

In vitro Antioxidant, Antibacterial activity and Phytochemical Characterization of *Senecio nudicaulis* Buch. -Ham. er D. Don leaves extracts

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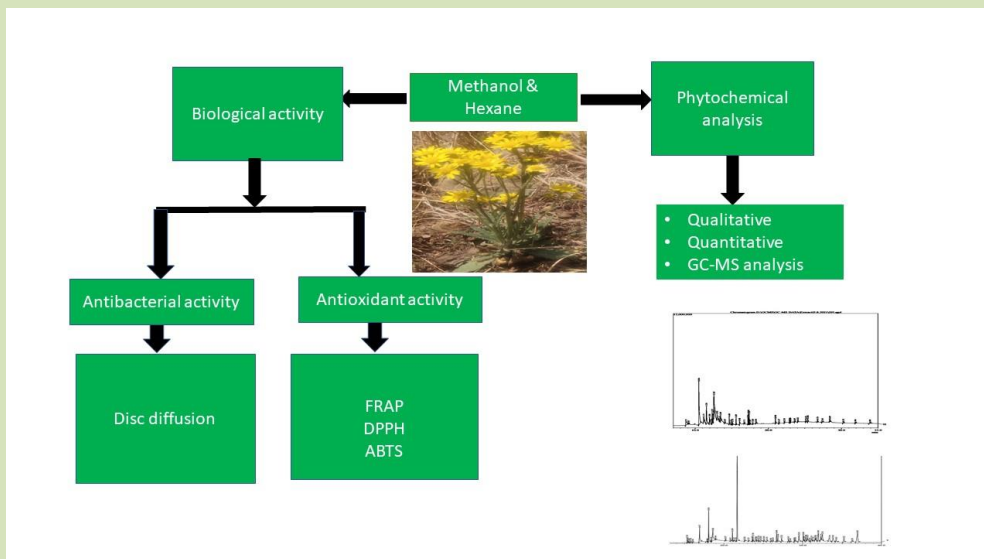
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ABSTRACT: The antioxidant and antibacterial activities of *Senecio nudicaulis* (Asteraceae) are elucidated in the current paper, along with chemical characterization using its methanol and hexane extracts. The highest levels of phenolics (78.26 ± 0.86 mg GAE/g dry weight) and flavonoids (32.10 ± 0.24 mg QE/g dry weight) were detected in the methanol extract, which also displayed remarkable antioxidant potential in DPPH (IC_{50} - 30.74 ± 1.93 μ g/mL), FRAP (120.11 ± 2.21 mg AAE/g dry weight), and ABTS (IC_{50} - 28.61 ± 0.25 μ g/mL) assays. Methanol extract showed the highest level of inhibition (13.66 mm) against *A. tumefaciens*, followed by hexane extract (12.5 mm) against *P. aeruginosa* and *A. tumefaciens*. Methanol extract against *A. tumefaciens* and hexane extract against *P. aeruginosa* both had substantial MIC values (12.5 mg/ml). GC-MS analysis revealed that 6-Methyl-3,5-heptadiene-2-one (27.9%), 2,5-dihydroxy benzene acetic acid (15.63%), and hexahydroindanin (11.37%) were dominated in methanol extract, whereas phytyl palmitate (9.98%), 9,19-Cyclo-27-norlanostan-25-one, 3-(acetyloxy)-24-methyl-(3.β.,24R) (5.77%), stearic acid (8.08%), dihomog-γ-linolenic acid (9.40%), and phytol (8.21%) were found to be the major constituents in hexane extract. While non-polar extract did show significant antibacterial activity, polar extract exhibited strong antioxidant potential. Results clearly demonstrated antioxidant and antibacterial activities due to the presence of bioactive phytochemicals. The present study makes a foundation for development of new and effective antibiotic and antioxidant drugs.

Keywords: *Senecio nudicaulis*, GC-MS, Phytochemicals, Antioxidant, Antibacterial, Medicinal plants.



INTRODUCTION

An imbalance between the antioxidant defence system and reactive oxygen species production creates oxidative stress (Betteridge, 2000). Redox stress causes immune cells to activate and emit reactive oxygen, which disrupts physiological and pathological processes and leads to the appearance of numerous diseases such as cardiovascular problems, atherosclerosis, cancer, diabetes, Alzheimer's, and Parkinson's (Lonkar and Dedon 2011). Several *in vivo* studies have demonstrated that consuming antioxidants is a crucial approach for promoting health by reducing oxidative stress (Watz, 2008).

Consumption of synthetic antioxidants such as butylated hydroxytoluene (BHT), tert-butylated hydroquinone (TBHQ), propyl gallate (PG), butylated hydroxyanisole (BHA), and octyl gallate (OG) has been associated with various health hazards (Grice, 1986; Wichi, 1988). Beside this, several synthetic antibiotics are employed in the treatment of infections and communicable diseases, but the emergence of multidrug resistance as well as the appearance of undesirable side effects of certain antibiotics (WHO, 2002) have created alarming clinical situations in the treatment of infectious diseases. Thus, it became essential to search for new alternatives to antioxidant and antibacterial agents from plant sources.

To ascertain the therapeutic effects of traditional medicinal plants, numerous pharmacological investigations have been carried out recently (Qasim *et al.*, 2010; Sati *et al.*, 2015; Pant *et al.*, 2018; Verma *et al.*, 2020; Ahmad *et al.*, 2021; Rawat *et al.*, 2022; Debbarma *et al.*, 2022; Magadelin *et al.*, 2023; Bhavadharaniparkavi and Abirami 2023). Due to the diverse range of secondary metabolites that medicinal plants create, they are excellent sources of potential antioxidant, antibacterial, anticancer, anti-inflammatory and related potentials (Ali *et al.*, 2005). Hence, there is growing understanding of the relationship between a medicinal plants phytochemical components and its pharmacological efficacy. Consequently, it is a logical to screen plant extracts for active compounds, which led to the development of novel medicines with effective protection and treatments against various infectious and non-infectious diseases.

