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ORIGINAL ARTICLE

Medicinal Plant's Potential Activity against Skin Disease-causing Bacteria and their Phytochemical Assessment

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ABSTRACT

Plants are the oldest remedies to cure ailments of mankind. They are a storehouse of bioactive compounds which serve as a lead for the development of therapeutics against many diseases including skin diseases. In the present study, leaf extracts of *Aegle marmelos*, *Nerium indicum*, *Ricinus communis,* and *Ziziphus nummularia* were selected and tested against common skin pathogens, namely *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli,* and *Pseudomonas aeruginosa.* The methanolic extracts of all the four plants were subjected to an assay for antibacterial activity and minimum inhibitory concentration. As antioxidants play a significant role in skin disease treatments, all the extracts were also evaluated for their antioxidant activity. Preliminary phytochemical screening and estimation of total phenolic content were carried out to establish its correlation with All the methanolic extracts showed good activity against the selected skin pathogens with significant minimum inhibitory concentration (MIC) values. *N. indicum* and *A. marmelos* showed the highest zone of inhibition against all tested organisms. The extracts possessed potential antioxidant activity against reactive oxygen species with *N. indicum* exhibiting most potent activity. Further, preliminary phytochemical screening indicated presence high amount of alkaloids, terpenoids, flavonoids, tannins, and saponins in *A. marmelos* and *N. indicum*. *R. communis* and *Z. nummularia* had the highest amount of phenolic content. The results of the study indicate that traditional knowledge can serve as a guideline to provide leads for further testing of potentially interesting plants to be used as modern treatment alternatives.

Keywords: Antibacterial, antioxidant, minimum inhibitory concentration, phenolics, skin disease, zone of inhibition.

INTRODUCTION

The prevalence of skin disorders such as acne, eczema, psoriasis, wart, and ringworm has increased due to rapid emergence of multidrug-resistant bacteria and environmental pollutants.[1] A number of tropical and systematic therapies are available for skin disorders. However, excessive use of these therapies over a long time can lead to the rising resistance of bacteria. These therapies have limitations with respect to toxicity and also have a lot of side effects. To overcome these limitations, there is an urgent need for the development of safe, effective, and low-cost therapeutics.[2] Skin diseases are majorly caused due to bacteria, virus, fungi, or other microorganisms. Bacteria such as *Staphylococcus aureus, Streptococcus pyogenes, Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae,* and *Escherichia coli* are some of the main causative agents of many skin diseases.[3]

Plants are the incredible reservoir of diverse and potential bioactive compounds which can be developed as the new future drugs. Traditional medicine from natural resources in particular from plants has proved to be effective and relatively less toxic than the exciting drugs. Therefore, people are still depends on natural remedies and their products. Exploration of herbal resources may give new drug scaffolds which combat skin infections and disorders.[4-7] From the prehistoric times, the use of different parts of medicinal

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plants was practiced to cure specific ailments evidently due to the presence of some bioactive compounds such as alkaloids, flavonoids, essential oil, glycosides, tannins, terpenoids, and steroids.[8] Isolation of chemical compounds from plants with efficient antimicrobial activities can have enormous impact in the health care.[9] Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and determine the parameters associated with it. The effects of plant extract on bacteria have been studied by many researchers in different parts of the world.[10]

The aim of the current study was to evaluate the biological activity of the selected medicinal plants and assess the major bioactive group of a compound which might be responsible for these activities. Accumulated evidences have proved the potential of traditional plants in providing effective antimicrobial agents including skin pathogens. *Aegle marmelos*, *Nerium indicum*, *Ricinus communis,* and *Ziziphus nummularia* were selected for the study as they have been reported for wide pharmacological activities in Indian system of medicine. These selected plants are reported for their anticancer, antiviral, antifungal, antipyretic, antifertility, antibacterial, and many other pharmacological activities. At present, the potential of plant extracts was evaluated against the common skin pathogens. Many diseases including skin disorders are associated with the generation of reactive oxygen species (ROS). Nascent oxygen is the critical factor for the growth of bacteria.[11] Compounds with antioxidant properties can reduce the effects of ROS on disease development. Therefore, the free oxygen scavenging activity of these plants has also been checked. Further, qualitative phytochemical testing of the potential extracts was carried out which helps to recognize a major group of bioactive compounds.

