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Pharmacognostic, phytochemical analysis and *in-vitro* antioxidant activity of *Senecio edgeworthii* Hook plant

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The objective of the present study was to investigate pharmacognostic, phytochemical and evaluate *in vitro* antioxidant properties of *Senecio edgeworthii* hook Plant. The study of leaf macroscopic features, microscopic features was undertaken and physicochemical parameters were evaluated using standard procedures as per WHO guidelines. *In vitro* antioxidant activity of *S. edgeworthii* plant was determined by application of standard methods. In microscopy of *S. edgeworthii*, leaf is having Upper epidermis, cuticle, multicellular covering trichomes. The transverse view of the lamina shows a single layer of closely packed palisade cells below the upper epidermis. In powder characteristics analysis, lignified xylem parenchyma, lignified fibres, stomata, epidermis, cork cell, xylem vessels, spiral vessels and trichomes were observed. A low amount of total ash acid insoluble ash and water-soluble ash indicate that the inorganic matter and non-physiological matter such as silica is less *S. edgeworthii* plant. The estimation of total phenolic content in the examined extracts showed SE-Chloro and SE-alcohol extract contains high phenolic content than SE-Oil and SE-Pet ether. The results of antioxidant evaluation based on the three models (DPPH, H_2O_2, and NO) used in this study revealed that chloroform and alcohol extract and flower oil of *S. edgeworthii* plant possess interesting antioxidant activity. The pharmacognostic, phytochemical analysis can contribute to the development of the quality control norms for this species. This plant may possess considerable antioxidant activities.

Keywords: Antioxidant activities, Pharmacognostic study, Phytochemical analysis, Quality control, *Senecio edgeworthii*

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Introduction

Scientific investigation in the ground of pharmacognosy had been preceded on numerous traces masking morpho-anatomical characterization of plant elements used as a crude drug, their physicochemical parameters, phytochemical screening and biological assay and on many other various procedures. It's far very important to make definite the quality and purity of herbal drugs to be able to maximize their efficacy and decrease the unfavourable side outcomes. WHO also emphasizes the want to ensure high-quality management of medicinal plant products utilizing the use of modern techniques and suitable requirements[1]. Also, improper authentication of herbal pills, their adulteration, infection with microorganisms, pesticides and heavy metals, has made standardization of plants a simple necessity. So, instruction of the pharmacognostic standards for the correct identity of the crude plants and detection of adulteration is handled as a critical step towards herbal product research[2]. The biological examination of medicinal plants is important not only for gaining novel natural products from the medicinal plants but also for validation of the ethnomedicinal claims of those therapeutically powerful plants even as curing exclusive health conditions[3].

A huge variety of biological interest studies like anticancer, antioxidant, and so forth, are being executed to identify the active compound or compounds from medicinal plants and to standardize the effectiveness from those bioactive Phyto-molecules[4]. It's been mounted that oxidative strain is some of the essential causative elements inside the induction of many continual and degenerative sicknesses such as atherosclerosis, ischemic heart disease, getting older, diabetes mellitus, cancer, immune suppression, neurodegenerative diseases and others[4]. The most realistic way to combat degenerative diseases is to increase antioxidant

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consumption in our body and that could be finished via consumption of vegetables, fruits, cereals and different meals with good content of antioxidant substances. There is an enormous look for exogenous antioxidants from natural sources possibly because they are much less high-priced, effortlessly available and believed to have lesser side consequences while in comparison to their synthetic opposite numbers. Several phytochemical groups from plant assets are recognized for their antioxidant ability and among them, the phenolics, flavonoids, tannins, etc. were installed as robust antioxidants displaying an excellent capability to inhibit the free radicals[5,6].