Senecio L. is the major genus of the Asteraceae family, which comprises around 1500 species distributed all over the world (Loizzo, 2004). Previous studies indicated that several species of *Senecio* exhibit various phytochemicals with antimicrobial and antioxidant activities (Üçüncü, 2010; Sharma, 2015; Albayrak *et al.*, 2015; Lawal *et al.*, 2016; Faraone, 2018; Joshi *et al.*, 2019; Ramadan *et al.*, 2020). The literature search reveals that still few works have been done on chemical compositions and bioactive studies of extracts from *Senecio nudicaulis* inspired us to make this research. The present study focused on antioxidant, antibacterial, and phytochemical characterization using polar and non-polar extracts of *S. nudicaulis*.

MATERIAL AND METHODS

Collection and extract preparation of plant.

Following Jain and Rao (1977), fresh *S. nudicaulis* Buch. -Ham. ex D. Don leaves were procured from the Central Himalaya in India (1627 m asl; 29.58 N, 80.22 E). The specimen (Acc. No.390) was given taxonomic documentation by B.S.I. Dehradun, Uttarakhand, India. Plant leaves were harvested, air dried in the shade, then ground using a mixer. 10g of plant powder was shocked in 60 ml of methanol and hexane for 72 hours before being filtered using Whatman filter paper No. 1. The obtained extracts were dried to a powdery consistency at a low temperature (40 to 50°C) and stored in a tightly sealed container. Next, stock solutions of 1.0 mg/ml concentration and dried extracts that had been diluted with the appropriate solvents were made for additional analysis (Handa *et al.*, 2008).

Qualitative phytochemical analysis. The methanol and hexane extracts of *S. nudicaulis* were subjected to preliminary phytochemical screening to determine the various chemical ingredients (Harborn *et al.*, 1998). With slight adjustments, the same approach was used to screen for the presence of flavonoids, phenols, steroids, glycosides, alkaloids, tannin, terpenoids, saponin, resin, volatile oil, carbohydrate, and protein.

Chemical characterization via (GC-MS) analysis. *S. nudicaulis* preparations in methanol and hexane were analysed using Agilent Technologies' [Model QP 2010 Ultra] GC-MS system. The electron ionisation voltage was 70 V, while the ion source's temperature was 230°C. At the stationary phase, HP-5MS (30mm × 0.25mm × 0.320 m) was employed as a highly pure helium gas (99.9% purity). The oven's initial temperature was 80°C for 2 minutes before rising to 300°C at a rate of 3.5°C/min. By comparing the spectra of the peaks with the equivalent standard mass spectra from NIST-MS and the Wiley library, the chemical components were identified (Mallard *et al.*, 2008).

Quantitative phytochemical analysis

Total phenolic content. Using Singleton and Rossi's (1965) folin-ciocalteu reagent (FCR) method (with a small modification), total phenolic content (TPC) was ascertained. Gallic acid in various concentrations (10–100 g/ml) was used to plot the standard curve. 0.2 ml of FCR and 0.5 ml of 20% Na₂CO₃ were combined with the sample (0.5 ml). After 30 minutes of incubation at room temperature, a 765 nm absorbance was observed in comparison to a blank. The gallic acid calibration curve ($Y=0.003x$; $R^2 = 0.995$) was used to determine the concentration of total phenolic content, which was expressed as mg GAE (gallic acid equivalent)/g dry weight.

Total flavonoid content. With little adjustment, the aluminium chloride method was used to assess the total flavonoid concentration (Christ and Muller, 1960). Various concentrations (10-100 µg /ml) of standard (quercetin) and extracts were prepared. Each sample (0.5 ml) was combined with 0.1 ml of 10% AlCl₃, 0.1 ml of potassium acetate (1mole), and adding distilled water, raising the final volume to 5.0 ml. After

incubation for 30 minutes at room temperature, the absorbance was noted at 415 nm. The flavonoid content of the extracts was determined and reported in terms of mg QE (quercetin equivalents)/g of dry weight using the calibration curve of the standard ($Y= 0.006x$; $R^2=0.983$).

Antioxidant activity

FRAP (Ferric reducing antioxidant power) method.

The FRAP experiment was conducted with a few minor variations by Benzie and Strain (1999). 1.5 ml of FRAP reagent was combined with 100 μ l of extracts. Plant extract absorbance were measured at 593 nm against a blank after 10 minutes at 37°C, of incubation. Furthermore, ascorbic acid (standard) with concentrations ranging from 10 to 100 g/ml was developed. Values were reported as mg AAE (ascorbic acid equivalent)/g of dry weight based on the calibration curve of the standard ($Y= 0.022x$; $R^2=0.997$).

DPPH(2, 2 -Diphenyl-1-picryl-hydrazyl)method.

Using the approach of Xie *et al.* (2010) with only minor adjustments, the antioxidant activity of *S. nudicaulis* was evaluated based on its ability to scavenge the DPPH (2, 2 -Diphenyl-1-picryl-hydrazyl) free radical. 2.0 ml of various concentrations of plant extracts (10–100 g/ml) were combined with a freshly made DPPH solution (1 millimole) that was prepared in methanol. As a control, 2 ml of DPPH in 2 ml of methanol were used. The mixtures were incubated in the dark at room temperature for 30 minutes before being spectrophotometrically examined at 517 nm in comparison to a control. The standard used was ascorbic acid. The concentration at which free radicals are 50% inhibited is represented as the IC₅₀ value and was determined using Hatano *et al.* (1988).

