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Determination of Antioxidant and Anti-quorum Sensing Activity of *Aegle marmelos*, *Picrorrhiza kurroa,* and *Swertia chirayita*

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ABSTRACT

From the ancient period, humans have been fighting pathogenic microorganisms for survival purposes and in this context, man has developed antibiotics as a powerful weapon to treat various infections caused by pathogens. Nevertheless, the need to discover new antimicrobial agents is increasing at an alarming rate. This is because the microorganisms have developed various mechanisms to resist the action of antibiotics. One such mechanism is the production of biofilm. Infections caused by biofilm-forming pathogenic microorganisms are very difficult to treat, even using potent antibiotics. However, in folk medicine, many plants are found to be helpful to treat certain infectious diseases. This is because of the synthesis of a variety of bioactive compounds by plants with high medicinal value. Hence, in the present study, three different plants were used viz *Aegle marmelos*, *Picrorrhiza kurroa,* and *Swertia chirayita* to determine their antioxidant and anti-quorum sensing activities. According to the literature, antioxidants delay the oxidation process and nullify the effect of free radicals that cause damage and accelerate aging. Quorum sensing is the chemical way of communication between biofilm-forming microorganisms. Among the alcoholic extracts, the methanolic extract of *P. kurroa* showed the highest DPPH radical scavenging activity of 82.11%. All the plant extracts under investigation exhibited anti-quorum sensing activity against the standard culture of *Chromobacterium violaceum* MTCC 2656; however, the plant extracts of *A. marmelos* were found to be more potent as compared to *P. kurroa* and *S. chirayita*. Plant extracts *P. kurroa* and *S. chirayita* showed almost similar anti-quorum sensing activity. This confirms the pharmaceutical importance of plant materials of interest, which might prove to be useful to treat damage caused by free radicals and biofilm-related infections, after due consideration of clinical trials for safety issues.

Keywords: Biofilm; *Aegle marmelos; Picrorrhiza kurroa; Swertia Chirayita*; Antioxidant activity; Anti-quorum sensing activity

1. INTRODUCTION

In today's world, pathogenic microorganisms are one of the serious threats to public health care. Infections caused by these microorganisms are found to be recalcitrant and recurrent due to the ability of the pathogens to acquire resistance against antibiotics used in the treatment. One of the most common mechanisms among the various ways of gaining resistance against antibiotics by pathogenic microorganisms is the formation of biofilm.

Biofilm is an inter/intra-dependent community of surface-associated microorganisms[1]. There are five stages of biofilm development which include initial attachment, irreversible attachment, maturation I, maturation II and the final stage of dispersion. Dispersal of cells from the biofilm colony is an essential stage of the life cycle of biofilm. It enables biofilms to spread and colonize on new surfaces, including living tissues and indwelling medical devices like catheters, valves, lenses, prostheses and so on[2]. Biofilms are majorly responsible for causing persistent, robust, and re-emerging infections because microorganisms within the biofilm are resistant to antibiotics and host immune defence mechanisms. This is overall due to the failure of the antibiotics to penetrate the biofilm, slow growth rate, heterogeneity of the population, physiological changes and the quorum sensing activity of the pathogens. Quorum sensing is the way of communicating and coordinating among the microorganisms involved in the biofilm. It was found that during quorum sensing; microorganisms release signaling molecules called Acyl Homoserine Lactone (AHL) and autoinducers[1].

In developing and underdeveloped countries, traditional medicine plays an important role in providing medical care. Such therapies involve the use of plant extracts or their active principles. This is because plants have been used as a natural source of medicine across the globe. Over the last few years, many plant species have been evaluated for antimicrobial or various biological activities. Indian flora offers huge opportunities for the discovery of potent bioactive compounds with medical application in combating

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infection and strengthening immunity. The antimicrobial agents found in plants may prevent pathogenic infections by different mechanisms, other than commercial antibiotics, hence may have clinical value in pharmaceutical science.

Furthermore, plants are often considered an important source of antioxidants for human beings. Free radicals are produced exogenously as well as endogenously by the living system which may lead to mutation, cancer, cardiovascular diseases, aging and so forth. Antioxidant compounds are generally produced by plants for normal growth, development, and defence against infection and injury. The antioxidant activity of phenolics is mainly due to their redox properties, which allows them to act as reducing agents, hydrogen donors, singlet oxygen quencher as well as a metal chelator, to decrease the potency of free radicals[3].

Aegle marmelos, *Picrorhiza kurroa,* and *Swertia chirayita* are ayurvedic plants with several ethnomedical applications. Based on the therapeutic potential of the plant species, the current study was designed to evaluate its antioxidant and anti-quorum sensing activity.