Senecio species is used to make medicine. Be careful not to confuse golden ragwort (*Senecio aureus*) with other species of ragwort, such as alpine ragwort and tansy ragwort. Despite serious safety concerns, people take golden ragwort to treat diabetes, high blood pressure, water retention, bleeding, chest congestion and spasms. *Senecio* is the largest genus in the family Asteraceae and includes over 1500 species widespread all over the world. Recent studies indicated that several species of *Senecio* exhibit antimicrobial, antifungal and cytotoxic activities[7-9]. *S. edgeworthii* hook vernacular name Sonaki of the family Asteraceae is an erect herbaceous, much-branched distributed in the Western Ghats of India. While this information validates its folk use, to date, study of its pharmacognostical character, phytochemical composition, antioxidant activity is lacking. The purpose of this work was to estimate the pharmacognostic, phytochemical, and antioxidant studies of the *S. edgeworthii* plant.

Materials and Methods

Collection of plant material

The plant *S. edgeworthii* was collected in November 2020 from the area of Kas pathar, Satara Maharashtra, India.

The specimen was authenticated by Dr Swapnaja M Deshpande**,** designation, Department of Botany, Institute name by comparing it with the voucher specimen (SENECIOE1) deposited earlier in the Department. A voucher specimen of the sample has also been deposited in the Department for future reference (VS no. 13688).

Pharmacognostic standardization

It includes the study of leaf macroscopic characteristics, microscopic features, and physicochemical parameters. Macroscopic features consist of the study of colour, odour, size, shape, taste, and special aspects including touch and texture etc of drugs with help of sensory organs. Microscopic aspects tell about tissue arrangement in the transverse section of leaf and type of stomata, trichomes, vascular bundle and different cells. Along with this, cell substance and crystalline structures were also studied. With the help of a photomicroscope, various leaf constants were verified. In physicochemical factors, different ash values, extractive values, loss on drying, foreign organic matter, swelling index and foaming index were studied. According to the WHO standards, these were all completed using standard operating procedures[10-12].

Extraction methodology

The plant material was dried in shade and powdered in the grinder. About 500 g of powdered plant material was extracted sequentially in the Soxhlet apparatus by using solvents in order of increasing polarity i.e., petroleum ether, chloroform, and ethanol. After every extraction, solvent was recovered using a rotary vacuum evaporator and dried extracts were stored in vacuum desiccators. These extracts were used in further phytochemical and antioxidant study[13,14].

Extraction of oil from the flower

Freshly collected flower of the plant *S. edgeworthii* were dried under room temperature for three weeks. The dried flower material was made into a coarse powder and a weighed quantity of the powder was extracted in a Soxhlet extractor, to which n-hexane was added for obtaining the total oil. During the extraction, the extracted lipids required the addition of 75% sodium chloride solution. The solvent was removed on a rotary evaporator at 50°C. Total oil was collected in a flask and stored at 4°C for further analysis[15,16].

Phytochemical screening

To determine various phytoconstituents, standard chemical tests were applied on plant extracts and flower oil. Observations were recorded to confirm the presence or absence of these constituents[17,18].

Determination of total phenolic content

Total phenol content (TPC) in the extracts was determined. Exactly 100 mg of extract was suspended in to 4.5 mL of distilled water with 0.5 mL of tween 80. About 0.5 mL (500 µL) of extracts (SE-chloro, SE-alcohol, SE-pet ether, SE-Oil), and Gallic acid

(Standard) solutions of strength (100-600 µg/mL) were pipette out. It was then mixed with 5 mL Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 5 mL (7.5 g/L) of sodium carbonate. The tubes were shaken vigorously for 15 seconds and at 40°C were kept to stand for 30 minutes for colour development. As per the standard method reported in earlier studies, absorbance was read at 765 nm using a spectrophotometer by putting 4 mL of the prepared mixture into a cuvette[19].

The percentage of TPC was calculated from the calibration curve of gallic acid plotted and TPC was expressed as mg gallic acid equivalent per g extract (mg GAE/g extract)[20].

