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Phytochemical Analysis, Antioxidant and Antibacterial Activities of Two Traditionally Used Indian Medicinal Plants

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Authors' contributions

This work was carried out in collaboration between all authors. Author RK performed the experiments. Author PM analysed and interpreted the data and wrote the manuscript. Author RS assisted in performing experiments and writing the manuscript. Author AP contributed to the concept, designed the experiment, analysed and interpreted the data and finalized the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of the present study was to determine the phytochemical constituents, antioxidant and antibacterial activities of crude extracts from leaves of two traditional Indian medicinal plants *Catharanthus roseus* and *Ocimum basilicum*.
Study Design: The methanol extracts of the leaves were used to study the antioxidant activity using DPPH and superoxide radical scavenging assays. The disc diffusion method was employed to

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evaluate the antibacterial activities on four major infective pathogenic agents.
Place and Duration of Study: The study was carried out at Post Graduate & Research Department of Biochemistry, Mohamed Sathak College of Arts and Science (Affiliated to University of Madras), Chennai - 600 119, Tamil Nadu, India during January 2017-August 2017.
Methodology: DPPH and superoxide radical scavenging assays were performed using standard methods. The disc diffusion method was employed to evaluate the antibacterial activities on four major infective pathogenic agents, namely, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis.*
Results: Both the extracts exhibited strong DPPH and superoxide radical scavenging activities in a dose-dependent manner. The DPPH scavenging activity by *Catharanthus roseus and Ocimum basilicum* extracts was found to be 78.62±5.4% and 62.72± 4.2%, respectively. At the concentration of 100 µg/mL, the superoxide radical-scavenging activities of methanol extracts of *Catharanthus roseus and Ocimum basilicum* were found to be 72.36±3.21 and 68.76±3.16, respectively. Moreover, both the extracts showed a remarkable inhibition of bacterial growth at a concentration of 300 µg/mL compared to the two other doses tested (100 and 200 µg/mL).
Conclusion: Present findings provide experimental evidence that the leaves of *Catharanthus roseus* and *Ocimum basilicum* have potential antioxidant and antimicrobial activities which might be used as a functional food and safe remedy for the treatment of infectious diseases. This study also revealed that the plant could be a promising source for development of natural antioxidant and antibacterial agents.

Keywords: Total phenolic content; antibacterial; disk diffusion method; Catharanthus roseus; Ocimum basilicum.

1. INTRODUCTION

Plants have formed the basis of the traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies [1]. They have been used by several communities to treat a large number of diseases; also these plants constitute a potential source for the production of new medicines [2].

Catharanthus roseus is an important medicinal plant of the family: *Apocynaceae;* Genus: *Catharanthus*; Species: *C. roseus*; Binomial name: *Catharanthus roseus* L. known as Madagascar rosy periwinkle. Generally, it is known as *Vinca rosea, Ammocallis rosea* and *Lochnera rosea*. The plant is named on the basis of their flower colors. Pink: *rosea*, White: *alba* [3]. Traditionally, leaves of *Catharanthus roseus* are used as medicine for the treatment of menorrhagia, rheumatism, dyspepsia, indigestion, dysmenorrhea, diabetes, hypertension, cancer, menstrual disorders, skin diseases, bleeding diarrhea and it has been reported to have sedative and antiviral properties [4].

The leaves of *Catharanthus roseus* have been reported to contain more than 70 types of chemical constituents such as indole type of alkaloids, ajmalicine, serpentine and reserpine. Due to presence of those alkaloids in *Catharanthus roseus*, it has antihypertensive and antispasmodic properties [5]. The phenolic compounds present in the plant have redox properties that act as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators. It has multiple applications in food, cosmetics and pharmaceutical industries. Besides antioxidant activity, these compounds exhibit anti-allergic, anti-inflammatory, anti-micro, anti-thrombotic, cardio protective and vasodilatory effects [6].