MATERIALS AND METHODS

Selection and collection plant material

A. marmelos, *N. indicum*, *R. communis,* and *Z. nummularia* were selected to evaluate their potential activity against common skin pathogens. Leaves of the selected plants were collected from Ahmedabad during the month of March 2018.

Processing and extraction of plant material

Freshly collected leaves of *A. marmelos*, *N. indicum*, *R. communis,* and *Z. nummularia* were washed, dried in the shade and coarsely powdered. Powdered plant materials (10 g) were immersed in 100 ml methanol and shaken gently for 30 min. The flasks were kept in orbital shaker overnight at 120 rpm and 25°C. The extract was filtered through Whatman filter paper no. 1. The extraction process was repeated 3 times to ensure complete extraction. The filtered extract was evaporated to dryness. The dried extracts were used for the further experimental process.

Assay for antibacterial activity

Test microorganism and growth medium

The test microorganisms used in this study were procured from Microbial Type Culture Collection (MTCC), Chandigarh, India and National Collection of Industrial Microorganisms (NCIM), Pune, India. In this study, two Gram-positive bacteria *B. subtilis* (MTCC 736) and *S. aureus* (NCIM 2079) and two Gram-negative bacteria *E. coli* (NCIM 2065) and *P. aeruginosa* (NCIM 2200) were used. Nutrient broth and nutrient agar medium (HiMedia Laboratories Limited, Mumbai, India) were used as growth medium for bacterial cultures.

Antibacterial assay

Rapid and qualitative screenings of the antibacterial potential of plant extracts were carried out by agar well diffusion method. The 24-h grown fresh bacterial cultures (100 µl) were uniformly spread using a cotton swab on sterile nutrient agar plates. Four wells were made in each plate using sterile cork borer. 100 µl of plant extracts (100 mg/ml in dimethyl sulfoxide [DMSO]) were added in agar well under aseptic conditions. For positive control, an equal volume of streptomycin (10 mg/ml) was added in one well. For negative control, an equal volume of 1% DMSO was added in one well. The plates were kept in the refrigerator for 30 min and then incubated overnight in an incubator at 37°C. The experiment was performed in triplicate and results were recorded as the average zone of inhibition.[12,13]

Evaluation of minimum inhibitory concentration (MIC)

The lowest concentration of the bioactive extracts which inhibited the growth of tested microorganisms was measured by the MIC. For the quantitative analysis, broth microdilution method was used to measure MIC. The wells of columns were loaded with 50 µl sterile nutrient broth (except first well of each column). 100 µl of plant extract (10 mg/ml in 1% DMSO) was added to the first well of columns 5–12 (each extract was added in duplicate). Serial two-fold dilutions were made from first well of columns in the seven consecutive wells of the columns. Subsequently, 50 µl solutions from the last well of each column were discarded. The concentration of the extracts ranged from 10 to 0.0781 mg/ml. For positive control, streptomycin (10 mg/ml) was used and diluted serially. Subsequently, 50 µl of sterile nutrient broth was added in all the well of the plate. For negative control, sterile broth, sterile water, and culture (50 µl each) were used, and for sterility control, 150 µl of sterile broth was added to the wells. Next, 50 µl of the bacterial inoculum (10^6 cells/ml) was added to each well so that the final volume of each well was 150 µl. The plates were incubated at 37°C for 24 h. After incubation, 40 µl INT indicator dye (0.2 mg/ml in water) was added to each well. The plates were reincubated at 37°C for 30 min and visually assessed for color development. The presence of bacterial growth is indicated with the development of purple color, and the growth inhibition is indicated by no change in color.[12,13]

Assay for antioxidant activity

The antioxidant ability of plant extracts was evaluated by 1,1- diphenyl-2-picrylhydrazyl (DPPH) scavenging activity. A microplate reader (Thermo Scientific) and 96 well plate were used for the determination of spectral absorption values. In this method, 0.5 ml methanolic DPPH solutions (0.3 mM) were added to 0.5 ml of methanolic samples of different concentrations (200, 400, 600, 800, and 1000 µg/ml). Diluted solution of 1:1 methanol and DPPH was used as a control. Ascorbic acid was used as the positive control with similar concentrations as the samples. These solutions were gently mixed and incubated in the dark for 30 min at room temperature (25°C). Thereafter, the absorbance of the resulting solution was measured at 517 nm.[14] The scavenging capabilities of samples were calculated using the following formula:

DPPH scavenging effect (%) = Abs control – Abs sample/Abs control ×100

The EC_{50} (effective concentration at which ROS radicals were scavenged by 50%) were calculated by interpolation from the linear regression analysis.