DPPH radical scavenging activity

DPPH radical scavenging assay was performed using 1, 1 diphenyl-2-picrylhydrazyl (DPPH). Ascorbic acid and *S. edgeworthii* extracts (SE-Pet ether, SE-Chloro, SE-Alcohol, SE-Oil) of plants prepared solutions are of strength (50-500 µg/mL). Then 3 mL from each extract was mixed with 1 mL of freshly prepared 0.1 mM/L DPPH solution. The mixture was shaken vigorously and incubated at room temperature for 30 minutes in dark. The reduction of the DPPH free radical was calculated by reading the absorbance at 517 nm by a spectrophotometer[21].

Inhibition of DPPH free radical in percentage was calculated by the formula:

$$\text{DPPH radical scavenging activity (\%)} = [(A_{control} - A_{sample})]/(A_{control})] \times 100$$

where, $A_{Control}$ is the absorbance of DPPH radical + methanol and A_{sample} is the absorbance of DPPH radical + sample extract /standard[22].

Hydrogen peroxide scavenging activity

Various concentrations (20-100 µg/mL) of the ascorbic acid and *S. edgeworthii hook* extracts (SE-Pet ether SE-Chloro, SE-Alcohol, SE-Oil) were prepared in distilled water. Exactly 1 mL of each solution of different concentrations of extracts and standard was mixed with 2 mL of 0.1 M phosphate buffer solution and 600 µL of 100 mM H_2O_2 solution. After about 10 minutes, absorbance was measured at 230 nm[23,24].

The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples using the formula:

$$I\% = Ac\ (At.\ Ab)\ Ac \times 100$$

where, I%= Percentage inhibition, Ac= Absorbance of control (0.1 M phosphate buffer solution and H_2O_2), At= Absorbance of ascorbic acid/plant extract with H_2O_2 after 10 min, and Ab= Absorbance of ascorbic acid/plant extract without H_2O_2[25].

IC_{50} values were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm[26].

Nitric oxide scavenging activity

Ascorbic acid and *S. edgeworthii* extracts (SE-Pet ether SE-Chloro, SE-Alcohol, SE-Oil) solutions are of strength (20-100 µg/mL) prepared. Then 2.5 mL sodium nitroprusside (10 mmol/L) in phosphate buffer (pH 7.4) was mixed with different concentrations (20-100 µg/mL) of 0.5 mL ascorbic acid and extract and incubated at 25°C for 150 minutes. Add 0.5 mL of Griess reagent. After 30 minutes, the absorbance of standard solutions of ascorbic acid was measured at 546 nm by a spectrophotometer[27].

The percentage of inhibition was measured by the following formula:

$$\text{Radical scavenging activity (\%)} = (A_{control} - A_{test})/A_{control} \times 100$$

where, $A_{Control}$ is the absorbance of the control (without extract), and A_{test} is an absorbance in the presence of the extract/standard[28].

Results

Pharmacognostic standardization

Macroscopic parameters

Freshly collected the plant *S. edgeworthii* (Fig. 1) were dried at room temperature for three weeks. The morphological characteristic of the *S. edgeworthii* leaves shown in Fig. *2* it shows that the leaves have green colour on the front side and back side light whitish green, the petiole is 0.5 cm in length, the midrib is prominent, the venation of the leaves is not prominent, the leaves are 2-3 cm in length and 1 –1.5 cm in wide, it has a smooth surface with an elliptical shape and acute apex, the margin of the leaves is serrate, the base is petiolate, the leaves have characteristics odour and taste slightly bitter. Flowers are yellow in colour (Fig. 3) and have a characteristics odour.

Microscopic parameters

The microscopy study was performed on leaf, stem, root transverse section and plant powder

Fig. 1 — *Senecio edgeworthii* hook plant.