2. METHODOLOGY

2.1 Plant Materials

Fine powder of *A.marmelos* (Fruit), *P.kurroa* (Root), and *S.chirayita* (Stem) were purchased from an ayurvedic clinic in Mumbai and then subjected to authentication from the Agharkar Research Institute, Pune.

2.2 Preparation of the Plant Extracts

Powdered form of the plant material (10g) was subjected to hot extraction using six different organic solvents like Acetone, ethyl acetate, chloroform, ethanol, methanol, butanol, etc., and an inorganic solvent like distilled water of 300ml volume each with the help of Soxhlet apparatus. The extracts obtained were concentrated by using a rotary vacuum evaporator. Stock solutions were prepared in Dimethyl sulphoxide (DMSO) to get a final concentration of 100mg/ml. These extracts were stored in the dark at room temperature until further use[4].

2.3 Determination of the Antioxidant Activity of the Plant Extracts by DPPH Assay

To determine antioxidant activity, the method used was α,α-diphenyl-β-picrylhydrazyl (DPPH; $C_{18}H_{12}N_5O_6Mr$ = 394.33) scavenging assay. It was developed by Blois in 1958[5]. The absorbance was recorded at 520nm after 10min of incubation in the dark at room temperature and the DPPH radical scavenging activity was expressed in percentage. All analyses were performed in triplicates. The data were expressed as mean ± S.D. (Standard Deviation) and were evaluated with a one-way ANOVA test, with $P < 0.05$ considered statistically significant.

2.4 Qualitative evaluation of Anti-quorum Sensing Activity of the Plant Extracts using *Chromobacterium violaceum*

Quorum sensing inhibitory effects of the extracts of *A. marmelos, P. kurroa, and S. chirayita* were elucidated by agar cup diffusion assay[6]. *Chromobacterium violaceum* was cultivated on sterile Luria Bertani (LB) broth for 24 hr at 28 ºC. The 100 µl of test culture was inoculated in sterile LB soft agar (0.5 % agar) and poured over basal

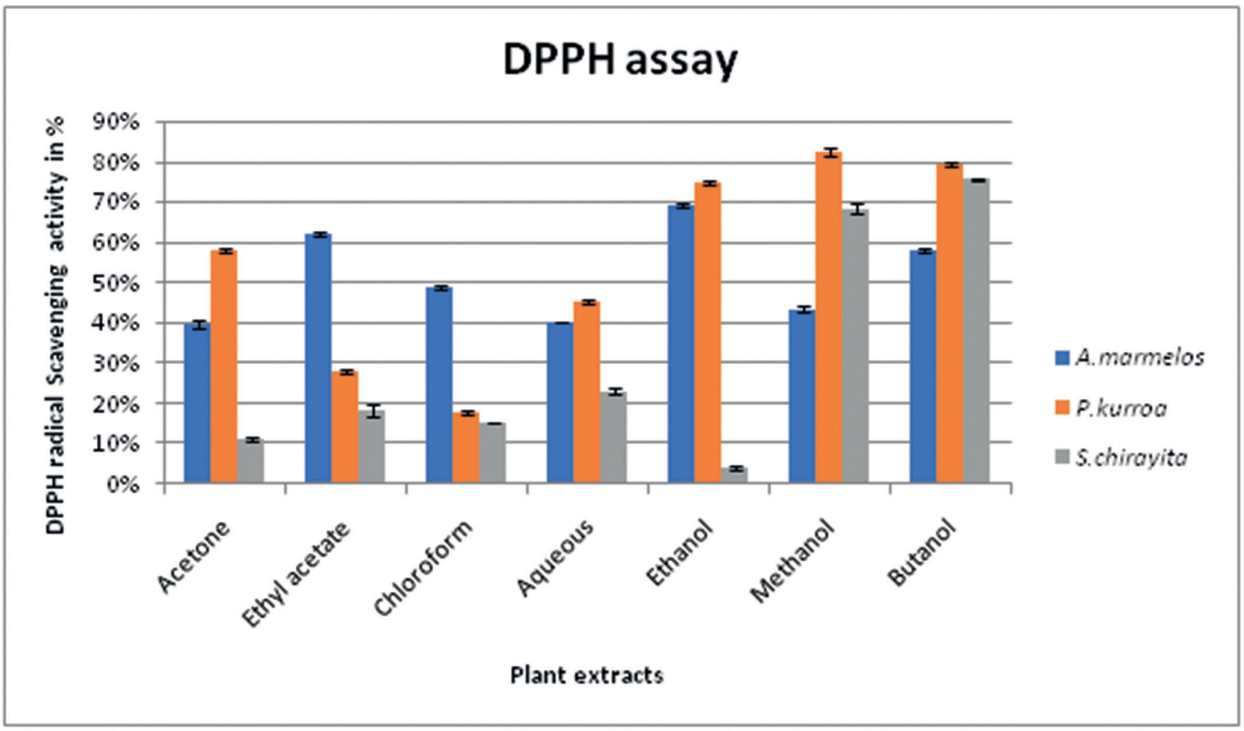

Figure 1. Screening of antioxidant activity of the plant extracts by DPPH assay.