Basil (*Ocimum basilicum*), also called great basil or Saint-Joseph's-wort, is a culinary herb of the family: *Lamiaceae* (mints); Genus: *Ocimum*; Species: *O. basilicum*; Binomial name: *Ocimum basilicum* L. It is also called the "king of herbs" and the "royal herb" [7]. It is possibly native to India [8] and has been cultivated there for more than 5,000 years [9]. According to Shafique *et al*., (2011) there are 30 to 150 basil species and there are a minimum of 60 cultivars of *Ocimum basilicum* [10]. Basil is native to India and Asia and has a long history of legend and use worldwide. It is also referred as sacred in India because it is used to disinfect the house contaminated with malaria, which kills the mosquitoes [11].

Fig. 1. Whole plant with flowers *Catharanthus roseus* L.

Fig. 2. The plant *Ocimum basilicum* L.

Chemical analysis of sweet basil has identified linalool (20-40%), methyl chavicol (20-25%) and eugenol as the three main constituents of the essential oil content of basil and thus aroma [12]. Other components of the oil include 1,8-cineole, camphor, pinene, ocimen, eugenol, geraniol, α-terpineol, β-caryophyllene methyl chavicol (terragon) and safrole [13]. A variety of biologically active compounds have been detected in the leaves including ursolic acid, apigenin, leuteolin, calcium, β-carotene and Vitamin C [14]. The difference types of basils have different scents due to their difference in essential oils composition.

Basil oil is extensively used as flavouring for confectionary, baked goods, tomato pastes, pickles, flavoured vinegars, spiced meats, sausages and beverages. It is also used for scenting dental and oral preparations. The oil is used in traditional medicine for coughs, colds and chest congestions mosquito-repellent, pesticide and flavouring agent [15]. It has been used medicinally to cure coughs and fevers in West Africa. The roots of basil are chewed to alleviate stomach aches and colds in Eastern Africa. Basil is a treatment for gonorrhea in India and a basil paste is used as a skin disease remedy in Sudanese [16].

The objective of the present study was to evaluate the *in vitro* antioxidant and antibacterial activity of methanol extracts of *Catharanthus roseus* and *Ocimum basilicum*. To the best of our knowledge, there is no literature documenting the comparative *in vitro* antioxidant and antibacterial activities of *C. roseus* and *O. basilicum*. This is the first study where we are reporting the comparative antioxidant and antimicrobial activity of methanol extracts of the plants.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material and Preparation of Extract

The fresh leaves of *Catharanthus roseus* and *Ocimum basilicum* were collected from southern part of India (Kanchipuram District, Tamil Nadu) and the pharmacognostic authentication was done by Dr. KN Sunil Kumar, RO and HOD, Pharmacognosy and Dr. M. Kannan, RO

(Siddha) and In Charge, Siddha Central Research Institute, Arignar Anna Government Hospital Campus (Central Council for Research in Siddha, Department of AYUSH, Ministry of Health and Family Welfare, Government of India) Chennai-600 106. Voucher specimens of *Catharanthus roseus* (L.) G. Don and *Ocimum basilicum* L. are C17112401R and O17112402B, respectively. The plant leaves were washed with tap water to remove soil and unwanted dust particles, then the leaves were shaded dried and then powdered by using mechanical blender and stored in air tight bottles.

The extract was prepared using the methods described by Olgica et al. [17] with minor modifications. Methanol extract was chosen because it has been reported to be the best solvents for the extraction of antioxidant compounds [17]. The dried powdered plant leaves of *Catharanthus roseus* and *Ocimum basilicum* material was extracted by maceration with methanol. Fifty g of plant material was soaked with 500 mL of the solvent for 24 h at room temperature in a shaker. The sample was filtered through filter paper. The residue from the filtration was extracted again, twice, using the same procedure. The filtrates obtained were combined and then evaporated to dryness using a rotary evaporator at 40°C. The obtained extract was stored in sterile sample tube at -20 ±°C. The extracts were used for both qualitative and quantitative phytochemical screening and for analysis of antioxidant and antibacterial activities.

2.2 Phytochemical Screening Tests

Preliminary qualitative phytochemical analysis was performed on the ethanolic extract using standard procedures to identify phytoconstituents as described by Harborne; Trease and Evans; Sofowara and Kokate et al. [18-21].