The calculation for the plant extract's free radical scavenging ability (%) was as follows:

Percentage of inhibition = [(Absorbance of Control - Absorbance of test Sample) / (Absorbance of Control)] × 100.

ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) method. To ascertain the amount of free radical scavenging capacity of the plant sample, the ABTS radical cation decolorization assay (Re *et al.*, 1999) was utilised with certain modifications. 7 mM ABTS in water (1:1) and 2.45 mM potassium persulfate reacted to produce the ABTS+ cation radical, which was then kept at room temperature in the dark for 12 to 16 hours before use. To obtain an absorbance of 0.700 at 734 nm, the ABTS + solution was then diluted with methanol and DMSO separately. After combining 5 ml of extracts with 3.99 ml of diluted ABTS+ solutions, the absorbance was measured at 734 nm after 30 minutes. Ascorbic acid is used as a positive control. IC₅₀ value, or the concentration at which 50% of free radicals are inhibited, is calculated using (Hatano *et al.*, 1988).

The free radical (ABTS+) scavenging potential (%) of the plant extracts, formula was:

Percentage of inhibition = [(Absorbance of Control - Absorbance of test Sample) / (Absorbance of Control)] × 100.

Antibacterial assay

Bacterial strains. Five bacterial pathogen strains from the microbial type culture collection-IMTECH, Chandigarh, India, were used: *Listeria monocytogenes* MTCC657, *Proteus mirabilis* MTCC3310, *Klebsiella pneumoniae* MTCC7028, *Pseudomonas aeruginosa* MTCC3542 and *Agrobacterium tumefaciens* MTCC609. For further investigation, a single stock culture of chosen bacteria was inoculated in nutritional agar broth and activated (at 37 °C for 18 h) (Andrews, 2005).

Screening for antibacterial activity. Antibacterial tests for selected micro-organisms are done using the disc diffusion method (Bauer *et al.*, 1966). Each agar plate (diameter: 9 cm) was prepared, cooled to 20°C, inoculated with bacteria broth that had been sitting on the counter for 24 hours, and swabbed with sterile cotton swabs. Next, discs made of 6 mm sterilised filter paper are put on top of the agar medium. Plant extract at a concentration of 100 mg/ml was put onto the paper disc.

Minimum inhibitory concentration (MIC). After screening for positive results, more research was done to determine the minimum inhibitory concentration (MIC) of samples consisting of 20 μ l of plant extracts loaded with a micropipette at four distinct concentrations (100, 50, 25, and 12.5 mg/ml). On plates were two discs each of the conventional drug gentamycin and ampicillin (10 mcg), as well as a control. All plates were incubated in triplicate for 24 hours at 37°C. The inhibitory zones' diameter was measured, and the mean value was presented (Vineela and Elizabeth 2005).

RESULTS AND DISCUSSION

Qualitative phytochemical analysis. The presence of carbohydrate, flavonoid, resin, glycoside, terpenoid, alkaloid, phenol, quinone, tannin, and volatile oil was revealed by qualitative phytochemical analysis of methanol and hexane extracts of *S. nudicaulis* (Table 1). Both the utilized extracts contained flavonoids, resins, glycosides, terpenoids, and phenols. Out of the twelve classes of phytochemicals that were tested, methanol extract of *S. nudicaulis* (SN_M) had the most positive results (9 classes) followed by Hexane extract of *S. nudicaulis* (SN_H) (7 classes).

Phytochemical studies reveal that the presence of diverse phytoconstituents is accountable for fending of sickness and endorsing health. Like, alkaloids have antibacterial, antifungal, antihypertensive, antimalarial and anticancer activities (Molyneux *et al.*, 1996; Wink *et al.*, 1998). Similarly, terpenoids have anticarcinogenic, antimalarial, anti-ulcer, antimicrobial and diuretic properties (Langenheim *et al.*, 1994; Dudareva *et al.*, 2004). Along with this tannin-containing plant extracts are effective against diarrhoea, stomach, and duodenal tumours. Similarly, flavonoids are reported to possess antimicrobial, antitumor,

cytotoxicity anti-inflammatory, antitumor activities (Tapas *et al.*, 2008) as well.

GC-MS analysis. Both extracts in the current study displayed a variety of compounds (Table 2 and 3). Compounds that were found in significantly lower concentrations (less than 1%), however, were disregarded. The Compounds identified in methanol extract are either pharmacologically active or valuable for various industries. Methyl linolenate and linoleic methyl ester have antimalarial activity (Melariri *et al.*, 2012) and are inhibitor of melanin synthesis, and are therefore employed in hyper pigmentation disorders (Ko *et al.*, 2018). Moreover, cosmetics contain Hydroquinone as skin whitening ingredient (Siddique *et al.*, 2012). Due to its antimutagenic properties research demonstrated that bis (2-ethylhexyl) phthalate can prevent DNA damage and has the potential to prevent frame shift and base pair substitution mutations (Cruz-Ramírez, *et al.*, 2020). Similarly, E-phytol has been reported with antimycobacterial and antischistosomal properties (Bhattacharya and Rana 2013; de Moraes *et al.*, 2014). Another key component of methanol extract, methyl heptadecenoic acid, serves as a mosquito repellent (Beschi *et al.*, 2021). Whereas, phloretic acid, a phenolic compound, have an ability to reduce oxidative stress (Owen *et al.*, 2003).