agar consisting of sterile LB with 1.5 per cent agar. A 60µl of each extract was added to the bored wells (6mm diameter) and plates were incubated at 28 ºC for 24hrs. Zone diameter (mm) was measured and recorded.

3. RESULTS AND DISCUSSIONS

The total yield of the residual components obtained from the extraction method used in this study was 5 g per cent. Twenty-one different plant extracts were utilized here to determine their antioxidant and anti-quorum sensing activity.

3.1 Antioxidant Activity of the Plant Extracts

In the present study among seven different plant extracts of *A. marmelos*, ethanolic extract showed the highest antioxidant activity. Gupta D et al. have reported the antioxidant nature of methanolic extract of dried fruit power of *A. marmelos* concerning radical scavenging activity in terms of percentage inhibition[7]; consistent with these findings, nearly similar radical scavenging activity was obtained with methanolic extract of *A. marmelos* in our study. In the present investigation, aqueous extract of *A. marmelos* showed 40 per cent radical scavenging activity; however, aqueous extract of leaves of *A. marmelos* exhibited significant DPPH scavenging activity[8]. Many researchers have documented the potent antioxidant activity of ethanolic extract of leaves of *A. marmelos*[9,10].

In the current study, concerning *S. chirayita*, butanolic extract exhibited maximum antioxidant properties, followed by methanolic and aqueous extract of the same part of the plant. Aqueous and methanolic extract of root, stem, and leaves of *S. chirayita* showed promising antioxidant activity[11]; in agreement with this, in our research work, methanolic and aqueous extract of stem of *S. chirayita* demonstrated significant DPPH radical scavenging activity. Some researchers have studied the antioxidant potential of aerial parts[12,13,14,15]as well as roots of *S. chirayita*[16] and it was found that methanolic extract showed excellent antioxidant activity.

It was observed that in our study alcoholic extracts of *P. kurroa* showed greater antioxidant activity as compared to others. Among the alcoholic extracts, the methanolic extract exhibited the highest radical scavenging activity, closely followed by butanolic and ethanolic extract. Various studies have indicated significant radical scavenging activity of ethanolic and methanolic extract of roots of *P. kurroa*[17,18,19,20,21].

The difference in the antioxidant activity of the extracts of *A. marmelos*, *P. kurroa,* and *S. chirayita* may be due to differences in the process of extraction, geographical location as well as part of the plant used in the studies by the other investigators mentioned (Figure 1).

3.2 Anti-quorum Sensing Activity of the Plant Extracts

Quorum sensing is one of the most essential aspects of biofilm development. To degrade the biofilm, it would be preferable to inhibit the mechanism of quorum sensing within the microorganisms involved. Therefore, the obtained plant extracts of *A. marmelos*, *P. kurroa* and *S. chirayita* were screened for anti-quorum sensing activity with the aid of standard culture of *Chromobacterium violaceum* MTCC 2656. It is very well known that the

Table 1. Determination of anti-quorum sensing activity of the plant

Plant Extract	Anti-quorum sensing activity of *S. chirayita* (Kirat tikta)	Anti-quorum sensing activity of *A. marmelos* (Bael)	Anti-quorum sensing activity of *P. kurroa* (Kutki)
Ethanol (E)	++	+++	+
Methanol (M)	+	+++	+
Butanol (B)	++	+++	+
Chloroform Chl)	++	+++	++
Ethyl acetate (EA)	-	+++	++
Acetone (Ace)	++	+++	++
Distilled water (D/W)	-	-	-
DMSO (D)	-	-	-

Figure 2. Anti-quorum sensing activity of extracts of *P. kurroa* (Kutki).

