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# Phytochemical studies, Chemical composition, antibacterial and antioxidant activities of *Stenosiphoniumrusselinium* Nees leaf extract

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## ABSTRACT

In this current study, the phytochemical class, antibacterial and its antioxidant activity of *Stenosiphonium russeliniu*mextracts. It's showed the presence of secondary metabolites and it was identified by GC-MS analysis showed the bioactive compounds in this extract was cyclohexanol (44.98%), hexadecanoic acid (28.66%), p-hydroxynorephedrine (14.95%) and 2-amininonadecane (11.42%) respectively. Methanolic extract showed dose-dependent activityand the maximum growth inhibition (35.3 mm) was observed against *E. coli*, which was followed by *P. aeruginosa* (34.1 mm), *B. subtilis* (33.3 mm) and *B. coagulans* (24.6 mm) and this methanolic extract at the concentration of 200 µg/ml showed significant growth of inhibition against all the human pathogenic bacterial strains. The DPPH assay of lowest concentration at 20µg/ml and high concentration100 µg/ml was measured with methanolic extract of *S. russelinium* at 100 µg /ml showed significant free radical scavenging activity with percentages of 68.3 %.

**KEYWORDS:** *S. russelinium*, Phytochemical, Antibacterial activity, Antioxidant activity, GC-MS

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## INTRODUCTION

Herbal medicine is the most widely used system of medicine has become more popularin the word and the treatment of many diseases[1-2]. About 80% of the individual from developed countries use traditional medicine, which has compounds obtained from medicinal plants[3]. In recent years the tendency of using natural products has increased and new drug discoveries have taken place by using the active plant extracts[4]. Secondary metabolites from plant serve as defense mechanisms against predation by many microorganisms, insects, and herbivores[5].The pharmacological action of crude plant extract is determined by the nature of its constituents. Plant drug has been a major resource for the treatment of infectious disease for a long time[6]. GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non-polar components and volatile essential oil, fatty acids, lipids, alkaloids, terpenoids and steroid[7-8].The results were also confirmed by the comparison of the compounds elution and order with their relative retention indices on non-polar phases reported in the literature. The name, molecular weight, and structure of the components of the test material were as curtained[9]. In the last few years, gas chromatography-mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in plant species.[10]

In recent year much attention has been devoted to natural antioxidant and their association with health benefits.[11] Antioxidant compounds like flavonoids, polyphenols and phenolic acid scavenge free radical and thus inhibit the oxidative mechanisms that can be lead to degenerative diseases.[12] Oxidative stress possible to the accumulation of free radicals effects in various alterations to biological macromolecules, such as lipid, protein, and DNA.[13] Therefore, the new discovery of the antioxidants derived from plant resource has always a great interest in upcoming research scientists.[14] The uses of different parts like leaves, flowers, stem, seeds, roots, and berries of the plants are known to prevent, treat and relieve various ailment and it also plays a vital role as antimicrobial properties.[15] The demonstration of antibacterial activity against gram positive and gram negative bacteria by the plant may indicative of the presence of a broad spectrum of antibiotic compounds.[16] Hence, the present study was aimed to find phytochemical classes and to analyze the total alkaloids, tannins and terpenoids contents, antibacterial activity, and antioxidant activities were showed the bioactive compound of *S. russelinium*

## MATERIALS AND METHODS

### *Chemicals and reagents*

Nutrient Agar (NA), Muller Hinton Agar (MHA) medium was purchased from Himedia Laboratories, Mumbai. Hexane, Acetone, Methanol, Aqueous, Ethyl Acetate, Dichloro Methane,

Sulphuric acid, Hydrochloric acid,Nitric acid, Sodium hydroxide, Chloroform, Fehling's solution, Acetic acid and Ammonia ($NH_3$) was procured from Ranbaxy fine chemicals Ltd, Mumbai. All the chemicals were used for an analytical grade. Distilled water was used for all the experimental work.

