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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF ALLAMANDA CATHARTICA LINN.

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ABSTRACT

Fresh flowers of Allamanda Cathartica Linn. were collected and was extracted with 90% methanol under reflux. The alcoholic extract was concentrated *in vacuo* and the aqueous concentrate was fractionated with benzene, peroxide free diethylether and ethylacetate successively. The ethyl acetate fraction was concentrated *in vacuo* and left in an ice chest for a few days. The yellow solid obtained, a quercitrin (quercetin 3-O-rhamnoside) derivative, was tested for its antibacterial and antifungal efficiencies against Staphylococcus aureus, Escherichia coli and strains of Candida albicans respectively. An explicit antibacterial effect was observed at 10µg concentration and as an antifungal agent at 800 µg.

KEYWORDS: **Allamanda Cathartic Linn., antibacterial, antifungal, E.coli, C.albicans.**

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I. INTRODUCTION

Plants are a great source of biologically active materials and have been a rich source of therapeutic agents. It is interesting to note that out of the 250,000-500,000 plant species identified on earth, roughly 10% have been studied chemically and pharmacologically for their innumerable medicinal values. *In-vitro* antifungal activities of extracts of various floral and leaf extracts have been widely studied and reported [1]. Many plants and their isolated products have been constantly screened for their possible antimicrobial activities [2-3]. For instance, extracts of various medicinal plants containing flavonoids have been reported to possess antimicrobial activity [4]. The activity of flavonoids is by inhibition of respiration and reproduction of microbes and has been proved by Powers et al [5]. The antimicrobial activity of isoflavonoids, flavonoids [6] and glycosides of luteolin and apigenin have been reported [7]. Floral extracts like quercetin, myricitrin are well known for their antiviral [8] and bactericidal activities [9-10]. Matricariachamomillais, another species widely reported to contain the flavonoids like apigenin, quercitrin and luteolin 7-O-glucosidepossesses substantial bactericidal activity particularly against gram-positive bacteria like Staphylococcus aureus and Candida albicans [11]. The root and rhizome oils of Kaempheria galangal showed similar activities against Staphylococcus aureus and Escherichia coli [12]. Apart from exhibiting antibacterial activities, several floral isolates also reveal antifungal properties. A wide variety of synthetic and natural compounds have been discovered largely over the past 40 years to inhibit growth of pathogenic fungi, which are usually non-photosynthetic eukaryotes, grow either as a colony of single cells or as filamentous multicellular aggregates. Most fungi live as saprophytes in soil or dead plant material and play a key role in mineralization of organic matter; unfortunately some species are parasites of terrestrial plants and cause serious damage to crops, humans and animals [13]. This has led to the exploration of compounds to initiate antifungal activities. Howard Mules et .al., isolated a new compound from dichloromethane extracts of the leaves of Psidiumacutangulum [14] and it revealed potent antifungal activity and tobacco budworm antifeedent activity. Candida albicans, a typical human pathogen fungus is found to be the least susceptible to the antifungal effects of the extracts of dormant fruits of Hyphaenethebaica [15]. In our current investigation, we have tested the antibacterial and antifungal activity of Allamanda Cathartica, belonging to the floral family of Apocynacea. This is a vast family which includes a population of around 250 genera and 2000 species of tropical trees, shrubs and vines. The choice of the plant in our work is based on the reports available regarding high biological activity and medicinal properties of similar species belonging to the same family [16]. The antibacterial activity of the flavonoid glycosides isolated from Allamanda Cathartica was evaluated using disc diffusion technique [17]. An antifungal activity of the extract was tested with pure strains of Candida albicans.

II. MATERIALS AND METHODS

The fresh flowers of Allamanda Cathartica Linn.were collected from places in and around Kumbakonam, India, during the months of May and December. The collected fresh flowers were brought to the laboratory for antibacterial and antifungal studies. An extract of the flowers were obtained with 90% of methanol. The combined alcoholic extract was concentrated *in vacuo*. The aqueous concentrate was fractionated with benzene, peroxide free diethyl ether and ethyl acetate successively. The ethyl acetate fraction was concentrated *in vacuo* and left in an ice chest for a few days. The yellow solid obtained was quercitrin. The isolation and characterization [18] of the extracted compound has been already reported by us and in the present work we have tested its antibacterial and antifungal efficiencies.