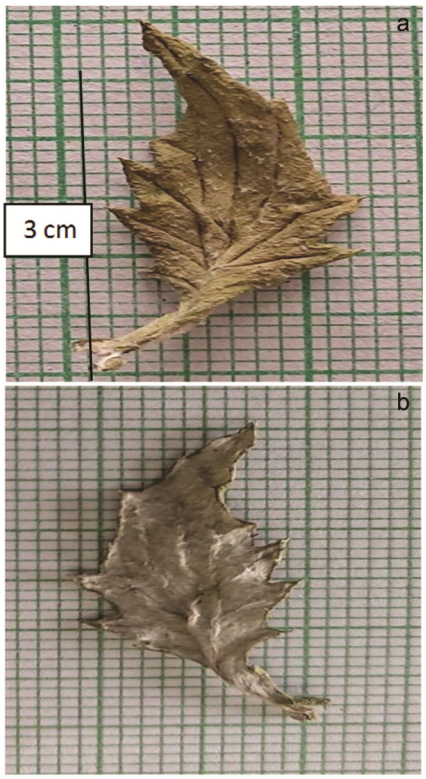

Fig. 2 — a) The front surface of leaf; and b) The back surface of leaf.

characteristics of *S. edgeworthii* plant. A transverse section of a fresh leaf is shown in Fig. 4a. Dorsi-ventral in nature. The upper epidermis is covered by a thin cuticle. Multicellular covering trichomes are ardently present on upper epidermis. The transverse

Fig. 3 — Flower of *Senecio edgeworthii*.

view of the lamina shows a single layer of closely packed palisade cells below the upper epidermis. Midrib show 5-7 layered thick wall having closely packed collenchyma on both surfaces. Spongy parenchyma, lignified vascular bundles are seen.

The transverse section of the *S. edgeworthii* plant stem is shown in Fig. 4b. Epidermis, the outermost layer of the stem is made up of compactly arranged cells. The cortex is composed of many layers of thin-walled parenchyma, with intercellular spaces. Phloem is present toward the epidermis. Xylem is radical. The transverse section of the root shown in Fig. 4c shows abundant lignified xylem vessels, cortex, and phloem.

Powder microscopy

Powder microscopy of the whole plant identified different components like lignified xylem parenchyma, lignified fibres, stomata, epidermis, cork cell, xylem vessels, spiral vessels, and trichomes (Fig. 5).

Leaf constants

Stomata are diacyctic nature. Approximately 29 to 35 stomata were found in 0.4 mm square (Fig. 6).

Physicochemical parameters

Total ash, acid insoluble ash, water-soluble ash, loss on drying, swelling index, foaming index, extractive value were performed as per standard operating procedures and the values observed for all these Physico-chemical parameters are given in Table 1.

Phytochemical screening

The observations of various chemical tests are shown in Table 2. *S. edgeworthii* alcohol extract showed the presence of flavonoid and glycosides.

