

**SCREENING FOR FOLKLORE FOR ANTIMICROBIAL ACTIVITY**Ekta Menghani* and Sita kumari sharma¹

*Department of Biotechnology, Mahatma Gandhi Institute of Applied Sciences, JECRC University, Jaipur, Rajasthan, India

¹Departments of Biotechnology Suresh Gyan Vihar University, Jaipur, Rajasthan, India***Corresponding author e-mail:** ektamenghani@yahoo.com**ABSTRACT**

Methanolic extracts of two Indian medicinal plants *Citrullus colocynthis*, and *Citrus medica*, were examined for their antimicrobial potential against selected bacteria and fungi. The purpose of screening is to justify and authenticate the use of Indian medicinal plants in ethno medicinal or folklore as traditional treasure to cure various ailments. In present investigations attempts were made to screen the Indian medicinal plants as antimicrobial agent. The extracts were tested against selected test bacteria and fungi through disc diffusion assay where Tetracycline and Mycostatin were used as standard. Indian medicinal plants have a traditional background that they have potentials to use as antimicrobial agents. The results showed that alcoholic extract possess good antimicrobial activity against selected test bacteria and fungi. The present results therefore offer a scientific basis for traditional use of the various extract of *Citrullus colocynthis*, and *Citrus medica*.

Keywords: *Citrullus colocynthis*, and *Citrus medica* Antimicrobial, Indian Medicinal Plants, Disc diffusion assay**INTRODUCTION**

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. Medicinal plants are a source of great cost-effective value all over the world. Nature has bestowed on us a very rich botanical prosperity and a large number of diverse types of plants grow in different parts of the country^[1]. Herbal medicine is still the core of about 75-80% of the whole population and the major part of traditional therapy involves the use of plant extract and their active constituents.

Following the advent of modern medicine, herbal medicine suffered a setback, but during last two or three decades, advances in photochemistry and in identification of plant compounds, effective against Certain diseases have renewed the interest in herbal medicines^[2].

The beneficial medicinal effects of plant materials typically result from the combinations of secondary

products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins and phenol compounds flavonoids, steroids, resins fatty acids gums which are capable of producing definite physiological action on body^[3].

The millenarian use of these plants in folk medicine suggests that they represent an economic and protected alternative to treat infectious diseases^[4]. The use of medicinal plants as a source for relief from illness can be traced flipside over five millennia to written documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind. Neanderthals living 60,000 years ago in present day Iraq used plants such as holly back, these plants are still widely used in ethno medicine around the world.^[5,6]

Therefore, in present study an attempts have been made to evaluate antimicrobial potential of two medicinal plants *Citrullus colocynthis*, and *Citrus medica* [Fig. 1(a), (b)] each belonging to different families.

MATERIALS AND METHODS

Collection: Plant samples (*Citrullus colocynthis*, and *Citrus medica*) were collected from various tribes living in tribal pockets of Mt. Abu, arid zone of Rajasthan. These plants were used by these tribes in their daily lives to cure various ailments and few from Chunnilal Attar Ayurvedic Store, Ghat Gate, and Jaipur in the month of May, 2011.

Identification: All the samples were authenticated and were given identification number *Citrullus colocynthis*, and *Citrus medica*. These samples were authenticated and submitted in Ethno medicinal Herbarium, Centre of Excellence funded by DST, MGiaS, Jaipur (Rajasthan).

Sources of Test Organisms: Pure culture of all test organisms, bacteria's namely *Enterobacter aerogenes*, *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Shigella flexneri*, and *Chryseobacterium gleum* and fungi *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus* were obtained through the courtesy of Mahatma Gandhi Institute of applied Sciences (MGiaS), Jaipur, which were maintained on Nutrient broth media.

Culture of Test Microbes: For the cultivation of bacteria, Nutrient Agar Medium (NAM) was prepared by using 20 g Agar, 5 g Peptone, 3 g beef extract and 3 g NaCl in 1 L distilled water and sterilized at 15 lbs pressure and 121°C temperature for 25-30 min. Agar test plates were prepared by pouring approximately 15 mL of NAM into the Petri dishes (10 mm) under aseptic conditions. A saline solution was prepared (by mixing 0.8% NaCl) in distilled water, followed by autoclaving and the bacterial cultures were maintained on this medium by regular sub-culturing and incubation at 37°C for 24-48 h. To prepare the test plates, in bacteria, 10-15 mL of the respective medium was poured into the Petri plates and used for screening. For assessing the bactericidal efficacy, a fresh suspension of the test bacteria was prepared in saline solution from a freshly grown Agar slant.

Preparation of Test Extracts: Crushed powder (50 g) of all the species were successively Soxhlet extracted with ethanol. Later, each of the homogenates was filtered and the residue was re-extracted twice for complete exhaustion, the extracts were pooled individually. Each filtrate was concentrated to dryness in vitro and redissolved in respective solvents, out of which 80 mg/10 discs i.e.