Hexane extract, like methanol, revealed the presence of a number of biologically active substances, including phytol, which has antioxidant, antimicrobial and antinociceptive effects (Islam *et al.*, 2015); stigmaterol, which has anti-inflammatory, antitumor, antioxidant, and antimutagenic properties (Kaur *et al.*, 2011); tetratriacontane, heneicosane, and stearic acid, which have antimicrobial activity (Hashem *et al.*, 2016; Vanitha *et al.*, 2020; Kanetsuna, 1985); vitamin E with antioxidant, anticancer, and antimicrobial activity (Burton, 1994), neophytadiene has antipyretic, analgesic, antimicrobial, anti-oxidant, and anti-inflammatory properties (Palic *et al.*, 2002, Sargunam *et al.*, 2021); pentacosane also has anticancer effect (Walia *et al.*, 2012); squalene has antioxidant, antitumor and hypocholesterolemic activities (Spanova *et al.*, 2011); lanosterol can lesser cataract in lens tissues (Zhao *et al.*, 2015) and Spinasterol has allelopathic activities (Ripardo Filho *et al.*, 2012).

The current study supports earlier discoveries on other *Senecio* species, including phytol in *S. giganteus* (Kenoufi *et al.*, 2018), *S. jacobaea* (Kenoufi *et al.*, 2018), and *S. stebianus* (Tundis *et al.*, 2012); pentacosane and heneicosane in *S. giganteus* (Chibani 2013) and *S. glaucus* (Ramadan *et al.*, 2020); vitamin E, squalene, stigmaterol, and neophytadiene in *S. stebianus* (Tundis *et al.*, 2012). However, neither the current investigation nor earlier studies (Roeder *et al.*, 2000; Joshi *et al.*, 2021) have indicated the presence of the toxic pyrrolizidine in *S. nudicaulis*. It is interesting because the majority of *Senecio* species have been shown in previous reports to have confirmed the alkaloid.

Quantitative phytochemical analysis. Total phenolic and flavonoid content in different extracts was

calculated using the regression equations of gallic acid ($Y=0.003x$; $R^2 = 0.995$) and quercetin ($Y= 0.006x$; $R^2=0.983$). Test results showed the highest TPC in SN_M (78.26 ± 0.86 mg GAE/g dry weight), while SN_H revealed a comparatively low TPC value (0.13 ± 0.96 mg GAE/g dry weight). Similarly, the highest TFC value was recorded in SN_M (32.10 ± 0.24 mg QE /g dry weight), followed by SN_H (4.33 ± 0.47 mg QE /g dry weight).

Phenolic and flavonoid compounds are powerful antioxidant because they donate their extra electrons and can break the destructive chain of free radicals (Ghasemnezhad *et al.*, 2011). Their antioxidant efficiency depends on the constancy in different systems, as well as number and positions of hydroxyl groups. In various trials they even exhibited greater antioxidant activity than did vitamins and carotenoids (Re, *et al.*, 1999 and Vinson *et al.*, 1995).

Antioxidant activity

In the present instance, the highest ferric ion reduction (120.11 ± 2.21 mg AAE/g dry weight) was observed in methanolic extract SN_M while hexane extract SN_H showed comparably low potential (0.68 ± 0.04 mg AAE /g dry weight).

DPPH free radical scavenging activity was analysed using different concentrations (10-100µg/ml) of plant extracts. DPPH is a stable free radical that on accepting hydrogen from a corresponding donor, loses its characteristic deep purple colour and can be monitored spectrophotometrically. The highest free radical scavenging activity was recorded in methanolic extract (IC_{50} , $30.74 \pm 1.93\mu\text{g/ml}$) followed by hexane extract (IC_{50} , $196.79\pm 1.47\mu\text{g/ml}$). At different concentrations used (10-100µg/ml), the free radical inhibition range was maximum in methanolic extract (22.55% to 92.25%) and lowest (13.53% to 25.41±0.19 %) in hexane extract. Whereas, at similar concentrations used, the reducing capacity of standard (ascorbic acid) ranged from 91.1% to 98.17%, with an IC_{50} 5.4µg/ml (Table 7).

The highest ABTS free radical scavenging activity was recorded in methanolic extract (IC_{50} - 28.61 ± 0.25), whereas a low activity (IC_{50} - 59.66 ± 0.58) was observed for hexane extract. At different concentrations used (10-50 µg/ml), the free radical inhibition range was highest in SN_M (20.4% to 70.25%) and comparatively low in SN_H (9.57% to 41.9%) while at similar concentrations the standard (ascorbic acid) ranged from 43.9% to 97.16% inhibition with an 11.39 IC_{50} .

When compared to other species, such as *S. trapezuntinus*, *S. integrifolius* (Albayrak *et al.*, 2008), *S. stebianus* (Tundis *et al.*, 2012), *S. fluviatilis*, and *S. pseudo-orientalis*, the current study likewise revealed considerable antioxidant activity (Albayrak *et al.*, 2015). Nonetheless, the current discovery is consistent with findings from *S. pandurifolius*, *S. hypochionacus*, *S. lorentii*, *S. gibbosus*, and *S. chrysanthemoids* (Conforti *et al.*, 2006; Albayrak *et al.*, 2008; Shahwar *et al.*, 2012). Whereas (Kumar *et al.*, 2019) discovered that *S. nudicaulis* essential oil had a relatively low FRAP content, the current study indicates a substantial

antioxidant activity in FRAP experiment. Similar to this study, earlier research on *S. nudicaulis* essential oil and butanol extract showed efficient antioxidant activity with DPPH and ABTS assays (Sharma *et al.*, 2015), but the findings are not directly comparable due to the different methodologies employed.