### *Sample collection and extraction*

Leaves of *S. russelianum* were collected from Tamil Nadu, India and leaves were washed and rinsed distilled water and then dried. Extraction was made using 25g of the sample was extracted with using Soxhlet apparatus. The extracts were concentrated with a rotary evaporator and dried to obtain yield dark brown residues. The obtained residues were subjected to antibacterial and antioxidant activity.

### *Phytochemical Screening*

Since the *S. russelianum* is a rare medicinal plant, only leaves were collected and shade dried. The previous method adopts analyzing and extracting the plant required a high quantity of raw materials hence, a modified procedure of ,[17] was applied for the analysis and extraction of phytochemical screening as well as quantification, antibacterial and antioxidant activity.

### *Total alkaloids content*

Quantitative estimation of total alkaloids was measured by,[18] 200 mg of 10% of acetic acid in ethanol was added to the extract (2.5 ml) and stand for 4h followed by addition of 1.5 ml of ammonium hydroxide to the extract. After 3h of mixture sedimentation was washed with 0.1M of ammonium hydroxide. The residues were dried in an oven and percentage (%) of alkaloids was calculated mathematically as % Alkaloids = weight of alkaloids/weight of the sample x 100. The total alkaloids contents were expressed as mg of AE/g of the extract.[19]

### *Total tannins contents*

The total tannins were determined by the method of polyvinylpolypyrrolidone (PVPP).[20] 100 mg of polyvinylpolypyrrolidone were added to the test tube containing 0.5 ml of distilled water and then added 0.5 ml of extract. The mixture allowed to stand for 40 min. and kept the test tube at $4^oC$ for 4 h. Centrifuged for 3000 rpm for 10 min. and the supernatant was collected and it was measured at 725nm. The content of the non-tannins phenolics on dry matter basis, the total tannins content was calculated as the following formula: Tannins (%) = Total phenolics (%) – Non-tannin phenolics (%). The total tannins content was determined as mg of TAE/g of the extract.[21]

### *Totalterpenoids content*

Total terpenoids in the extracts were determined by,[22] 1g of the sample was taken in a test tube and soaked in ethyl alcohol for 1 day. Then the filtrate was extracted with petroleum ether.

After the extract was calculated and a measure of total terpenoids the following formula as Total terpenoids content = (Final weight of the sample – Initial weight of the extract) / Weight of the sample x 100.

### *Gas Chromatography-Mass Spectrometry (GC-MS)*

The methanol extract was performed GC-MS analysis previously developed a method with minor modification,[23] using Agilent Technologies (GC) 5975C Agilent with mass selective detector (MS), HP-5MS (5% phenyl methyl siloxane) capillary column of dimension 30m x 250µm x 0.25 µm and used helium carrier gas 1 mL /min. and the column temperature was programmed initially at 500C for 10 min, followed by an increase of 30C/ min to 2400C and then it was kept isothermally for 5 min. The mass spectrometry was working with 70eV. The active components were identified with a comparison of their mass spectral data analysis by those from NIST 0.5 spectral library matches.

### *Antibacterial assay*

### *Test Organisms*

The extracts were assayed against the following test organisms; gram-negative bacteria *Escherichia coli* (MTCC-40) and *Pseudomonas aeruginosa* (MTCC-422); gram-positive bacteria *Bacillus coagulans* MTCC-492) and *Bacillus subtilis*(MTCC-864). All the stock cultures were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. The test organisms were maintained in glycerol stock and stored at -20$^0$C.

### *Culture media and inoculum preparation*

The component of Muller Hinton Agar (MHA) medium was dissolved in distilled water and volume was brought to 300 ml. the media was autoclaved for 15 min. at 15 psi pressure at 121$^0$C. The agar plates were prepared by pouring 30 ml of sterile media in sterile Petri plates and the plates were allowed to solidify. 0.1 ml of inoculums were swabbed uniformly and allowed to dry.