Analysis of phytoconstituents

Preliminarily phytochemical analysis of plant extracts was performed with the standard methods. Ethanolic extracts of all plants were evaluated for the group of compounds such as alkaloids, triterpenoids, saponins, flavonoids, and phenols.[15]

Estimation of total phenolics

The phenolic content in extracts of the selected plants was determined using spectrophotometric method.[16] The determination of the total phenol content employed the Folin–Ciocalteu method, where 0.1 ml of extract and 0.9 ml of distilled water were mixed in a 25 ml volumetric flask. To this mixture 0.1 ml of Folin–Ciocalteu phenol reagent was added and the mixture shaken well. 1 mL of 7% sodium carbonate (Na_2CO_3) solution was added to the mixture. After 5 min, the volume was made up to 2.5 ml with distilled water. A set of standard solutions of gallic acid (0.0625, 0.125, 0.25, 0.5, and 1 mg/ml) were prepared as described above. The mixtures were incubated for 90 min at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an ultraviolet/visible spectrophotometer. Total phenol content was expressed as mg of gallic acid equivalents/g of extract.

RESULTS

Collection and extraction of plant material

The plant materials (leaves) were collected from different parts of Ahmedabad depending on its availability. The collected plant species were authenticated based on their morphological characteristics such as arrangement, shape, texture, and appearance of leaves [Figure 1].

Antibacterial activity of plant extracts by agar well diffusion method

Four plant species were investigated to evaluate their antibacterial activity against two strains of Gram-positive bacteria (*B. subtilis* and *S. aureus*) and two strains of Gram-negative bacteria (*E. coli* and *P. aeruginosa*) using agar well diffusion method [Figure 2]. Evaluation of antibacterial activity of these plant extracts is illustrated in Figure 3. The diameter of zone of inhibition for all the extracts against tested bacteria ranged from 9 to 28 mm. *N. indicum* and *A. marmelos* showed the highest zone of inhibition (27.33 and 25.00 mm, respectively) against *B. subtilis*. This result is in accordance with the earlier studies indicating the efficient inhibition of *B. subtilis* by methanolic extract of leaves of *N. indicum* and *A. marmelos*.[17] Results of antimicrobial activity of the four plant extracts suggested that *P. aeruginosa* was the most resistant strain to plant extracts and *B. subtilis* was the most susceptible strain to the extracted plants. The results revealed that all plant extracts were potentially effective in suppressing microbial growth with variable potency. Hence, experiments were conducted to determine their MIC against all the bacterial strains.

MIC of plant extracts by broth microdilution method

The MIC of the plant extracts was evaluated by broth microdilution method in 96-well polystyrene microtitre plates. Following the addition of INT dye, all sterility control wells for all tested bacteria remained colorless after 30 min incubation. In contrast, all wells in the negative control column (containing growth medium and bacteria) of all tested bacteria changed from colorless to violet indicating the growth of bacteria. The MIC of positive control, streptomycin (10 mg/ml) was observed to be <0.078 mg/ml against all the four bacterial strains studied. The MIC concentration of methanolic extract of plants ranged from 0.078 to 5.0 mg/ml [Table 1]. The extracts of *A. marmelos*, *N. indicum,* and *R. communis* were very active against *B. subtilis* among all the bacterial strains tested with a MIC of 0.078, 0.078, and 0.156 mg/ml, respectively. The extract of *Z. nummularia* was most active against *E. coli* with a MIC of 0.313 mg/ml.

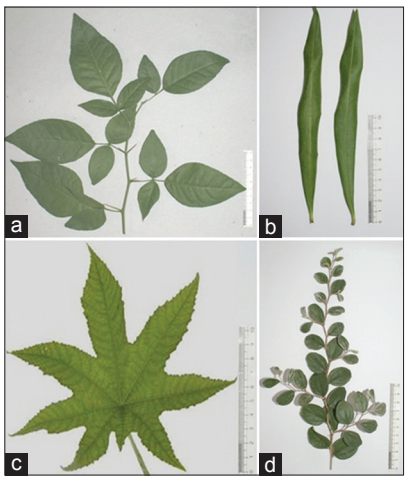

Figure 1: Morphological characteristics of collected plant material. (a) *Aegle marmelos*, (b) *Nerium indicum*, (c) *Ricinus communis*, and (d) *Ziziphus nummularia*