II.1. Antibacterial activity

An Agar medium 24hour cultures of Staphylococcus aureus, a gram positive bacteria and Escherichia coli, a gram negative bacteria were chosen for the microbial

screening of antibacterial activity. The bacterial cultures were maintained on slants consisting of nutrient. 5% w/v test solution of extract was prepared by dissolving 250 mg of extract in 5ml of sterile dimethyl formamide. A nutrient agar medium was prepared and sterilized by an autoclave. In an aseptic room, it was poured into sterile petridishes to a uniform depth of 4 mm and then allowed to solidify at room temperature. After solidification, the test organisms were inoculated with the help of a sterile swap soaked in a bacterial culture or suspension. This enables the uniform surface growth of bacterium and is used for antibacterial sensitivity studies. Then the sterile filter paper discs (6 mm) containing sample (100 µl) were immersed in floral extracts and was placed over the solidified agar in such a way that there is no overlapping of zone of inhibition [19]. Plates were kept at room temperature for half an hour for the diffusion of the sample into the agar media. The organisms inoculated in the petridishes were incubated at 37°C for 48 hours. On completion of incubation, the zone of inhibition produced by the sample with different organisms in different plates were measured and recorded immediately by using a zone reader [20].

II.2. Antifungal activity

Pure strains of Candida albicans, a fungus was tested with the drug. Four compartments of a sterile petridish were loaded with 200 µg, 400 µg, 600 µg and 800 µg of the flavonol glycosides isolated from Allamanda Cathartica. Muller-Hinton agar used in double strength was chosen as medium. 2.5 ml of double strength Muller-Hinton agar was mixed with 2.5 ml of different concentrations of the flavones as already cited. Candida albicans was injected (0.5 McFarland scale 0.001ml). The growth of the fungi was observed.

III. RESULTS AND DISCUSSION

The antibacterial activity of the compound quercitrin isolated from Allamanda Cathartica Linn. was tested with Staphylococcus aureus and Escherichia coli micro organisms. The very usual method of zone inhibition of testing was followed here. The reason for choosing the method is that, it is highly reliable, informative, fast and qualitative. If substantial antibacterial activity is present in the testing material, a zone of inhibition appears around the test product. The zone of inhibition is identified as the area on the agar plate that remains free from microbial growth. In our testing, the activity of quercitrin was checked with doses of 10 µg and 20 µg of the drug. The action of isolated compound over the micro organisms is as shown in Figure 1. It was observed from the snap shots, that the inhibition capacity of the isolated compound increased in case of Staphylococcus aureus, a gram positive bacteria with an increase in dosage of the drug (Figure 1a.). The antibacterial effect was quite explicit even at a very low dosage of 10µg. The above tests were carried out using Penicillin as a standard drug. It was observed that the growth of microbial organisms was completely contained in case of gram positive bacteria. However, the effect of the isolated compound was rather less prevalent against Escherichia coli, a gram negative bacterium. The testing here was done with Norflaxin as the standard drug. The activity was tested with a specimen of Escherichia coli (Figure 1b), but, it was observed that the growth of the microbial organisms could not be controlled even at a dosage level of 20 µg. This may be attributed to thick walls of E.coli that interrupts the permeation of the quercitrin and hence may be the reduced efficiency of antibacterial behavior of the isolated compound.

The behavior of the isolated compound was also tested for its antifungal activity against the species Candida albicans and the observation is as shown in Figure 2. As indicated, the antifungal activity of the quercitrin was tested with various dosages from 200 µg to 800 µg shown as compartments I to IV, respectively. It was practically observed that the population of the fungal organisms was maximum at the dosage of 200 µg (Compartment I) but decreased continuously with an increase in applied dosage of the isolated compound. The population was reduced critically at the dosage level of 800 µg (compartment IV). Thus the antibacterial activity of quercitrin isolated from Allamanda Cathartica Linn. was tested successfully with gram positive and gram negative microbial organisms as well as

fungal species. The isolated compound was shown to exhibit substantial antibacterial action against S. aureus and antifungal action against C. albicans. However, the effect was less prominent with a gram negative bacterial species, E. coli. Nevertheless, an enhanced activity of the drug can very much be anticipated at higher concentrations of the drug.