### *Antibacterial activity*

*In vitro* antibacterial activity was done by agar well diffusion method,[24] to determine the zone of inhibitory activity of the extracts. The varying concentration of extract (100µg/ml and 200µg/ml) was added into the agar well. The agar plates were incubated at 37$^0$C for 18-24 h. Positive control (Chloramphenicol) and negative solvent control (Dimethyl sulfoxide) were also incubated and the diameter of the zone of inhibition was measured in mm and compared with control values. Triplicates were maintained and the experimental work was repeated thrice.

### *2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay*

Free radical scavenging activity of the extract was determined by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH; Himedia, Mumbai) with slightly modified.[25] Freshly prepared DPPH

solution was taken into the test tubes and the extract was added (20 to 100 µl) each test tube and final volume was adjusted with 100µl with methanol. The reaction was carried out at $27^0$C in dark condition for 20 min. the absorbance was measured at 517 nm. The DPPH radical scavenging activity was calculated as the mathematically:

DPPH Radical scavenging activity = $A_{control} - A_{sample} / A_{control} \times 100\%$

## RESULTS AND DISCUSSION

The plant extracts were tested for its various phytochemicals. Phytochemical screening of plant extracts revealed the presence of alkaloids, flavonoids, steroids, terpenoids, glycosides, saponins, phlobatannins, proteins, amino acid and carbohydrates (Table 1). Protein was observed with only aqueous extract followed by saponins and phlobatannins were observed in methanol extract since other extracts didn't contain this compound. The tannins were observed with methanol and aqueous extracts; whereas steroids were observed with acetone and methanol extracts. In all the extracts flavonoids were not present which indicated that either the non-existence of this compound in the plant or the need for study of extraction with other solvents for confirming of the compound. Alkaloids, terpenoids, amino acids, and carbohydrates were observed with all the extracts, confirming the presence of alkaloid bioavailability in this plant.

**Table 1 Phytochemical screening of *S.russelianum* extracts**

| Secondary metabolites | Hexane | Acetone | Methanol | Aqueous | Ethyl Acetate |
|---|---|---|---|---|---|
| Alkaloids | + | + | + | + | + |
| Tannins | - | - | + | + | - |
| Flavonoids | - | - | - | - | - |
| Saponins | - | - | + | - | - |
| Steroids | - | + | + | - | + |
| Carbohydrates | + | + | + | + | + |
| Phlobatannins | - | - | + | - | - |
| Terpenoids | + | + | + | + | + |
| Glycosides | + | + | + | - | + |
| Proteins | - | - | - | + | - |
| Amino acids | + | + | + | + | + |

Present (+); Absent (-)

The total alkaloids, terpenoids, and tannins contents were measured with different extracts and presented in the (Table 2). The alkaloids content was obtained in different extracts and expressed in terms of atropine equivalent as mg of AE/g of extract. The alkaloids were reported to have antibacterial activity against gram negative and gram positive bacteria.[26] The high amount of alkaloids were measured with (31.28 mg/g) in methanol extract. This compound was comparatively less with acetone (13.07mg/g) and ethyl acetate (11.59mg/g) extract. The total tannins were obtained maximum amount in aqueous (30.10mg/g) whereas ethyl acetate (26.71mg/g). The fewer amounts of tannins were obtained from hexane extract (17.25mg/g).

**Table 2 Total alkaloids, terpenoids, and tannins contents of the *S.russelinium***

| Extracts | Total Alkaloids mg AE/g extract | Total Tannins mg TAE/g extract | Total Terpenoids mg QE/g extract |
|---|---|---|---|
| Aqueous | 12.08 ± 0.01 | 30.10 ± 0.21 | 18.26 ± 0.12 |
| Methanol | 31.28 ± 0.21 | 24.09 ± 0.00 | 14.91 ± 0.08 |
| Ethyl acetate | 11.59 ± 0.10 | 26.71 ± 0.11 | 17.51 ± 0.16 |
| Acetone | 13.07 ± 0.31 | 19.42 ± 0.01 | 13.10 ± 0.01 |
| Hexane | 15.12 ± 0.11 | 17.25 ± 0.03 | 15.90 ± 0.20 |