8 mg/disc concentration were stored at 4°C in a refrigerator, until screened for antibacterial activity.

Antimicrobial Assay by Disc Diffusion Method: For both, bactericidal in vitro Disc diffusion method was adopted [7] because of Reproducibility and precision. The different test organisms were proceeded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibition were measured around sterilized dried discs of Whatman No. 1 paper (5 mm in diameter), which were containing 1mg, 5mg and 10mg of the test extracts and reference drugs (tetracycline and Mycostatin for bacteria and fungi, respectively) separately. Such treated discs were air-dried at room temperature to remove any residual solvent, which might interfere with the determination, sterilized and inoculated.

These plates were initially placed at low temperature for 1 h so as to allow the maximum diffusion of the compounds from the test disc into the agar plate and later, incubated at 37°C for 24 h in case of bacteria and °C in case of fungi, after which the zones of inhibition could be easily observed. Five replicates of each test extract were examined and the mean values were then referred. The Inhibition Zone (IZ) in each case were recorded and the Activity Index (AI) was calculated as compared with those of their respective standard reference drugs (AI = Inhibition Zone of test sample/Inhibition zone of standard).

RESULTS AND DISCUSSION

The profile of the medicinal plants used in the present investigation. The results of antimicrobial activity of the crude extracts of Selected Indian Medicinal Plants (*Citrullus colocynthis*, and *Citrus medica*) showed good Antimicrobial activity against selected test bacteria and fungi (Table-1). Overall, this extract showed appreciable activity against selected test bacteria and fungi and hence, it justify their use in our traditional system of medicine to cure various diseases (Fig.2, 3)

***Citrullus colocynthis*:** While screening the extracts of *Citrullus colocynthis* the, good antimicrobial activity against the selected bacteria and fungi was observed. The alcoholic s extract was found active against all the bacteria and fungi tested. Results, comparable to the standard, the maximum activity was shown, *Staphylococcus aureus* (8.3 mm) in methanolic extract whereas the minimum activity was shown by *E.aerogenes* (6.3 mm).in the case of fungi the maximum activity was shown by *A.niger*

(7.6 mm) and minimum activity was shown by *C.albicans* (8 mm).

Citrus medica: While screening the extracts of *Citrus medica* the, good antimicrobial activity against the selected bacteria and fungi was observed. The alcoholic s extract was found active against all the bacteria and fungi tested. Results, comparable to the standard, the maximum activity was shown *Staphylococcus aureus* (6.3 mm) in methanolic extract whereas the no activity was shown by *P. vulgaris*.in the case of fungi the maximum activity was shown by *A.niger* (6.3 mm) and minimum activity was shown by *A.flavus* (3 mm).

CONCLUSION

The versatile medicinal plants are the unique source of various types of compounds having diverse chemical structures. Very little work has been done on the biological activity and plausible medicinal

applications of these compounds and hence extensive investigation is needed to exploit their therapeutic utility to combat diseases. The present results therefore offer a scientific basis for traditional use of the various extracts of *Citrullus colocynthis*, *Citrus medica*. These results explain that Indian Medicinal Plants have potentials as antimicrobials. Further, more or less both the selected Indian Medicinal Plants have also possessed antimicrobial potential against all test bacteria and fungi which explains that their use in daily life will generate a resistance or immunity to fight against microorganisms.

ACKNOWLEDGEMENT

Authors acknowledge with thanks the financial support from Department of Science and Technology, Government of Rajasthan, in the form of Centre with Potentials for Excellence in Biotechnology, sanction no.f. 7(17) (9) Wipro/Gaprio/2006/7358-46(31/10/2008).



Fig.1: (a) *Citrullus colocynthis* (b) *Citrus medica*,

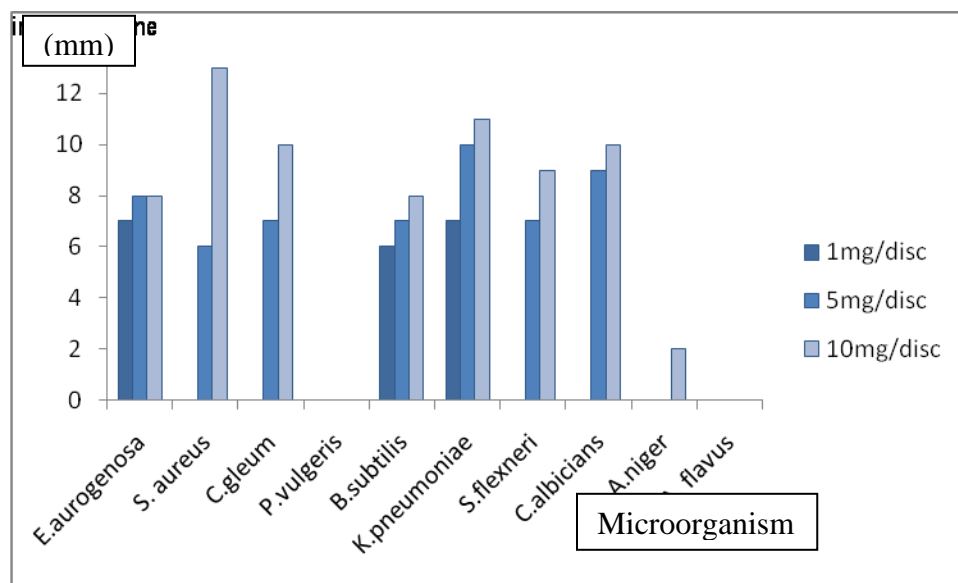


Figure 2: Antimicrobial potential of *citrus medica* against selected test microorganisms in terms of inhibition zone