Antibacterial activity. The results confirm that among all the five bacteria tested, *K. pneumonia* was found non-sensitive to methanol, and this similarly corroborates with previous findings of Ndom *et al.* (2007); Albayrak *et al.* (2015) in another *Senecio* species, including *S. mannii*, *S. fluviatilis*, *S. nemorensis*, *S. pseudo-orientalis*, and *S. racemosus*. The methanol extract showed the highest efficiency against *A. tumefaciens* (ZOI-13.66±0.33 mm), followed by *L. monocytogenes* and *P. aeruginosa*, against which moderate activity (ZOI-11±0.57mm in both cases) was observed. The least inhibition was observed against *P. mirabilis* (10±0.57).

All five bacteria were found to be susceptible to hexane extract. SN_H exhibited moderate efficiency, with the maximum zone of inhibition against *P. aeruginosa* (ZOI-12.5±0.28) followed by *A. tumefaciens* (ZOI-11.6±0.44), and lowest against *P. mirabilis* (ZOI-10.83±0.44). The same value of zone of inhibition, i.e., ZOI-11±0.57, was recorded for both *K. pneumoniae* and *L. monocytogenes*.

Out of nine tests, the lowest concentration of methanol and hexane (12.5 mg/ml) was recorded as MIC against *A. tumefaciens* and *P. aeruginosa*, respectively. A MIC value of 25mg/ml for methanol extract was found against *L. monocytogenes*, whereas for hexane extract, the same concentration was recorded as MIC against *K. pneumoniae* and *A. tumefaciens*.

Table 10 depicts the MIC value of 50mg/ml for methanol found against *P. aeruginosa* and for hexane extract against *L. monocytogenes* and *P. mirabilis*. In only one instance, i.e., SN_M against *P. mirabilis*, no dilution could establish an inhibitory effect.

Previously, (El-Amier *et al.*, 2014; Arancibia *et al.*, 2010) reported the *P. aeruginosa* to be resistant to the essential oils of *S. glaucus* and *S. mustersii*. The earlier

work by (Kumar *et al.*, 2017), which found that *S. nudicaulis* had moderate activity against all pathogens, including *P. aeruginosa* and *K. pneumonia*, also lends credence to the current findings. However, variations in the inhibitory zone can be linked to the following elements: the methods employed for analysis, the extraction procedures, plant locality, and the time of harvest. The results of the current investigation suggest that the presence of antimicrobial substances including phytol, heneicosane, stearic acid, tetratracontane, cholestenone, and vitamin E may be the cause of the efficient antibacterial activity of hexane extract.

Traditionally, uses of *S. nudicaulis* include treating ear issues, stomach problems (Singh *et al.*, 2017), cough and cold (Kaur *et al.*, 2017), skin conditions, wounds, and ocular conjunctivitis (Pala *et al.*, 2010). Moreover, *S. nudicaulis* is utilised as an antipyretic in conventional medicine (Singh *et al.*, 2017; Bisht *et al.*, 2010). Several studies have demonstrated that antioxidants control cyclooxygenase-2 (COX-2), which catalyses the creation of prostaglandins and promotes fever (Jiang *et al.*, 2000). Because of its antioxidant action, the traditional use of *S. nudicaulis* in fever can be attributed to its antioxidant activity. The current investigation confirms the efficacy of these traditional ethnomedical practises as sources of antibacterial and antioxidant formulae.

Table 1: Qualitative Phytochemical Analysis of *S. nudicaulis*.

Sr. No.	Phytochemicals	Methanol	Hexane
1.	Carbohydrate	+	-
2.	Protein	-	-
3.	Flavonoid	+	+
4.	Resin	+	+
5.	Glycoside	+	+
6.	Terpenoid	+	+
7.	Alkaloid	-	+
8.	Phenol	+	+
9.	Saponin	-	-
10.	Quinone	+	-
11.	Tannin	+	-
12.	Volatile oil	+	+

+ = Present; - = Absent;

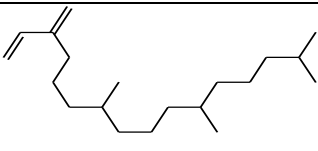
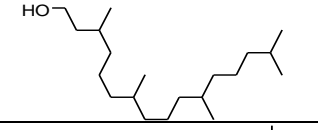
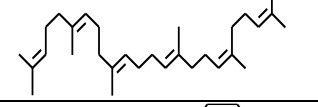
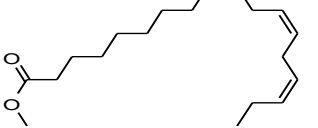
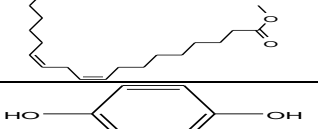
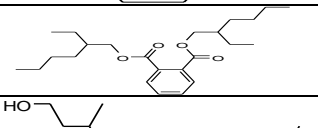
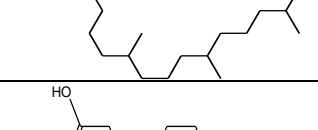
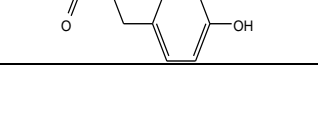

Table 2: Phytochemical compounds identified in the methanol extract of *S. nudicaulis* using GC-MS.