Each value is expressed as mean ± Standard deviation (SD) of triplicate values. TAE – Tannic Acid Equivalent; AE – Atropine Equivalent; QE – Quercetin Equivalent

Quantification of total terpenoids in the different extract of *S. russelinium* showed in (Table 2). The high amount of total terpenoids contents were obtained (18.26 mg/g) in aqueous extract, whereas ethyl acetate (17.51 mg/g) extract. The fewer amounts of terpenoids were obtained from acetone (13.10 mg/g) extract.

The chemical composition analysis by GC-MS chromatogram showed the presence of 4 major bioactive compounds in the methanolic extract of *S. russelinium*was shown in (Table 3) and (Figure 1). The percentage (%) composition of each and every compound was corresponding based on a percentage (%) of the peak area and it provides the information about the molecular weight (MW) of each bioactive compound. From this analysis, it was also observed that the plant contained many bioactive metabolites such as alkaloids, vitamins, steroids, fatty acid, and phytol.

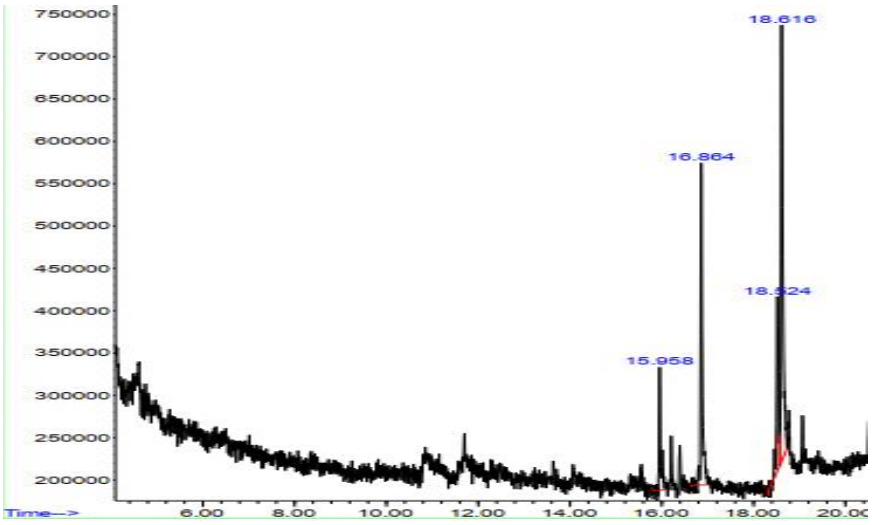
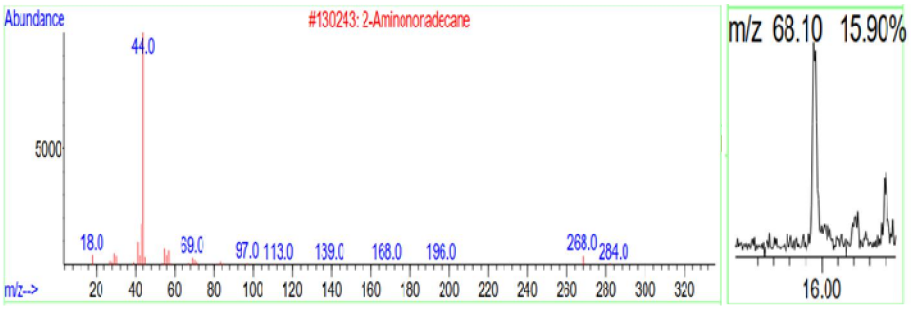
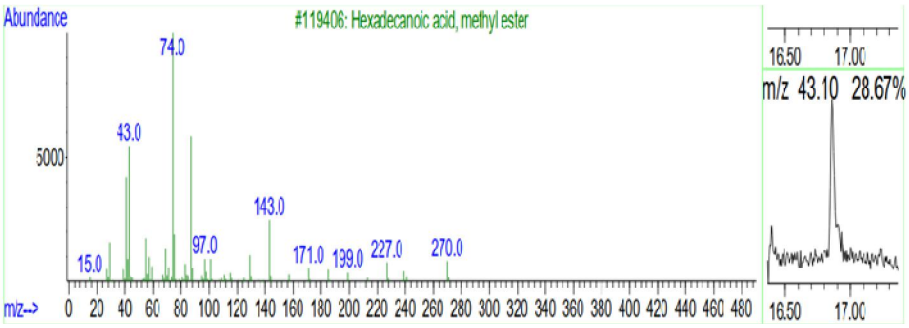