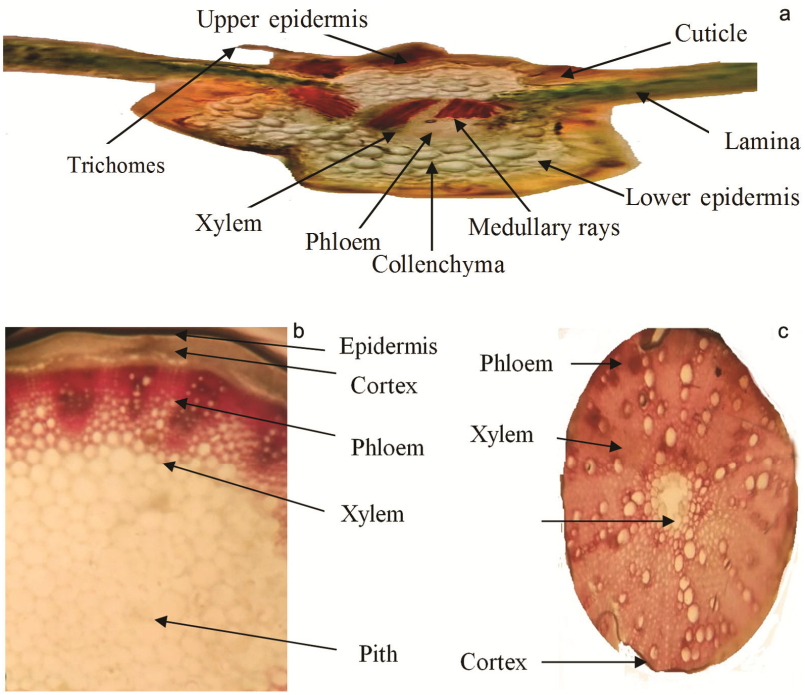

Fig. 4 — a) T. S. of leaf; b) T. S. of stem; c) T. S. of root of *Senecio edgeworthii*.

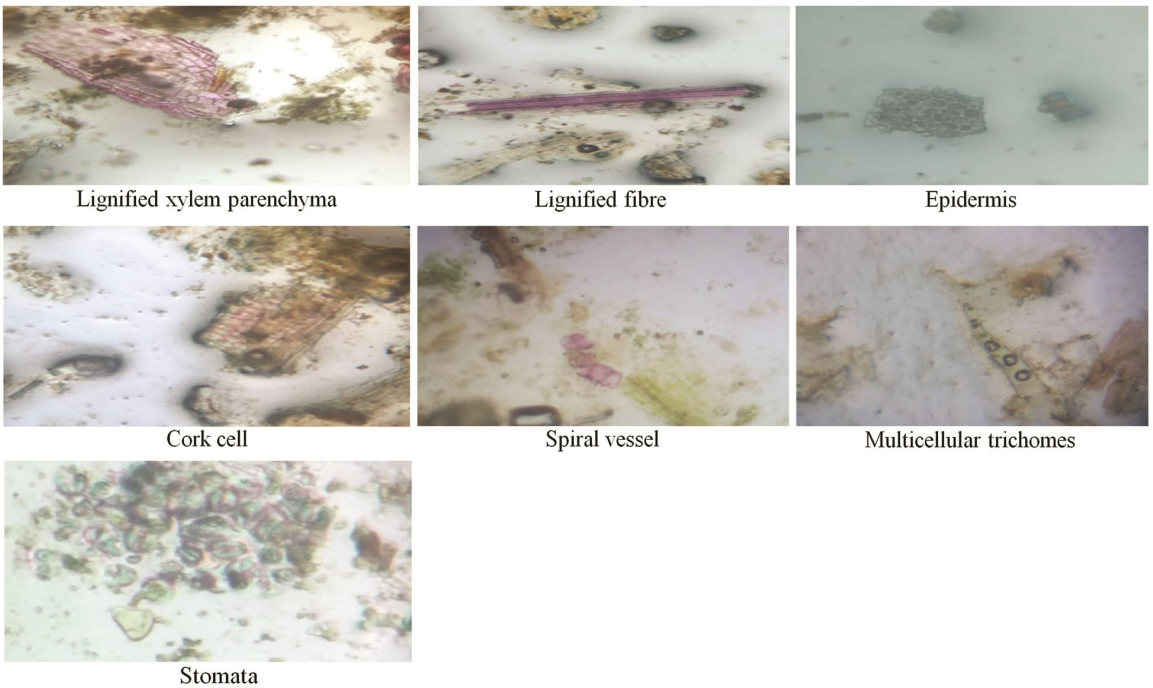

Fig. 5 — Powder microscopy of the leaves observed under microscope. All the components are observed using ×10 and ×45.

S. edgeworthii chloroform extract showed the presence of various phytochemical constituents like tannins, flavonoid and alkaloid. *S. edgeworthii* in petroleum, extract showed the presence of saponin. *S. edgeworthii* flower oil is showed the existence of triterpenes.