Peak	R. Time	Name of compounds	Molecular Formula	Molecular weight	Area % age	Nature of Compounds
1.	8.775	Hydroquinone	C ₆ H ₆ O ₂	110	4.40	Phenol
2.	10.504	6-Methyl-3,5-heptadiene-2-one	C ₈ H ₁₂ O	124	27.90	Ketones
3.	11.171	Phloretic acid	C ₉ H ₁₀ O ₃	166	3.80	Phenol
4.	11.534	Hexahydroindan	C ₉ H ₁₆	124	11.37	Hydrocarbon
5.	11.956	1,3-Diazabicyclo [3,2,2] nonane-4-thione	C ₇ H ₁₂ N ₂ S	156	1.66	Ketone
6.	12.327	Dopamine-4-0-sulphate	C ₁₄ H ₂₃ NO ₃	281	4.81	Aryl sulfate
7.	12.565	2,5-dihydroxy benzene acetic acid	C ₈ H ₈ O ₄	168	15.63	Phenolic acids
8.	12.993	Trans-4-(3-acetylamino propyl) cyclohexanol	C ₁₁ H ₂₁ NO ₂	199	4.06	Alcohol
9.	13.469	6,9-Dioxobicyclo [3,3,1] non-3-yl acetate	C ₁₁ H ₁₄ O ₄	210	3.81	Ketone
10.	14.660	Phytol acetate	C ₂₂ H ₄₂ O ₂	338	1.50	Diterpenealcohol
11.	15.589	Methyl heptadecanoate	C ₁₈ H ₃₆ O ₂	284	1.49	Fatty acid methyl ester
12.	17.224	Linoleic acid, methyl ester	C ₉ H ₃₄ O ₂	294	1.20	Fatty acid
13.	17.286	Methyl linolenate	C ₉ H ₃₂ O ₂	292	2.26	Fatty acid methyl ester
14.	17.391	E- Phytol	C ₂₀ H ₄₀ O	296	2.91	Diterpene alcohol
15.	20.980	Bis- (2-ethylhexyl) phthalate	C ₂₄ H ₃₈ O ₄	390	1.26	Phthalate ester

Table 3: Phytochemical compounds identified in the hexane extract of *S. nudicaulis* using GC-MS.

Peak	R. Time	Name of compounds	Molecular Formula	Molecular weight	Area%	Nature of Compounds
1.	15.339	Neophytadiene	C ₂₀ H ₃₈	278	1.35	Diterpene
2.	16.917	Stearic acid	C ₁₈ H ₃₆ O ₂	284	8.08	Fatty acids
3.	18.074	Phytol	C ₂₀ H ₄₀ O	296	8.21	diterpenoid
4.	18.602	Dihomo- γ -linolenic acid	C ₂₀ H ₃₄ O ₂	306	9.40	Fatty acids
5.	21.089	Hexanoic acid, 2-ethyl-, dodecyl ester	C ₂₀ H ₄₀ O ₂	312	1.95	Fatty acids
6.	23.701	Squalene	C ₃₀ H ₅₀	410	1.18	Triterpenoid
7.	24.690	β -Progesterone	C ₂₁ H ₃₀ O ₂	314	1.09	Steroid
8.	26.696	Pentacosane	C ₂₅ H ₅₂	352	3.03	Hydrocarbon
9.	26.860	Heptanoic anhydride	C ₁₄ H ₂₆ O ₃	242	1.98	Acid anhydride
10.	27.325	VitaminE	C ₂₉ H ₅₀ O ₂	430	1.68	fat-soluble vitamin
11.	28.191	Heneicosane	C ₂₁ H ₄₄	296	1.01	Hydrocarbon
12.	29.541	Stigmasterol	C ₂₉ H ₄₈ O	412	4.97	Phytosterol
13.	30.094	Tetratetracontane	C ₄₄ H ₉₀	618	3.10	Hydrocarbon
14.	30.408	23-(Phenylsulfanyl) lanosta-8,24-dien-3-ol	C ₃₆ H ₅₄ OS	534	1.99	Tetracyclic terpinol
15.	30.613	Otochilone	C ₃₀ H ₄₈ O	424	1.26	Steroidal ketones
16.	31.135	Lanosterol	C ₃₀ H ₅₀ O	426	2.09	Tetracyclic triterpenoid
17.	31.725	Cholestenone	C ₂₇ H ₄₄ O	384	1.17	Steroids
18.	31.991	9,19-Cyclo-27-norlanostan-25-one, 3-(acetyloxy)-24-methyl-, (3. β .,24R)	C ₃₂ H ₅₂ O ₃	484	5.77	Triterpenoid Sterol
19.	32.315	Spinasterone	C ₂₉ H ₄₆ O	410	1.99	Steroids
20.	32.511	Cycloartenol 3-acetate	C ₃₂ H ₅₂ O ₂	468	4.14	Triterpenoid
21.	33.416	Stigmasta-4,22-dien-3-one	C ₂₉ H ₄₆ O	410	3.85	Steroid
22.	33.853	Androstane-3,11,17-triol	C ₁₉ H ₃₂ O ₃	308	3.81	Steroidal terpenoid
23.	34.264	Fucosterone	C ₂₉ H ₄₆ O	410	1.62	Triterpenoid Sterol
24.	35.252	Tetratetracontane	C ₄₄ H ₉₀	618	2.60	Hydrocarbon
25.	36.974	Phytyl palmitate	C ₃₆ H ₇₀ O ₂	534	9.98	fatty acid

Table 4: Biological active phytochemicals of *S. nudicaulis* in methanol and hexane extract