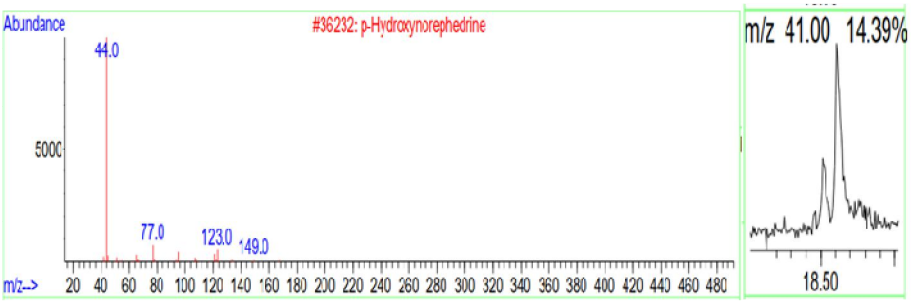

**Fig 1 GC-MS chromatogram of bioactive compounds identified in the methanolic extract of *S. russelinium***



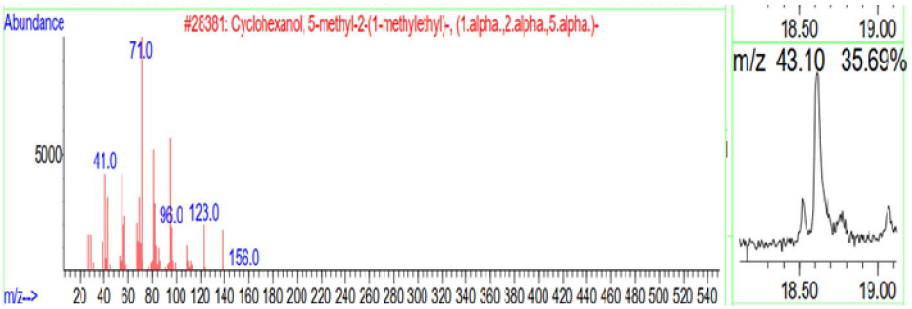

A) Mass spectrum of 2-Aminononadecane




B) Mass spectrum of Hexadecanolic acid




C) Mass spectrum of p-Hydroxynorephedrine




D) Mass spectrum of Cyclohexanol

**Fig 2 GC-MS analysis of methanolic extract from *S. russelinium***

The major phytochemical examined in this extract was cyclohexanol(44.98%), hexadecanoic acid (28.66%), p-hydroxynorephedrine (14.95%) and 2-aminononadecane (11.42%) was represented in the (Figure 2)

**Table 3 Bioactive compounds identified in the methanolic extract by GC-MS analysis**

| Peak % | RT | Name of the compound | Molecular Formula | Molecular Weight |
|---|---|---|---|---|
| 11.42 | 15.96 | 2-Aminononadecane | $C_{19}H_{41}N$ | 283.5 |
| 28.66 | 16.86 | Hexadecanolic acid | $C_{16}H_{32}O_2$ | 256.4 |
| 14.95 | 18.52 | p-Hydroxynorephedrine | $C_9H_{13}NO_2$ | 167.2 |
| 44.98 | 18.61 | Cyclohexanol | $C_6H_{12}OH$ | 100.1 |

