples in 3 cows in 2 batches. Finally, the filtrate was filtered through membrane filter (pore size 0.45 lm) and stored at 4^0 C till used.

2.4 Preparation of Bio-diffusion disc

Sterilized 10 mm diameter disc were cuted from Whatman's filter papers. The discs were deeped in to a extract of *O*. sanctum and *C. asiatica* separately. Discs were allowed to air dry and then placed in to four sub-corners of the Petridis.

2.5 Disc diffusion methods

Nutrient agar (N – agar) was used for cultivation of bacteria. Total 27 Petridis were prepared. The inoculums of *B. subtilis*, *E. coli* and *S. aureus* were spread out over N – agar plate (9 + 9 + 9). Four Bio-diffusion discs of *O*. sanctum were placed in sub-corner of Petridis (3 + 3 + 3). Likewise discs of *C. asiatica* were also placed in triplicate. Simultaneously a Petridis which act as a control (without any bio-diffusion disc) were also prepared in triplicate.

2.6 Direct measurement of Growth inhibition

20 mg of extracted drugs of *O*. sanctum and *C. asiatica* was tested against the growth of *B. subtilis*, *E. coli* and *S. aureus Streptococci* culture. The experimental design was prepared in such a way (Table - 1) that three sets of culture medium were prepared with 100 ml nutrient broth medium in 250 ml conical flask in triplicate. Total 27 flasks were prepared – 9 for Control "A" (without drug one ml inoculums of respective bacterial strains – 3 for *B. subtilis*, 3 for *E. coli* and 3 for *S. aureus).* Other 9 flasks were tested for growth inhibition "B" (with 20 mg drugs of

O. sanctum and 3 for *B. subtilis*, 3 for *E .coli* and 3 for *S. aureus* and 1 ml inoculums). Rest 9 flasks were tested with 20 mg drugs of *C. asiatica* with same preparation. All the preparation was carried out under aseptic condition to overcome the contamination problem. Initial O.D. was measured for all the flasks. Flasks were placed on rotatory shaker at room temperature for 24 hours. After 24 hours incubation O.D. were measured for growth of bacteria.

The antibacterial activities of extracts of *C. asiatica* were tested upon *B. subtilis* and *E. coli* and O. sanctum were tested upon *B. subtilis*, *E. coli* and *S. aureus* by disc diffusion method [3] [4].

RESULT AND DISCUSSION

Table – 1: Shows effect of drugs on growth of *B. subtilis*

Flask	O.D. at 0 Hours				O.D. at 24 Hours			
	1	2	3	SD	1	2	3	SD
A	0.06	0.03	0.04	0.043 ± 0.01	1.35	1.44	1.31	1.367 ± 0.02
B	0.05	0.03	0.06	0.047 ± 0.01	0.08	0.04	0.09	0.070 ± 0.01
C	0.06	0.05	0.03	0.047 ± 0.01	0.08	0.05	0.04	0.057 ± 0.01

Table – 2: Shows effect of drugs on growth of *E. coli*

Flask	O.D. at 0 Hours				O.D. at 24 Hours			
	1	2	3	SD	1	2	3	SD
A	0.06	0.03	0.04	0.043 ± 0.01	1.55	1.56	1.66	1.59 ± 0.02
B	0.05	0.03	0.06	0.047 ± 0.01	0.06	0.09	0.08	0.077 ± 0.01
C	0.06	0.05	0.03	0.047 ± 0.01	0.09	0.05	0.05	0.063 ± 0.02

Table – 3: Shows effect of drugs on growth of *S. aureus*

Flask	O.D. at 0 Hours				O.D. at 24 Hours			
	1	2	3	SD	1	2	3	SD
A	0.06	0.03	0.04	0.043 ± 0.01	1.22	1.12	1.31	1.220 ± 0.02
B	0.05	0.03	0.06	0.047 ± 0.01	0.06	0.04	0.06	0.053 ± 0.01
C	0.06	0.05	0.03	0.047 ± 0.01	0.06	0.05	0.03	0.047 ± 0.01

A result (Table 1 – 3) clearly suggests that flasks with drug were remaining as such without bacterial growth but control flaks show the measurable growth. So, our drug product is effective against the diseases causing bacteria *B. subtilis*, *E. coli* and *S. aureus* by preventing the growth of them.

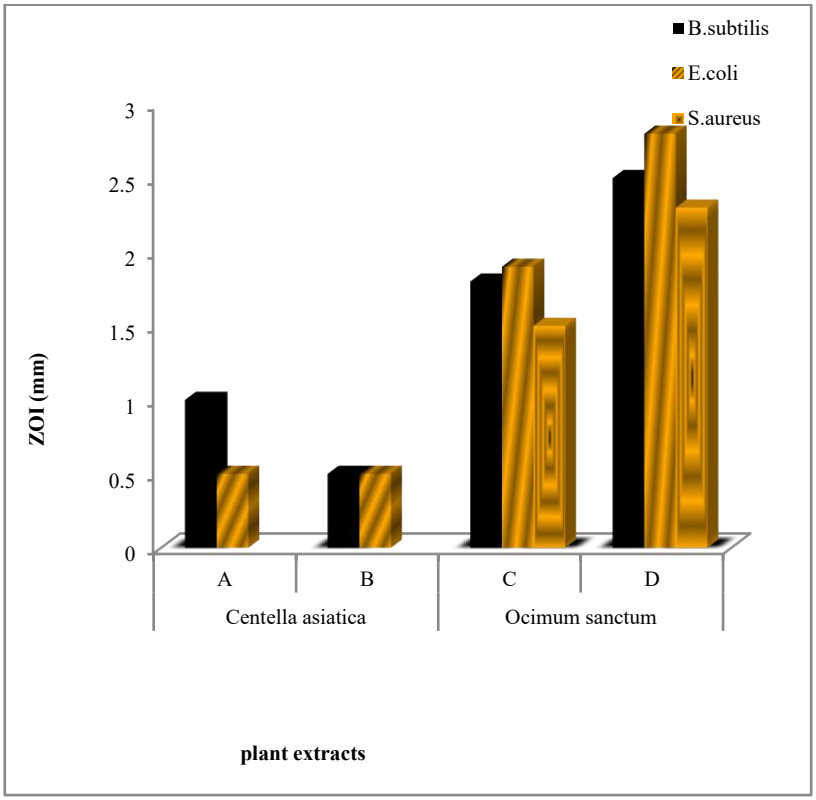

FIGURE 1. *Antibacterial activity of medicinal plants using disc diffusion method.*
Where, A:20 mg extract, B: 10 mg *extract, C:10 mg extract, D: 20 mg extract.*

The result of the extracts of *C. asiatica* exhibited antibacterial activity against all the tested strain viz. Gram-positive bacteria *B.subtilis* and Gram-negative bacteria *E. coli.* The Gram-positive bacteria *B.subtilis*, *S.aureus* and Gram-negative bacteria E. *coli* (Fig. 1). The zones of inhibitions (ZOI) were observed by the 10 mg and 20 mg extracts against all the test organisms. But quantity shows the greater effect in form of greater zone of inhibition. The zones of inhibition were ranging from 0.5 – 2.8mm in diameter. The highest zones of inhibition (2.8 mm) noted in aqueous extract of 20 mg of *O.sanctum* against *Escherichia coli* in 0.5 ml concentration and least activity recorded in 10 mg extract of *C.asiatica.*

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