Compounds	Biological importance	References	Structure
Neophytadiene	antipyretic, analgesic, antimicrobial, anti-oxidant, anti-inflammatory properties	(Palic, 2002), (Sargunam, 2021)	
Phytol	Antimicrobial, anticancer, anti-inflammatory properties	(Islam, 2015)	
Squalene	Anti-cancerous, antioxidant and hypocholesterolemic activity	(Spanova, 2011)	
Methyl linolenate	Antimalarial activity inhibitor of melanin synthesis	(Melariri <i>et al.</i> , 2012); (Ko, 2018)	
Linoleic methyl ester	Antimalarial activity inhibitor of melanin synthesis	(Melariri <i>et al.</i> , 2012); (Ko, 2018)	
Hydroquinone	Skin whitening agent	(Siddique, 2012)	
Bis- (2-ethylhexyl) phthalate	Antimutagenic activity	(Cruz-Ramirez, 2021)	
E- phytol	Antimycobacterial Anti-schistosomal	(Bhattacharya 2013, de Moraes, 2014)	
Phloretic acid	Antioxidant activity	(Owena <i>et al.</i> , 2003)	

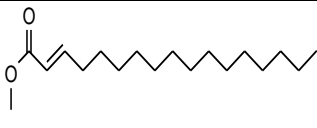


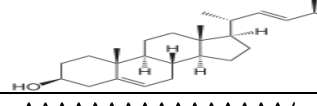
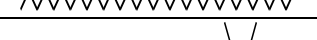
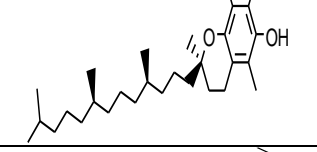
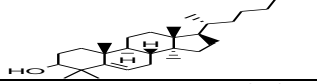
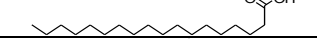
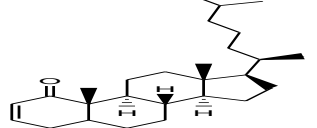

Methyl Heptadecenoic acid	Mosquito repellent	(Beschi <i>et al.</i> , 2021)	
Pentacosane	Anticancer	(Walia <i>et al.</i> , 2012)	
Henicosane	Antimicrobial	(Vanitha <i>et al.</i> , 2020)	
Stigmasterol	Antioxidant, Antitumor	(Kaur <i>et al.</i> , 2011)	
Tetratriacontane	Antimicrobial	(Hashem <i>et al.</i> , 2016)	
Vitamin E	Antioxidant, Anticancer, Antimicrobial	(Burton, 1994)	
Lanosterol	Used as Cataract reductant	(Zhao <i>et al.</i> , 2015)	
Stearic acid(n-octadecanoic acid)	Antimicrobial	(Kanetsuna, 1985)	
Cholestenone	Antimicrobial	(Kobayashia, 2021)	
Dihomo-γ-linolenic acid	Anti-inflammatory	(Yazawa <i>et al.</i> , 2007)	

Table 5: Total Phenolic (TPC) and Total Flavonoid content (TFC) (mg /g dry weight) in *S. nudicaulis* leaf extract.

Chemical class	Methanol	Hexane
TPC (mg GAE/gm dry weight)	78.26 ±0.86	0.13±0.96
TFC (mg QE / gm dry weight)	32.10 ±0.24	4.33±0.47

Table 6: Ferric reducing antioxidant potential of *S. nudicaulis* with respect to Ascorbic acid.

Extracts	FRAP (mg AAE/gm dry weight)
Methanol	120.11 ±2.21
Hexane	0.68±0.04

Table 7: Percentage inhibition of DPPH radical and IC₅₀ value of two extracts of *S. nudicaulis* and standard.

Concentration	Ascorbic acid	IC ₅₀	Methanol	IC ₅₀	Hexane	IC ₅₀
10	91.14±0.97	5.48±0.5	22.55±1.82	30.74 ±1.93	13.53±1.00	196.79±1.4
30	92.44±0.19		49.15±2.93		17.16±0.19	
50	93.88±0.58		76.3±4.76		19.0±0.6	
70	94.92±0.30		84.79±0.17		22.44±0.5	
90	97.26±0.30		91.44±0.11		24.09±0.19	
100	98.17±0.44		92.25±0.23		25.41±0.19	

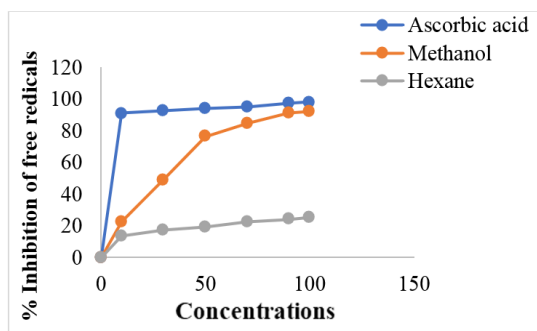


Fig. 1. Percentage of inhibition of DPPH by standard and plant extracts.

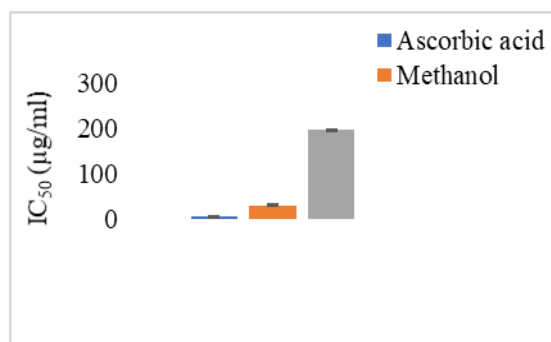


Fig. 2. IC₅₀ value for DPPH by standard and plant extracts.

Table 8: Percentage of inhibition of ABTS radical and IC₅₀ value of both extracts of plant sample and standard.