*In vitro* antibacterial activities of the tested methanolic extract of *S. russelinium* showed significant reduction of bacterial growth and derived from different concentration against the four bacterial strains in terms of zone of inhibitions (Figure 3). The methanolic extract was revealed antibacterial activity against two gram-positive (*Bacillus coagulans* and *Bacillus subtilis*) bacteria and two-gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria. This extract was exhibited antibacterial activity against all the tested bacterial strains (Table 4)

**Table 4 Antibacterial activities of *S. russelinium* against human infectiousdisease**

| Concentration (µg/mL) | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| Organisms | *Bacillus coagulans* (MTCC-492) | *Bacillus subtilis* (MTCC-864) | *Escherichia coli* (MTCC-040) | *Pseudomonas aeruginosa* (MTCC-422) |
| 100 | 19.2 ±1.10 | 26.2 ± 1.1 | 27.2 ± 1.5 | 25.6 ± 0.5 |
| 200 | 24.6 ± 0.5 | 33.3 ± 1.5 | 35.3 ± 1.0 | 34.1 ± 0.7 |
| Chloramphenicol | 12.1 ± 0.8 | 22.1 ± 0.2 | 21.2 ±1.0 | 22.8 ± 1.0 |

Each value is expressed as mean ± Standard deviation (SD) of triplicate

These extract showed dose-dependent activity i.e., while an increase in the dose concentration of the extract, the zone of inhibition also increased. In the current study, maximum growth inhibition (35.3 mm) was observed against *E. coli*, which was followed by *P. aeruginosa* (34.1 mm), *B.subtilis* (33.3 mm) and *B. coagulans* (24.6 mm).

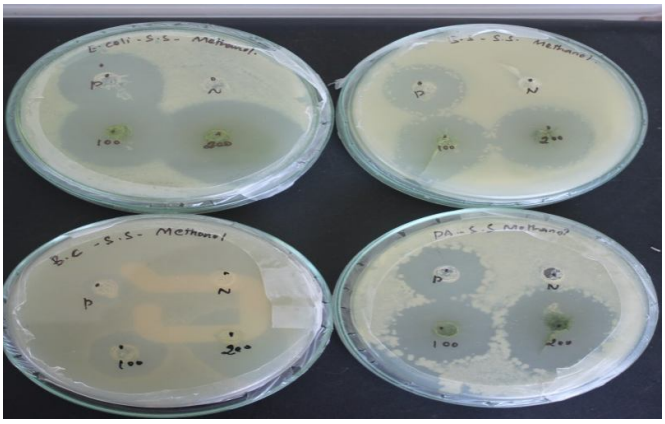


**Fig 3. *In vitro* antibacterial activities against tested bacterial strains**

### *3.5. DPPH free radical scavenging activity*

Antioxidant activity determined by DPPH based upon the reduction of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity. The free radical scavenging activity of the methanolic extract was examined by their capability to reduce the DPPH, a constant free radical activity. DPPH is a purple color dye having absorption at 517 nm and it reacted with a hydrogen donor the purple color disappears due to the conversation of it to 2, 2-diphenyl-1-picrylhydrazine resulting in the decrease in absorbance. Methanolic extract showed DPPH scavenging activity was increased when increasing the concentration of the extract and it was observed dose-dependent manner of antioxidant activity. It was inferred by a gradual increase in free radical activity of*S. russelinium* extract with a low concentration of extract 20 µg/ml to high concentration at 100 µg/ml. The DPPH assay of lowest concentration at 20 µg/ml and high concentration100 µg/ml was measured (Figure 4).