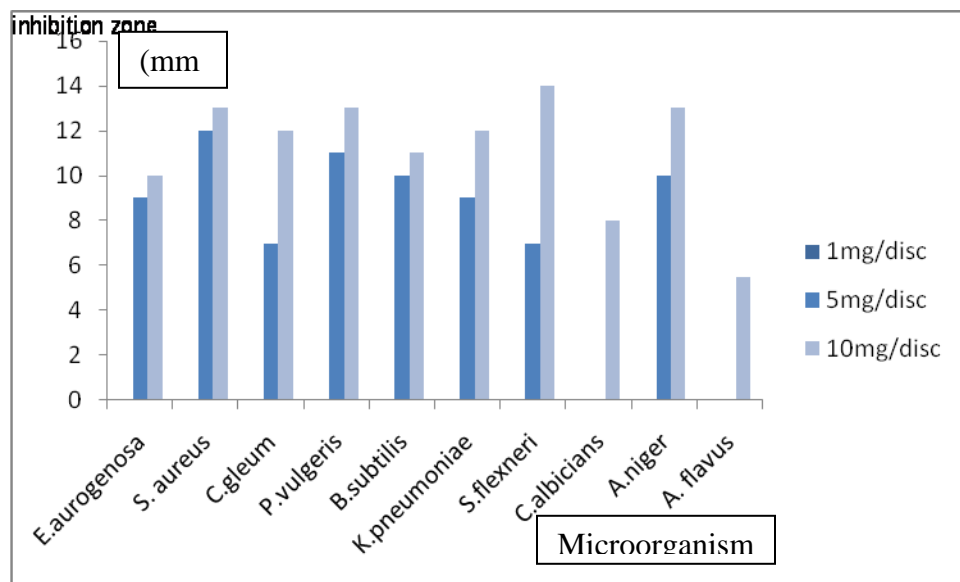


Figure- 3: Antimicrobial potential of *Citrullus colocynthis* against selected test microorganisms in terms of inhibition zone

Table 1: Antibacterial efficacy in terms of inhibition zone of *Citrullus colocynthis*, *Citrus medica* against selected bacteria and fungi

| | Measure | Bacteria | | | | | | | Fungi | | |
|------------------------------|---------------|--------------------|------------------|--------------------|-----------------|----------------------|--------------------|---------------------|--------------------|-----------------|------------------|
| | | <i>P. vulgaris</i> | <i>S. aureus</i> | <i>B. Subtilis</i> | <i>C. gleum</i> | <i>K. pneumoniae</i> | <i>S. flexneri</i> | <i>E. aerogenes</i> | <i>C. albicans</i> | <i>A. niger</i> | <i>A. flavus</i> |
| Methanol | Standard I.Z. | 19 | 22 | 14 | 13 | 18 | 16 | 21 | 12 | 16 | 9 |
| <i>C. colocynthis</i> | I.Z. (mm) | 8 | 8.3 | 7 | 6.3 | 7 | 7 | 6.3 | 8 | 7.6 | 5.5 |
| | A.I. | 2.375 | 2.650 | 2 | 2.166 | 2.571 | 2.285 | 0.317 | 1.5 | 2.105 | 1.636 |
| <i>C. medica</i> | I.Z. (mm) | 0 | 6.3 | 7 | 5.3 | 9.3 | 5.3 | 7.6 | 4.3 | 6.3 | 3 |
| | A.I. | 0 | 3.492 | 2 | 2.452 | 1.935 | 3.018 | 2.763 | 2.790 | 2.539 | 3 |

REFERENCES

1. Ahmed L, Mohammed Z, Mohammed F. J Ethnopharmacol, 1998: 62; 183-93.
2. Arora, D, Kaur J. Int J Antimicrobial Agents, 1999: 12; 257-62.
3. Bishnu J, Sunil L, Anuja S. J Sci Engineering Technol, 2009: 5; 143-50.
4. Toama MA, El-Alfy TS, El-Fataty HM. Antimicrobial Agents and Chemotherapy, 1974: 6; 225-6.
5. Thomson WAR. Medicines from the Earth Maidenhead, United Kingdom. McGraw-Hill Book Co. 1978.
6. Stock Well C. Nature's pharmacy. London United Kingdom. Century Hutchinson Ltd... 1988.
7. Bowie JH, Gould JC. J Clin Pathology, 1952: 5; 356.