Total phenols content

The TPC of various extracts of *S. edgeworthii* plant was calculated as shown in Table 3 and the standard curve as Fig. 7. The TPC SE-pet ether, SE-chloro, SE-alcohol, SE oil extracts of *S. edgeworthii* respectively, 287.4, 364, 487.6, 364 and 255.8 mg/mL.

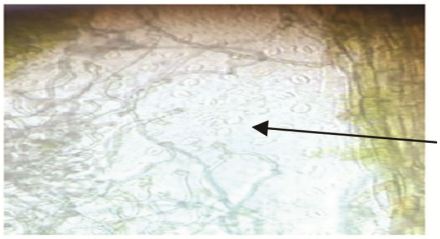

Fig. 6 — Stomata.

Table 1 — Observation of various physicochemical parameters for of *Senecio edgeworthii* plant

Parameter	% Ash value
Total ash	12% w/w
Acid insoluble ash	2.5% w/w
Water-soluble ash	4.5% w/w
Loss on drying	11.5% w/w
Swelling index	5 cm
Foaming index	Less than 100
Extractive values	
Ethanol soluble extractive value	0.5% w/w
Water soluble extractive value	2% w/w

Table 2 — Preliminary phytochemical screening of various extracts of *Senecio edgeworthii*

Chemical constitutes	Pet. Ether extract	Chloroform extract	Ethanol extract	Flower oil
Alkaloids	-	++	-	-
Glycosides	-	-	+	-
Flavonoids	-	++	+	-
Amino acid and Protein	-	-	-	-
Carbohydrate	-	-	-	-
Starch	-	-	-	-
Tannins	-	++	-	-
Saponins	++	-	-	-
Terpenoids	-	-	-	++

Table 3 — Total phenolic content of *Senecio edgeworthii* plants extracts

Plant extracts	Total phenolic content (GA equivalent)
SE-Pet. ether	287.4 mg
SE-Chloro	364.2 mg
SE-Alcohol	487.6 mg
SE – Oil	255.8 mg

Antioxidant activity

DPPH scavenging effect

The antioxidant effect of various extracts of *S. edgeworthii* Plant by DPPH % scavenging is shown in Table 4 and Fig. 7a. IC_{50} value of the *S. edgeworthii* plant extracts, SE-Oil, SE-alcohol, and SE-chloro was 531.597, 351.714, and 291.611 µg/mL respectively.