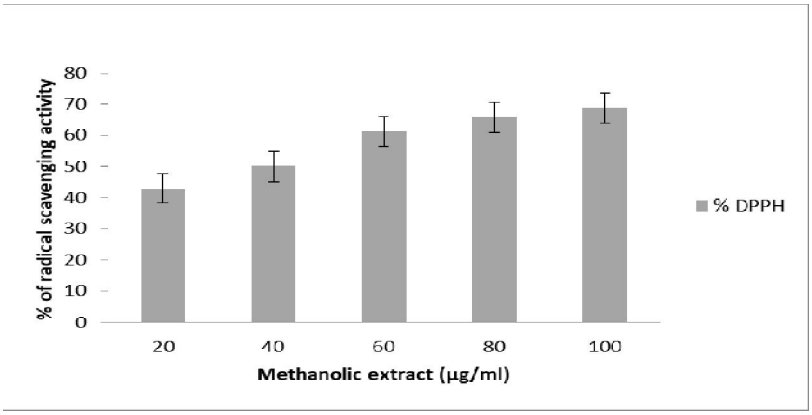



**Fig 4. Free radical scavenging potentials of methanolic extract from *S. russelinium***

Therefore, when the above extract was tested for the DPPH free radical scavenging ability, the methanolic extract of *S. russelinium* at 100µg /ml showed strong radical scavenging activity with percentages of 68.3 %. The current study suggests that plant can be used as a source of antioxidant.

## *Discussion*

The presences of these phytochemical compounds have significant application against human pathogens and are reported against several pathogens and therefore might suggest their use in the treatment of infectious diseases.[27] In addition, the total phenolic compounds found in the leaf are the major sources to the antioxidant properties of the plant.[28]Terpenoids are reported to have beneficial for antibacterial activity and it can be applied against bacterial infections like E. coli, S. aureus, Pasteurellamultocida, Shigellaflexneri, and salmonella and etc.[29] Glycosides work as defense mechanisms against several microorganisms, herbivores, and insects.[30] Alkaloids are reported to be responsible for antibacterial activity.[31]

Hence the current study showed that the tannins content was high when compared with hexane extract, therefore the extract showed significant antioxidant activity. These plants contain rich sources for naturally occurring antioxidant properties particularly tannins, alkaloids and phenolic contents.[32] Terpenoids are natural compound found in plant species and which is having rich sources of flavor and fragrance.[33] Medicinal plant terpenoids serve as antibacterial, anticancer, antioxidant properties.[34] Hence, based on phytochemical results the plants were rich antioxidant properties and it might be further examined by *in vitro* free radical scavenging activity.

Among the four bioactive compounds isolated,hexadecanoic acid has significant anticancer and antioxidant properties.[35-36] The cyclohexanol compounds have been proved clinically as cholesterol reducer was studied by, [37] and it was also proved as novel metabolites against rheumatoid arthritis and chronic inflammatory disease was reported by,[38]

Hence, the methanolic extract at the concentration of 200 µg/ml) showed significant growth of inhibition against all the human pathogenic bacterial strains,[39]reported that methanolic extract showed moderate antibacterial activity against human pathogenic bacterial strains. Similarly, aqueous, ethyl acetate extract against *P. aeruginosa*, which was compared with the chloramphenicol as a positive control. Therefore, the aqueous extract showed less antibacterial at lower concentrations.[40] Above the pathogens, usually grow on mucus and skin of the human and it causes a serious of illness.[41]

DPPH free radical scavenging assay revealed that the ability of the tested methanolic extract act as antioxidant activity and it was based on the role of 2, 2-diphenyl-1-picrylhydrazyl, stable free radical to be decolorized due to the presence of antioxidant properties.[42] This property has been widely used to estimation the free radical scavenging activity of natural antioxidant.[43] The present investigation about medicinal plants to screen phytochemical compounds is very important hence these plants show good activity against bacterial infection and heart diseases by scavenging free radicals .

## CONCLUTION

From the above study, the different extracts of *S. russelinium* were found in promising antibacterial and antioxidant activity. DPPH free radicals scavenging which exhibited that methanolic extract showed significant antioxidant properties and it was exhibited the best antibacterial activity; this was subjected to GC-MS analysis, that can be evidenced for the presence of the antibacterial compound. The current study will be useful for scientists who engage in new drug development against infectious diseases.

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