Concentration	Ascorbic acid	IC ₅₀	Methanol	IC ₅₀	Hexane	IC ₅₀
10	43.9±0.93	11.39±0.24	20.4±0.53	28.61±0.25	9.57±1.01	59.66±0.58
20	67.19±1.06		32.67±0.31		16.09±0.66	
30	80.98±0.33		52.42±0.47		29.88±0.60	
40	87.12±0.47		61.75±0.51		35.01±0.75	
50	97.16±0.33		70.25±0.26		41.90±0.40	

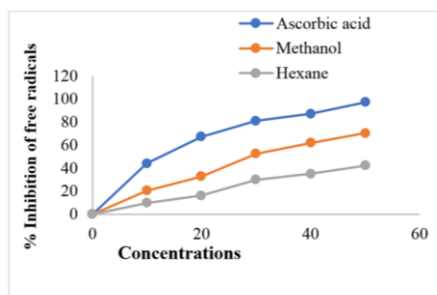


Fig. 3. Percentage of inhibition of ABTS free radicals by standard and plant extracts.

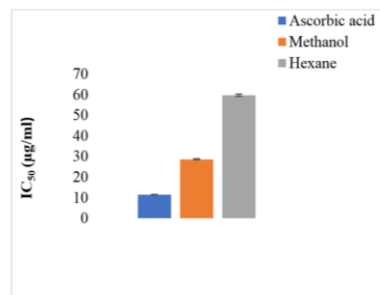


Fig. 4. IC₅₀ value for ABTS scavenging activity of standard and plant extract.

Table 9: Antibacterial screening of *S. nudicaulis* and standards.

Bacteria	ZOI (mm) at 100 mg/ml		Standard 10 mcg	
	SN _M	SN _H	G	A
LM	11±0.57	11±0.57	26±2.08	14±0.57
PM	10±0.57	10.83±0.44	31±2.08	16.6±2.3
KP	NA	11±0.57	30±1.1	15.33±2.6
PA	11±0.57	12.5±0.28	29±2.08	20.3±0.88
AT	13.66±0.33	11.6±0.44	27.5±2.04	19±0.81

Values are mean ± SE of three independent observations; NA- Not Active; G- Gentamycin; A- Ampicillin; LM- *Listeria monocytogenes*; PM- *Proteus mirabilis*; KP-*Klebsiella pneumoniae*; PA *Pseudomonas aeruginosa*; AT- *Agrobacterium tumefaciens*; SN_M-*S. nudicaulis* methanol extract; SN_H- *S. nudicaulis* hexane extract, ZOI- Zone of inhibition

Table 10: Minimum inhibitory concentration (MIC) of *S. nudicaulis*.

Sr. No.	Bacteria	SN _M	SN _H
		MIC (mg/ml)	MIC (mg/ml)
1.	LM	25	50
2.	PM	100	50
3.	KP	-	25
4.	PA	50	12.5
5.	AT	12.5	25

LM- *Listeria monocytogenes*; PM-*Proteus mirabilis*; KP-*Klebsiella pneumoniae*; PA *Pseudomonas aeruginosa*; AT- *Agrobacterium tumefaciens*; SN_M-*S. nudicaulis* methanol extract; SN_H- *S. nudicaulis* hexane extract; - not tested

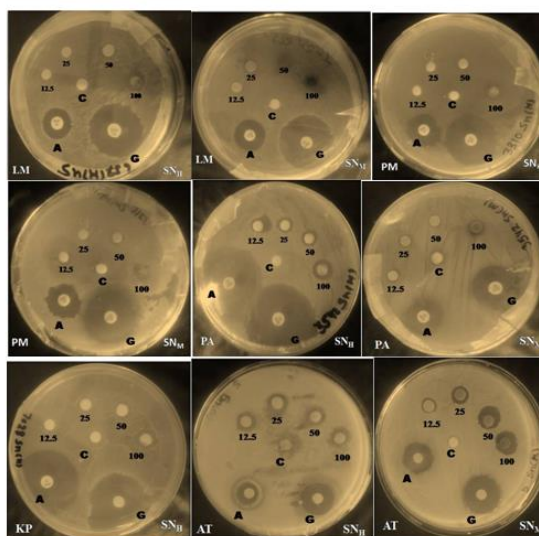


Fig. 5. Photographs of antibacterial activity of *Senecio nudicaulis* against susceptible bacteria: Representing ZOI (mm) at MIC (mg/ml), SN_H-*Senecio nudicaulis* hexane extracts, SN_M-*Senecio nudicaulis* methanol extract, G – Gentamycin, A- Ampicillin, C- negative control (solvent), LM- *Listeria monocytogenes*, PM- *Proteus mirabilis*, PA- *Pseudomonas aeruginosa*, KP- *Klebsiella pneumoniae*, AT- *Agrobacterium tumefaciens*.

CONCLUSIONS

The current paper is the first to detail the chemical makeup and antioxidant and antibacterial effects of *S. nudicaulis* methanol and hexane extracts using in-vitro experiments. The present study reports the presence of many medicinally valuable and industrially relevant compounds that have previously been linked to biologically active functions. Tested plant extracts showed antioxidant activity in the DPPH, FRAP, and ABTS assays. Furthermore, bacterial strains were also found to be susceptible to plant extracts.

FUTURE SCOPE

These investigations offer a great basis for the purification of particular important components as well as the conduct of rigorous clinical trials and pharmacological screening, which are all necessary to determine their efficacy and explore their therapeutic uses. Further research is needed to determine the extract's toxicity in order to maximise the health advantages for humans. This work reveals that *S. nudicaulis* extracts in methanol and hexane are strong reservoirs of active principles that can be further investigated to create novel therapies.

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Conflict of Interest. None.

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