2.3 Determination of Total Phenolic Content

The total phenolic content (TPC) of the extract was estimated using the Folin's-Ciocalteu colorimetric method according to Cai et al. (2004) with minor modifications [22].

Appropriately diluted test sample (0.2 ml) or standard solution was reacted with 1.5 ml of Folin's-Ciocalteu reagent for 4 min at room temperature. The reaction was then neutralized with saturated sodium carbonate and allowed to stand for 2 h in the dark at room temperature. Later the absorbance of the resulting blue colour was measured at 760 nm with a spectrophotometer (Shimadzu, UV-Pharma Spec-1700). Quantification was done on the basis of a standard curve with gallic acid. *Total phenolics content of the extracts is expressed as milligram of gallic acid equivalent (mg GAE) per g of extract (dry weight).*

2.4 Determination of Flavonoid Content

Flavonoid contents were measured using a modified colorimetric method of Jia et al. [23]. An aliquot (1.0 ml) of extract or standard solution of quercetin were added to 10 ml volumetric flask containing 4 ml of double distilled water. Then, 0.3 ml of sodium nitrite solution was added to the flask and after 5 min, 0.3 ml aluminium chloride was also added. At 6^{th} min, 2 ml of 1 M sodium hydroxide was added and the total volume was made up to 10 ml with double distilled water. The solution was mixed completely and the absorbance level was measured versus prepared reagent blank at 510 nm using a spectrophotometer (Shimadzu, UV-Pharma Spec-1700). *The flavonoids content is expressed as milligram of quercetin equivalent (mg QE) per g of extract (dry weight).*

2.5 Assay of DPPH Radical Scavenging Activity

DPPH radical-scavenging activity of methanol extracts of the plants and the standard antioxidant butylated hydroxyanisole (BHA) were determined according to the method described by Archana et al. [24]. Various concentrations (10, 20, 40, 60, 80, 100 and 200 µg/ml) of test extract were added to 2.9 ml of a 0.004% (w/v) methanol solution of DPPH. After 30 min of incubation period at room temperature, the absorbance was measured against a blank at 517 nm using a spectrophotometer (Shimadzu, UV-Pharma Spec-1700). Controls containing methanol instead of the sample and blank containing methanol instead of DPPH solution were also measured.

DPPH scavenging activity (%) =

$$\frac{(A_{517} \text{ of control} - A_{517} \text{ of sample}) \times 100}{A_{517} \text{ of control}}$$

2.6 Assay of Superoxide Radical-Scavenging Activity

Measurement of superoxide anion-scavenging activity of the extracts was based on the method

described by Liu et al. [25] with minor modifications. The reaction mixture consisted of 1.0 ml of NBT, 1.0 ml of NADH and 0.5 ml of an appropriately diluted sample (10, 20, 40, 60, 80, 100 and 200 µg/ml). The reaction was initiated by addition of 100 µl of PMS to the mixture. The tubes were incubated at ambient temperature for 5 min and the absorbance was measured at 560 nm in a spectrophotometer (Shimadzu, UV-Pharma Spec-1700). The absorbance of the control was determined by replacing the sample with methanol. BHA was used as positive control. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{(A_0 - A_1)}{A_0} \times 100$$

where A_0 is absorbance of the Control, and A_1 is absorbance of the Sample or Standard (BHA).

2.7 Antibacterial Studies

The bacterial strains *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis* were obtained from the Institute of Microbial Technology, Chandigarh, India, and were used for evaluating antimicrobial activity. All the bacterial strains were maintained on nutrient agar in slants or Petri Plates at room temperature (28±2°C). The extracts in the concentration range from 10 to 30µg/mL dissolved in 10% dimethyl sulfoxide (DMSO) were used in this study, and amikacin was used as a reference drug.

2.8 Statistical Analysis

The values are expressed as mean ± standard deviation (SD). The results were computed statistically using Statistical Package for Social Sciences (SPSS software package, Version 16).