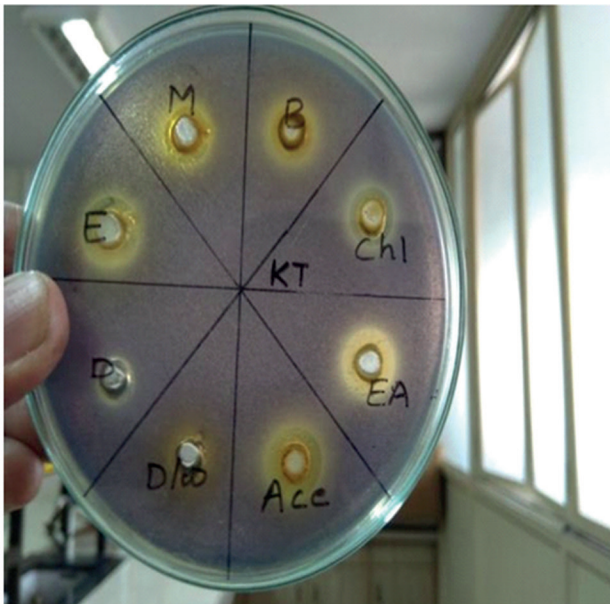

Figure 3. Anti-quorum sensing inhibition activity of extracts of S. chirayita (Kirat tikta).

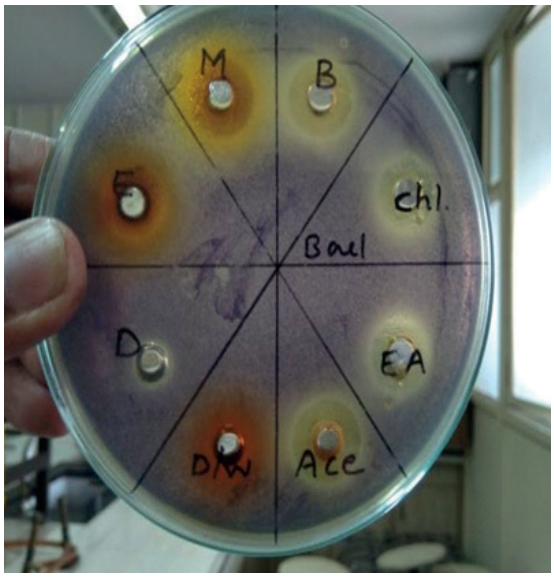

Figure 4. Anti-quorum sensing inhibition activity of extracts of A. marmelos (Bael).

strain of *Chromobacterium violaceum* produces biofilm and secretes a certain quorum-sensing-dependent pigment called violacein. Hence any extract which inhibits violacein producing ability of the *Chromobacterium violaceum* can be considered as having anti-quorum sensing ability[22].

In the case of *S. chirayita*, ethanolic, methanolic, butanolic, chloroform, and acetone extract showed a zone of clearance as well as a quorum sensing inhibition zone. However, ethyl acetate extract showed only a zone of clearance with no quorum sensing inhibition zone. With respect to *A. marmelos,* except for negative control and aqueous extract, all other extracts revealed an excellent zone of clearance as well as quorum sensing inhibition zone.

Viraj Gala et al have reported lower quorum sensing activity of aqueous, methanolic, ethyl acetate, and n-hexane extracts of *P. kurroa* stem as compared to other plant materials used in their study[23]. In our investigation, chloroform, ethyl acetate, and acetone extract of *P. kurroa* gave a significant zone of clearance as well as a quorum sensing inhibition zone. Though all alcoholic extract manifested zone of clearance, however, quorum sensing inhibition zone was comparatively lesser. Negative control and aqueous extract of all three plants demonstrated negligible antibacterial activity with no quorum sensing inhibition activity (Table.1).

Legends: (+, ++, +++) Indicates the presence of anti-quorum sensing activity in increasing order by visual observation, and (-) Indicates the absence of activity

Among the three different plant materials of interest, plant extracts of *A. marmelos* manifested prominently visible anti-quorum sensing activity against the test organism used, as compared to *P. kurroa* and *S. chirayita*. Hence it will be utilized further to estimate its violacein inhibition potency quantitatively[24].

4. CONCLUSION

The results of this study are very promising; indicating that the plant materials used should be studied further to explore its phytoconstituents. The plant extracts can be used in the future to determine their antimicrobial, antiviral and anticancer activity as well. Furthermore, the antioxidant and anti-quorum sensing activity of the plant extracts may be of great importance in modern clinical therapy. This is because the use of green medicine is always appreciated over synthetic drugs that are generally associated with chances of gaining resistance, high cost and adverse side effects. The current study of *A. marmelos*, *P. kurroa,* and *S. chirayita* stimulates pharmaceutical interest to identify the bioactive compounds and their mechanism in exhibiting biological activities as well as their synergistic potential to treat infections caused by biofilm-forming pathogenic microorganisms.

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In the current investigation, he was involved in the conceptualization of the work, literature search, experimentation, data acquisition and manuscript writing.

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