Antioxidant activity of plant extracts by DPPH method

Many disorders including skin diseases are associated with the generation of ROS.[18] Free radical scavenging action is considered to be one among the various mechanisms for antioxidation. Therefore, free radical scavenging activity of all four plant extracts was assessed by DPPH assay. The percentage values of antioxidant activity of the plant extracts at different concentration 200–1000 µg/ml are shown in Figure 4. In this assay, the standard ascorbic acid gave antioxidant activity with EC_{50} value of 0.371 µg/ml. The results of the present investigation indicated that the *A. marmelos*, *N. indicum, R. communis,* and *Z. nummularia* exhibited significant antioxidant activity with the EC_{50} value of 25.45, 2.16, 88.79, and 130.60 µg/ml, respectively. *N. indicum* was found to be exhibiting most potent while *Z. nummularia* was found to be exhibiting least potency in the assay.

Qualitative screening for presence of phytoconstituents in plant extracts

The qualitative phytochemical screening indicates the presence of major chemical groups in the plants. The presence and absence of these chemical groups were scored based on the visual observations of intensity of color produced during individual tests [Table 2]. The results of these experiments displayed that compounds of all major chemical groups were present in all

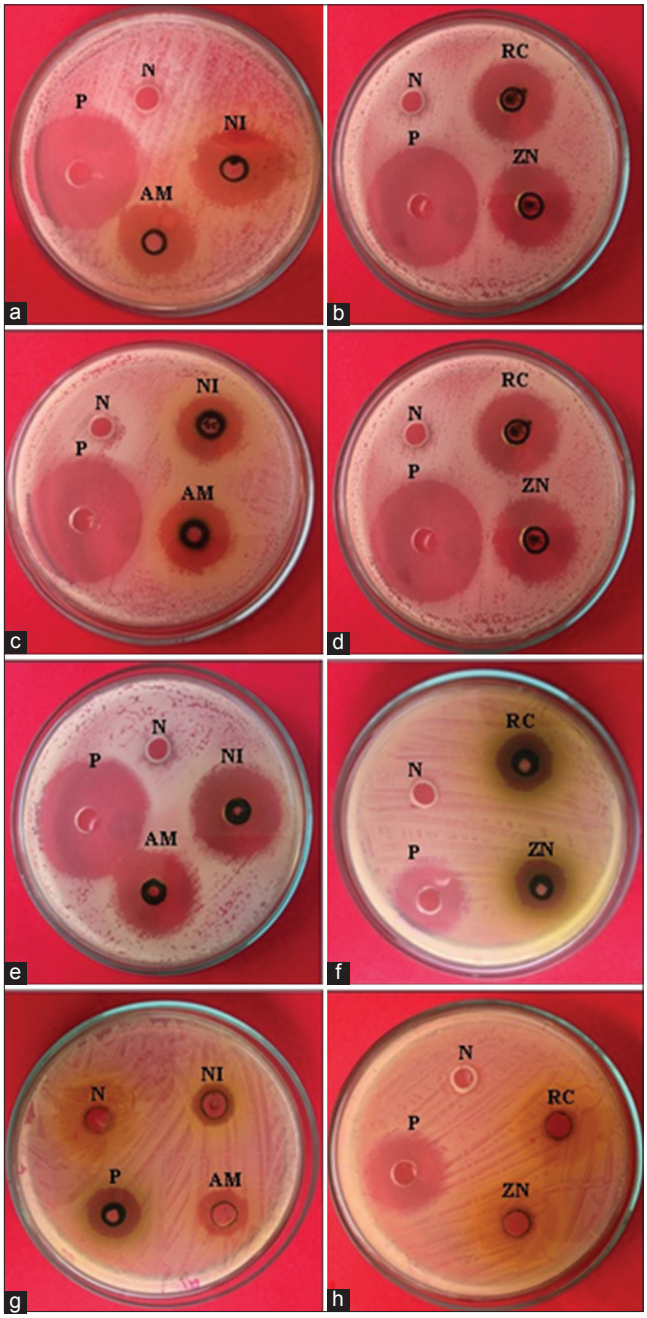

Figure 2: Antibacterial activities of plant extracts against selected test organisms. (a, b) *Bacillus subtilis*; (c, d) *Staphylococcus aureus*; (e, f) *Escherichia coli*; (g, h) *Pseudomonas aeruginosa*. N, Negative control; P, Positive control; AM, *Aegle marmelos*; NI, *Nerium indicum*; RC, *Ricinus communis*; ZN, *Ziziphus nummularia*