3. RESULTS

3.1 Preliminary Phytochemical Screening

The results of preliminary phytochemical screening tests carried out with extracts were recorded in Table 1**.** Phytochemical screening of the extract shows that the plant leaves are rich in flavonoids, alkaloids, glycosides, saponins, steroids, terpenoids, tannins and phenolic compounds.

3.2 Total Phenolic and Flavonoid Content

The amounts of total phenolic and flavonoid content of the extracts were quantified in this study. The yield of methanol extract, total phenolics and flavonoids content are shown in Table 2.

3.3 DPPH Free Radical-Scavenging Activity

It is well known that the antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture free radicals [35].

As shown in Fig. 3. the extracts exhibited dose-dependent DPPH radical scavenging activity. The extract, which contained the considerable

Table 1. Phytochemical analysis of *Catharanthus roseus* and *Ocimum basiliculum* leaves

S. No.	Phytochemical components	*Ocimum basilicum*	*Catharanthus roseus*
1.	Carbohydrates	+++	+
2.	Cardiac Glycosides	+++	+
3.	Proteins	+	++
4.	Terpenoids	+++	++
5.	Flavonoids	+++	+++
6.	Tannins	++	++
7.	Saponins	++	++
8.	Alkaloids	+	++
9.	Anthocyanin	++	+
10.	Phenol	++	++
11.	Quinines	+	++
12.	Sterols	-	-

+++ Highly present, ++ Moderately present, + Present, - Absent

Table 2. Contents of total phenolic and flavonoid in methanol extracts of *Catharanthus roseus and Ocimum basilicum*

Parameter	*Catharanthus roseus*	*Ocimum basilicum*
Yield of methanol extract (%)	16.32 ± 1.38	20.67 ± 1.75
Total phenolic content[a]	53.65 ± 3.64	48.24 ± 3.45
Flavonoid content	36.51 ± 2.85	32.76 ± 2.96

Each value is expressed as mean ± SD from minimum of three independent experiments.
[a] Data expressed as milligram of gallic acid equivalent (mg GAE) per g of extract (dry weight)
[b] Data expressed as milligram of quercetin equivalent (mg QE) per g of extract (dry weight)

amount of total phenolics and flavonoids, showed a significant effect in inhibiting DPPH, at a concentration of 100 µg/ml. The DPPH scavenging activity by *Catharanthus roseus and Ocimum basilicum* extracts was found to be 78.62±5.4% and 62.72± 4.2%, respectively.

The extracts had strong DPPH and superoxide radical scavenging activity but lower than BHA. These differences were not found statistically significant (p0.05). The percentage inhibition of superoxide generation by 25 mg/ml concentration of propofol was found as 73.3%. On the other hand, at the same concentration, BHA, BHT and a-tocopherol have 69.6, 82.2 and 75.4% inhibition of superoxide radical generation, respectively.

3.4 Superoxide Radical Scavenging Activity

Fig. 4. shows the superoxide radical scavenging activity of the tested plant extracts at different concentrations (10, 20, 40, 60, 80, 100 and 200 µg/ml). The extracts scavenged superoxide anions in a concentration dependant manner. At the concentration of 100 µg/mL, the superoxide radical-scavenging activities of methanol extracts of *Catharanthus roseus and Ocimum basilicum* was found to be 78.62±5.4% and 62.72± 4.2%, respectively.

3.5 Antibacterial Activities

The antibacterial activities of the extracts against both gram positive organisms *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis* at different concentrations ranging from 100, 200 and 300 µg/mL and their antibacterial activities were compared to those of the reference control (amikacin). The antibacterial activity of the extracts were found to increase with increasing concentration against all bacterial strains tested, as evidenced by the higher zones of inhibition at higher concentrations (Fig. 5). Moreover, both

