International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2015): 6.391

Antimicrobial Activity of Asiatic Acid against Bacteria and Fungi

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Abstract: ***The biologically active components of Centella asiatica improve human health through the herbal derived medicine as an alternative source. Asiatic acid is the pentacyclic triterpenes, in the form of genins, isolated from the Centella asiatica. In the present study, the asiatic acid is employed for testing its efficacy against the two gram-positive and gram-negative bacteria and against fungi. The result showed that the asiatic acid has potential activity against both the bacterial and fungal strains. Hence, this active compound is therapeutically active against microbes and, can be taken for further pre-clinical investigations.***

Keywords: Antibacterial, antifungal, *centella asiatica,* asiatic acid and, triterpenes.

1. Introduction

Centella asiatica (Linn.) belongs to the family of Umbelliferae (Apiaceae) in specific to the Hydrocotyle subfamily[1]. The plant is indigenous to south-East Asia, Sri Lanka, China, the Western South Sea Islands, Madagascar, South Africa, South-East United States, Mexico, Venezuela, Columbia and Eastern South America[2]. The plant is propagated by its seeds or from stolons. It grows in damp soils and characterized by long internodes and nodes, reniform-cordate leaves and sessile flowers in simple umbels[3]. *Centella asiatica* gained its popularity by its medicinal properties[4]. It has varied usage according to the people of different continents. In general, the plant is used topically or orally[5]. Even though the plant has been used in Madagascan, Indian, Chinese, American-Indian and Indonesian medicine for more than 3000 years, its appearance in modern medicine was in late 1884 and the first crude extract was produced in 1941[6]. The plant has therapeutically active components, which prevailed to treat the variety of afflictions such as skin diseases, wound healing, dehydration, and diarrhea[7,8]. The active constituents of *Centella asiatica* are pentacyclic triterpenes, in the form of genins; they are asiatic acid, and madecassic acid, and of heterosides; they are asiaticoside and madecassoside[9]. Albeit the clinical investigations are ongoing in *Centella asiatica,* the interest on promising constituent with high effectiveness and low toxicity for the benefit of human health are yet to be explored. Hence, in the present research we try to comprehend the insight of the single triterpene compound which is asiatic acid derived from the most potential herb *Centella asiatica* through investigating its efficacy on antibacterial, antifungal.

2. Materials and Method

Test-pathogenic microorganisms

Two Gram-negative *Klebsiella pneumonia*, *Pseudomonas aeruginosa,* and two Gram-positive *Bacillus subtilis, Staphylococcus aureus* bacterial pathogens and one fungal pathogen *Candida albicans* were used for *in vitro* antimicrobial activity. These selected pathogenic strains were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, Punjab, India.

In vitro antimicrobial activity

The antibacterial activity was determined by well diffusion methods[10]. About 25 mL of molten Mueller Hinton agar was poured into a sterile Petri plate (Himedia, Mumbai, India). The plates were allowed to solidify, after which 18 h grown (OD adjusted to 0.6) 100 µl of above said pathogenic bacteria cultures were transferred onto plate and made culture lawn by using sterile cotton swab. After five minutes setting of the pathogenic bacteria, a sterile cork borer was used to make 5 mm well on the agar. The test samples were dissolved in DMSO and loaded in to wells with various concentrations such as 25 µg/well, 50 µg/well, 75 µg/well and 100 µg/well. The streptomycin added well served as positive control for bacteria and clotrimazole served as control for fungi. The solvent alone served as negative control. The plates were incubated at 37°C in a 40 W florescent light source (~ 400 nm) for 24 h. The antibacterial activity was determined by measuring the diameter of the zone of inhibition around the well using antibiotic zone scale (Himedia, Mumbai, India).

3. Result

The Asiatic acid showed antibacterial activity for few of the isolates and it was less active against the fungal isolate. The highest zone of inhibition observed in *Klebsiella pneumonia* in which the lowest concentration of 25 µg/well showed 13mm and 100 µg/well showed 28 mm zone of inhibition. In case of *Candida albicans* 6mm zone of inhibition observed for the highest concentration of 100 µg/well.

Antimicrobial activity of Asiatic Acid

Test organism	Diameter of inhibition zone (mm)			
Bacteria				
Concentration per disc	25mg	50mg	75mg	100mg
Staphlococcus aureus	7	8	12	13
Bacillus	7	9	15	17

subltilis				
Klebsiella pneumonia	13	23	26	28
Pseudomonas aeruginosa	-	-	6	6
Fungi				
Candida albicans	-	-	6	6

4. Discussion

Researches on finding the new biologically active compounds from the medicinal plants have always been the interest among researchers. In regard with the crude extract of *Centella asiatica* against the antimicrobial activity observed to be positive. Antibacterial effects against *Pseudomonas pyocyaneus*, *Trichoderma mentagrophytes* and *Entamoeba histolytica* have been demonstrated[11]. Yet another study indicated that *Centella asiatica* has confirmed activity against the enteropathogens and proposed to be the possible treatment for antidiarrhoeal drug development[12]. Antiviral activity was also observed against type II *Herpes simplex virus* in the alcoholic and in aqueous extract of *Centella asiatica*[13]. Therefore, from the above-mentioned studies it is apparent that the screening of specific compound extracted from the *Centella asiatica* was not carried to validate the compound for drug development. In the present study, the experiment is scrutinized in targeting, the particular therapeutically active compound asiatic acid from *Centella asiatica* against the gram-negative *Klebsiella pneumonia,* which showed minimum zone of inhibition at 25ug/well with 13mm and the highest zone of inhibition at 100ug/well with 28mm. *Pseudomonas aeruginosa* against asiatic acid has zone of inhibition 6mm at the highest concentration of 100ug/well. The gram-positive *Bacillus subtilis* showed maximum zone of inhibition at 100ug/well with 17mm when compared with *staphylococcus aureus* with the maximum inhibition zone of 13mm at 100ug/well. *Candida albicans* was resistant at lower concentration against asiatic acid and at highest concentration the zone of inhibition with 6mm at 100ug/well. From these observations of asiatic acid against gram positive and gram negative bacteria with appreciable zone of inhibition, in particular the *Klebsiella pneumonia* had maximum zone of inhibition compared with other bacterial strains.

5. Conclusion

In the view of versatile medicinal properties, the asiatic acid derived from *Centella asiatica* showed activity against bacterial strains and fungal strain. Consequently, the asiatic acid can be taken to further antimicrobial activity with the broad spectrum to develop as a drug as the alternative treatment for bacterial and fungal infections with less adverse effect.

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