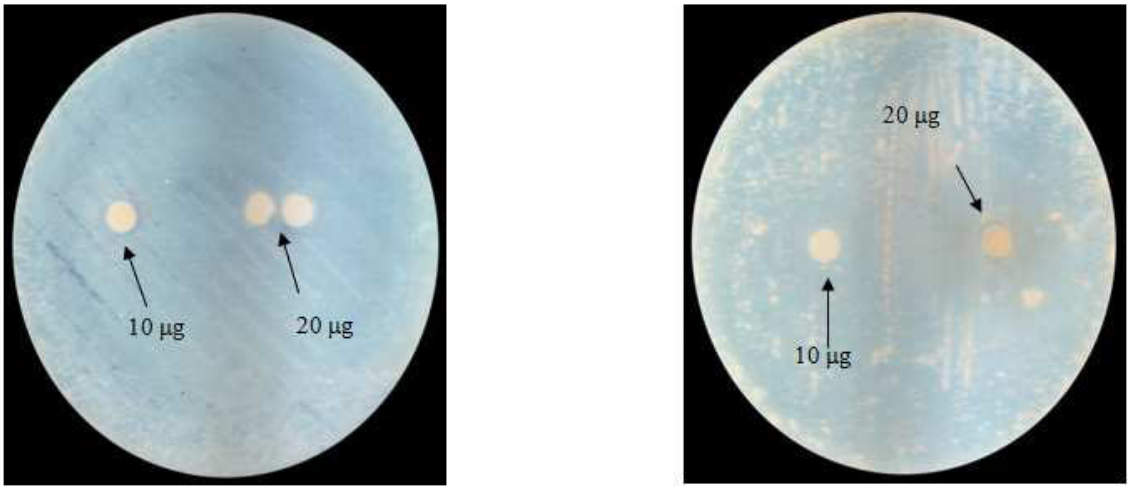

Figure 1
Antimicrobial activity of quercitrin over a) S. aureus b) E.coli

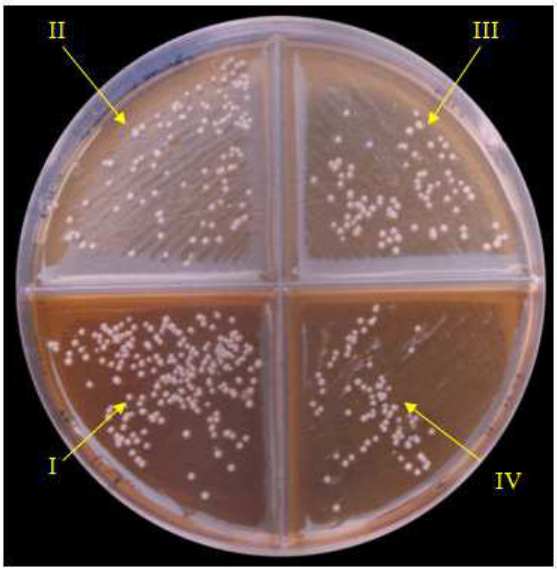

Figure 2
Antifungal activity of quercitrin over candida albicans at dosage levels of I) 200 µg, II) 400 µg, III) 600 µg and IV) 800 µg.

IV. CONCLUSION

The flavonoids isolated from the floral species Allamanda Cathartica Linn. was tested for its phytochemical activities. The testing species were Staphylococcus aureus, Escherichia coli and Candida albicans for antibacterial and antifungal activities respectively. The quercitrin thus tested proved to be a significant antibacterial drug even at a very

low concentration of 10 µg. Correspondingly, the antifungal activity of the drug was also explicit at a concentration of 800 µg. Thus, the isolated compound quercitrin was found to be very effective against the infected pathogens and hence they could serve as a fine secondary for antibiotics.

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