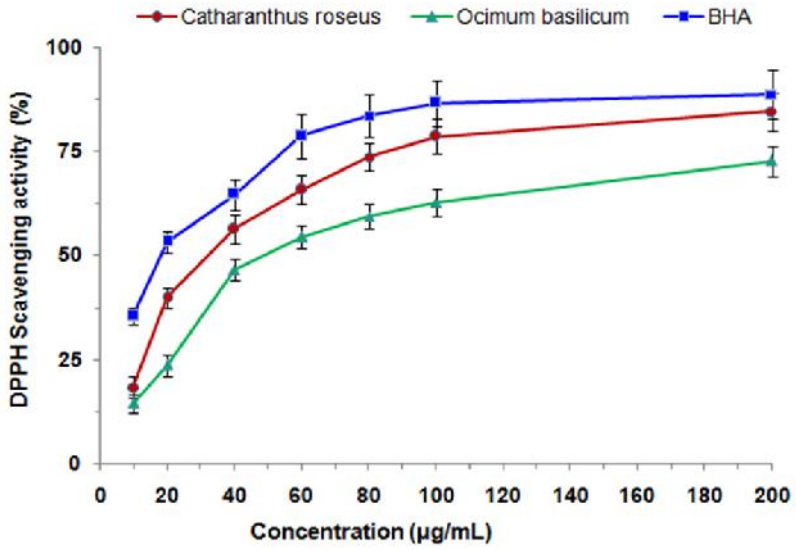

Fig. 3. DPPH radical scavenging activity of methanol extracts at different concentrations
Butylated hydroxyanisole (BHA) was used as a reference antioxidant
Values are expressed as mean ± standard deviation, (n = 3)

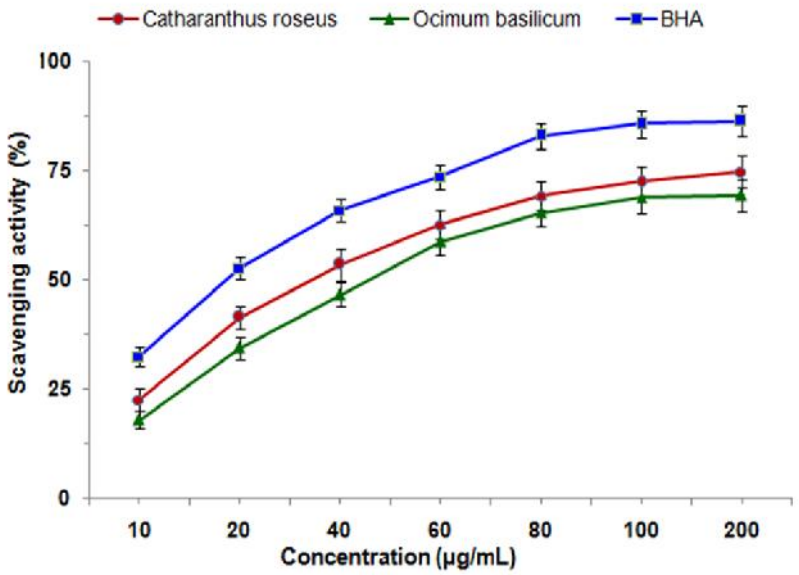

Fig. 4. Superoxide anion radical scavenging activity of methanol extracts at different concentrations. Butylated hydroxyanisole (BHA) was used as a reference antioxidant

Values are expressed as mean ± standard deviation, (n = 3)

the extracts showed a remarkable inhibition of bacterial growth at a concentration of 300 µg/mL compared to the other two doses (100 and 200 µg/mL) and the activities are comparable to amikacin, a commercially available antibiotic drug that was used as the reference control drug (Table 3).

4. DISCUSSION

Infectious bacterial diseases represent an important cause of morbidity and mortality worldwide. Therefore, the development of new antimicrobial agents for the treatment of bacterial infections is of increasing interest. A number of naturally occurring compounds found in plants, herbs, and spices have been shown to possess antimicrobial functions, and they may serve as sources of antimicrobial agents against pathogens [26]. The first step towards this goal is an *in-vitro* antibacterial activity assay [27-29].