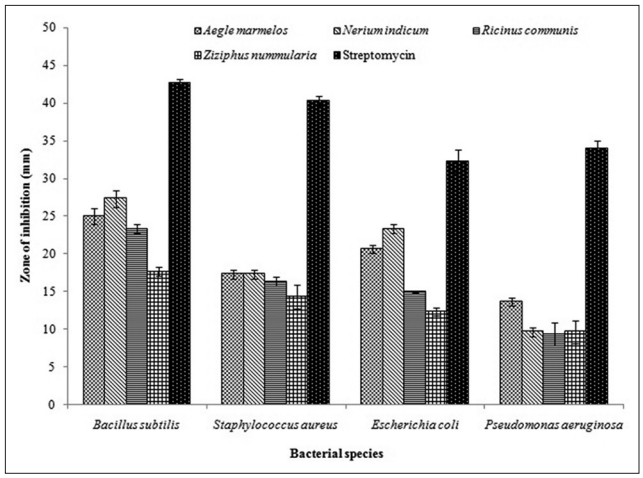

Figure 3: Zone of inhibition of selected medicinal plants against common skin pathogens. Data represented as mean ± SD (n = 3)

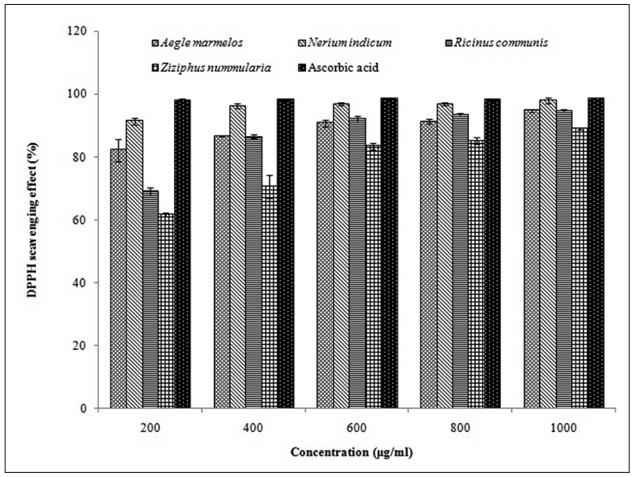

Figure 4: 1,1- diphenyl-2-picrylhydrazyl scavenging effect of selected medicinal plants. Data represented as mean ± SD (n = 3)

the plants. However, the concentration of these chemical groups varied, which was observed with reference to the intensity of color produced. The presence of alkaloids, terpenoids, flavonoids, tannins, and saponins was found in abundance in *A. marmelos* and *N. indicum* while their presence was trace to moderate in *R. communis* and *Z. nummularia.* The abundance of these chemical groups in *A. marmelos* and *N. indicum* may be the reason behind the potent antibacterial and antioxidant properties of these two plants. Various studies have reported the presence of alkaloids, terpenoids, flavonoids, tannins, and saponins in all the four plants screened in the present study.[19]

Quantitative determination of total phenolic content in plant extracts

The antimicrobial and antioxidant activities of the plants are positively associated with the amount of phenolic compounds present in the plants. Phenolic compounds have redox properties, which allow them to act as antioxidants. As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antimicrobial and antioxidant activity.[20]

The total phenolic content in *A. marmeloes*, *N. indicum*, *R. communis,* and *Z. nummularia*

Table 1: MIC of methanolic extracts (10 mg/ml) against bacterial strains

Sample	Minimum inhibitory concentration (mg/ml)			
	Gram-positive bacteria		Gram-negative bacteria	
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
Aegle marmelos	0.078	0.313	0.156	2.5
Nerium indicum	0.078	1.25	0.625	0.625
Ricinus communis	0.156	2.5	1.25	0.625
Ziziphus nummularia	0.625	1.25	0.313	5.0
Streptomycin (10 mg/ml)	<0.078	<0.078	<0.078	<0.078