The preliminary phytochemical screening of the extracts confirmed that the plants are rich in flavonoids, alkaloids, glycosides, saponins, steroids, terpenoids, tannins and phenolic compounds. *Catharanthus roseus* extract was found to have phenolics and flavonoids levels of 53.65 ± 3.64 milligram of gallic acid equivalents (mg GAE/g), 36.51 ± 2.85 milligram of quercetin equivalents (mg QE/g), respectively. Total phenolics and flavonoids concentrations of *Ocimum basilicum* were found to be 36.51 ± 2.85milligram of gallic acid equivalents (mg GAE/g), 32.76 ± 2.96 milligram of quercetin equivalents (mg QE/g), respectively. Phenolic compounds have been proved to be responsible for the antioxidant activity of plants [30,31]. Flavonoids are most commonly known for their antioxidant activity. They are transformers which modify the body's reactions to carcinogens, viruses and allergens [32,33]. They show anticancer, anti-inflammatory, antimicrobial and anti-allergic activities and are useful in therapeutic roles [34]. Hence, the phenolic and flavonoid compounds identified in the plant extract may contribute to the antioxidant activity.

It is well known that the antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture free radicals [35]. Scavenging activity of DPPH radical was found to rise with increasing concentration of the extracts. Additionally, it has been determined that the antioxidant effect of plant products is mainly due to radical scavenging activity of phenolic compounds such as flavonoids, polyphenols and tannins [36]. The antioxidant activity of phenolic compounds is mainly due to their oxidation reduction properties, which can play an important role in adsorbing and neutralising free radicals,

Table 3. Antimicrobial activity of *Catharanthus roseus and Ocimum basilicum* against various human pathogens

Particulars	Zone of inhibition (mm)							
	Catharanthus roseus				*Ocimum basilicum*			
	P. aeruginosa	***B. subtilis***	***S. aureus***	***E. coli***	***P. aeruginosa***	***B. subtilis***	***S. aureus***	***E. coli***
Positive control (25 µg/mL)	13.56 ±1.6[a]	13.6 ±1.3[a]	16.4 ±1.2[a]	19.5 ±1.3[a]	8.6 ±1.2[a]	7.41 ±1.1[a]	14.9 ±1.3[a]	22.8 ±1.5[a]
Extract (100 µg/mL)	NA	NA	NA	NA	NA	NA	NA	NA
Extract (200 µg/mL)	8.7 ±0.92[b]	8.3 ±0.64[b]	7.2 ±0.49[b]	11.6 ±0.42[b]	6.4 ±0.26[b]	4.4 ±0.31[b]	7.5 ±0.41[b]	8.9 ±1.12[b]
Extract (300 µg/mL)	11.7 ±0.72[a]	12.8 ±0.62[a]	11.6 ±0.43[a]	17.3 ±1.38[a]	7.5 ±0.86[a]	6.6 ±0.55[a]	13.6 ±0.83[a]	18.2 ±0.92[a]

Values are expressed as mean ± standard deviation.
NA, no activity exhibited against microorganism
Positive control, Amikacin (25 µg/mL)
Means in the same column followed by different letters are significantly different for the concentration tested and positive control ($p<0.05$)
All experiments were performed in duplicate and repeated three times

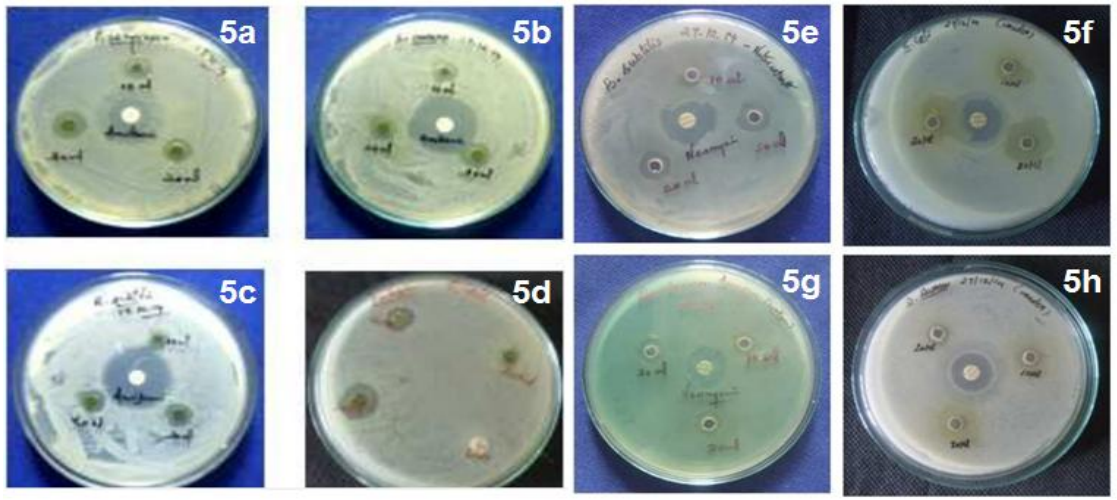

Fig. 5. Antimicrobial activity of *Catharanthus roseus* (5a-5d) *and Ocimum basilicum* (5e-5h) against four human pathogens viz. *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*

reducing singlet and triplet oxygen, or decomposing peroxides [37]. Oxidative injury now appears as the fundamental mechanism causing a number of human neurologic and other disorders such as autoimmune pathologies, inflammation, infections and digestive system disorders including gastrointestinal inflammation and ulcer [38]. It has been reported that free radical-scavenging activity is greatly influenced by the phenolic composition of the sample [39]. The results obtained suggested that some components within the extract were significantly strong radical scavengers. The results revealed that the tested plants can prevent damage *in vivo* system caused by free radicals in pathological conditions [40].

The extracts was significantly active, exhibiting antimicrobial activity against tested organisms *viz. Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. The antibacterial activity of the extracts was found to increase with increasing concentration against all bacterial strains tested, as evidenced by the higher zones of inhibition at higher concentrations. Both the extracts showed a remarkable inhibition of bacterial growth at a concentration of 300 µg/mL compared to the other two doses (100 and 200 µg/mL) and to amikacin, a commercially available antibiotic drug that was used as a reference positive control drug. Moreover, the antioxidant and antibacterial activity of *Catharanthus roseus* is significantly higher than that of *Ocimum basilicum*. This is interesting in view of the perspective of developing new antibacterial drugs from those plants.

To the best of our knowledge, this is the first report on comparative antioxidant and antimicrobial activities of antimicrobial activities of the *Catharanthus roseus* and *Ocimum basilicum*. The overall results of this study can be considered as very promising in the perspective of obtaining new drugs from plant sources, especially when the medical importance of the tested microorganisms is considered. *Staphylococcus aureus* is a major cause of community and hospital-associated infections, with an estimated mortality of around 7% - 10% [41]. Moreover, about 2% of patients in Cameroon are infected with *Staphylococcus* spp. [42]. Each year, some 500,000 patients in American hospitals contract a staphylococcal infection [41]. Such findings stress the importance of finding an antibiotic against which the *Staphylococcus aureus* organism is sensitive. This pathogen was found to be sensitive to both the plant extracts.

5. CONCLUSION

The present study attempted to explore the diverse phytochemical efficacy of two traditionally used Indian medicinal plants *Catharanthus roseus* and *Ocimum basilicum* against several diseases and oxidative stress, by evaluating the antioxidant and antibacterial activities of its methanolic extracts. Furthermore, to the best of our knowledge, this is the first comprehensive study of the antioxidant and antibacterial potential of the extracts. Generally, *Catharanthus roseus* extract showed a higher efficacy than the *Ocimum basilicum* extract did. In addition, the total phenolic and flavonoid content were higher in *Catharanthus roseus* extract than they were in the *Ocimum basilicum* extract, and these results were in agreement with the percentage radical inhibition results, which were higher for the *Catharanthus roseus* extract than they were for the *Ocimum basilicum* extract. Both extracts showed promising evidence of antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. Finally, the observed antioxidant and antibacterial activities of these extracts could be attributed to the high content of phenolics and flavonoids. Further studies are underway to elucidate and understand the antioxidants and antibacterial potential of these plants using various biochemical and molecular biology tools such as LC/MS and GC/MS.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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