MIC: Minimum inhibitory concentration

Table 2: Qualitative phytochemical screening of plant extracts

Test	*Aegle marmelos*	*Nerium indicum*	*Ricinus communis*	*Ziziphus nummularia*
Alkaloids	+++	+++	+	+
Terpenoids	+++	+++	+	++
Flavonoids	+++	+++	+	++
Tannins	++	+++	+++	++
Saponins	+++	+++	+	++

+: Trace, ++: Moderate, +++: Abundant

was estimated to be 0.263, 9.540, 11.397, and 10.6978 mg% (w/w), respectively. *R. communis* had highest amount of phenolic content followed closely by *Z. nummularia*. The results obtained in this study are not in agreement with that of antioxidant activity. The phenolic content *A. marmeloes* and *N. indicum* was found to be less but these plants exhibited high antioxidant activity. This could be due to the presence of non-phenolic compounds which have more potent antioxidant activities. In contrast, the high phenolic content and less antioxidant activity in *R. communis* and *Z. nummularia* can be attributed to the types of phenolic compounds present.

DISCUSSION

Unlike other chronic diseases, skin disorders are unique in the sense; they carry a high level of morbidity than mortality. Abatements and recerbations are regular with dermatological conditions. Medications which are powerful, time tried, practical, and without uncommon reactions are need of great importance. In that way, a rundown of single herbs and solutions which are prominent in conventional Ayurveda drugs are being looked into and recorded.[5] Herbals can possibly cure various types of skin maladies. More than 80% of individuals in India rely on customary human services and utilize distinctive plant-based items for curing skin related issues. In contrast to the routine allopathic medications, they have moderately to minimal side effects and can be of incredible advantage to the number of inhabitants in India. Herbals can be more secure and savvy treatment for skin sicknesses extending from rashes to loathsome skin disease.[21]

Accumulated results of antibacterial activity showed that leaf methanolic extract of all the four plants possesses a varied degree of antibacterial action against the four bacterial pathogens of skin diseases, i.e. *B. subtilis*, *S. aureus*, *E. coli,* and *P. aeruginosa*. The results are in agreement with reports which reveal that in plants most of the compounds having antimicrobial potential are soluble in methanol.[22] Leaf extracts of *A. marmelos* have been reported for its varying degree of antibacterial activity against various tested bacterial species.[23] Earlier studies have also reported that the crude extract of *N. oleander* possesses significant antibacterial activity against *S. aureus* and *E. coli*.[24] Methanolic leaf extract of *R. communis* showed maximum antimicrobial activity as compared to aqueous extract against *S. aureus*, *B. subtilis,* and *P. aeruginosa*.[25] *Z. nummularia* methanolic extract was also reported to possess effective antibacterial activity against *E. Coli*, *S. aureus,* and *P. aeruginosa*.[26]

In addition, the plant extracts also demonstrated potential antioxidant activities. Earlier findings have revealed that oxidative stress exists almost all the skin diseases including *Acne vulgaris* and

may play an important role in its pathogenesis.[27] It will be useful to combine at least one drug having antioxidant property along with treatment provided for skin diseases. Previous studies have reported the presence of high antioxidant components in the leaves of *A. marmelos*, *N. indicum*, *R. communis,* and *Z. nummularia*.[28-31]

Evidences suggest that secondary metabolites present in the plants might be responsible for biological activity such as antibacterial and antioxidant activity. Therefore, the presence of different chemical groups was identified the in-plant extracts to establish the correlation with the observed activity. The presence of alkaloids, terpenoids, flavonoids, tannins, and saponins was found in abundance in *A. marmelos* and *N. indicum*, thus justifying their potent antibacterial and antioxidant property.

Antipathogenic and antibacterial components derived from plants possess vast curative properties since they have fewer side effects as compared to synthetic antimicrobials drugs. The results of antibacterial and MIC study concluded that the leaf extracts of *A. marmelos*, *N. indicum*, *R. communis,* and *Z. nummularia* could be developed as an alternative source of antibiotic. Researchers have two reasons to be interested in the study of the antimicrobial nature of plant extracts. First, it is very likely that these phytochemicals will pave the way for discovering new antimicrobial drugs from plants as a substitute source for combating resistant pathogens. Second, the public is becoming increasingly aware of the problems with the overprescription and misuse of traditional antibiotics. The results of the antibacterial assay indicated that the presence of potential bioactive compounds that are antibacterial in nature can be screened against other skin pathogens also. This study reinforced the assumption that ethnomedicinal plants could be a promising an alternative resource of therapeutic compounds.

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