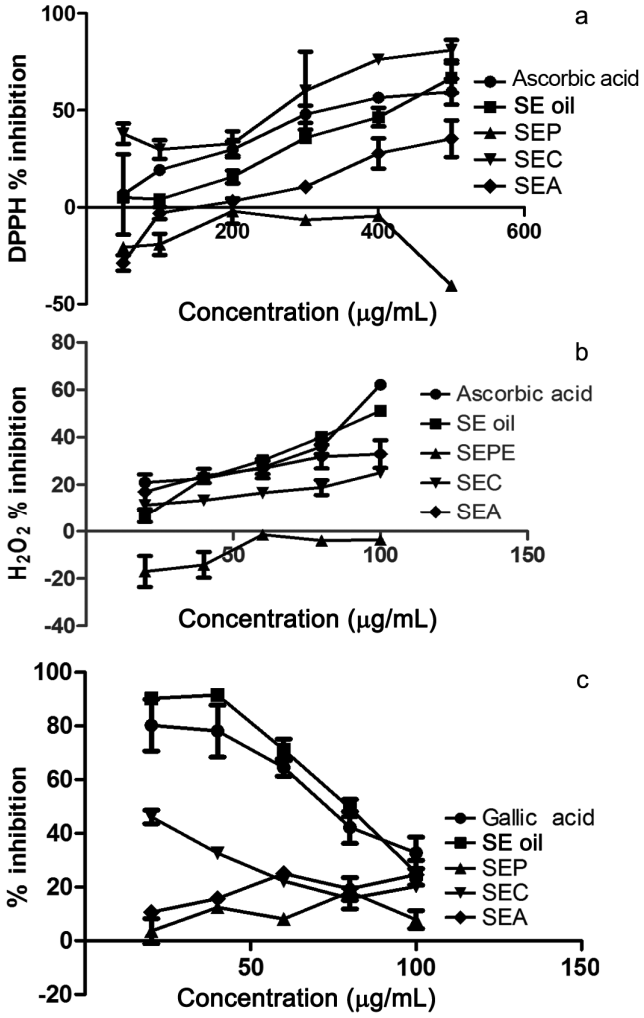

Fig. 7 — a) DPPH; b) H_2O_2; and c) Nitric Oxide % scavenging effect of various extracts of *Senecio edgeworthii*.

H_2O_2 scavenging effect

The antioxidant effect of various extracts of *S. edgeworthii* by H_2O_2 % scavenging is shown in Table 5 and Fig 7b. The IC_{50} values were calculated from the graph (ascorbic acid-193.011 µg/mL, SE-chloro 313.434 µg/mL, SE-alcohol 63.600 µg/mL, and SE- oil 313.434 µg/mL).

Nitric oxide scavenging effect

The antioxidant effect of various extracts of *S. edgeworthii* by nitric oxide % scavenging is shown in Table 6 and Fig. 7c. The IC_{50} values were calculated from graph (ascorbic acid 68.333 µg/mL, SE-chloro 40.454 µg/mL, SE-alcohol 40.625 µg/mL, SE- oil 83.736 µg/mL and SE-pet ether 21.732 µg/mL).

Table 4 — IC_{50} values and % inhibition of *Senecio edgeworthii* extracts and standard ascorbic acid obtained for DPPH radical scavenging activity

Conc. µg/mL	Ascorbic acid % Inhibition	SE- oil % Inhibition	SE-pet ether % Inhibition	SE-chloro % Inhibition	SE-alcohol % Inhibition
50	6.56±2.74	5.050±2.201	- 20.707±2.525	37.878±5.248	2.787±4.008
100	19.19±2.67	4.040±1.010	- 19.191±5.555	29.797±4.817	3.030±3.154
200	29.79±4.13	15.656±3.311	- 2.0202±6.565	32.828±6.328	3.030±1.749
300	47.97±4.48	35.858±2.201	- 6.565±0.505	60.151±20.124	10.606±2.314
400	56.56±2.02	46.464±4.817	- 4.545±1.515	76.262±1.010	27.777±7.840
500	59.59±6.6	66.66±7.626	- 40.404±2.525	81.060±5.303	35.353±9.435
IC_{50}	274.985 µg/mL	531.597 µg/mL	0	291.611 µg/mL	351.714 µg/mL

Table 5 — IC_{50} values and % inhibition of *Senecio edgeworthii* plant extracts and standard ascorbic acid obtained for Hydrogen peroxide (H_2O_2) scavenging activity

Conc. µg/mL	Ascorbic acid % Inhibition	SE- oil % Inhibition	SE-pet ether % Inhibition	SE-chloro % Inhibition	SE-alcohol % Inhibition
20	20.947±0.383	6.466±2.550	-17.006±6.573	11.337±0.190	16.958±7.372
40	22.627±0.965	22.603±1.131	-14.243±5.454	13.373±1.141	23.734 ±2.971
60	27.307±2.788	30.272±0.095	-1.405±1.155	16.529±2.125	26.819± 4.083
80	36.072±3.092	40.026±1.055	-3.977±1.024	18.816±3.165	31.820±4.982
100	62.176±0.626	51.184±1.478	-3.608±0.714	25.044±1.296	32.916±5.859
IC_{50}	193.01 µg/mL	313.434 µg/mL	0	244.966 µg/mL	63.6 µg/mL

Table 6 — IC_{50} values and % inhibition of *Senecio edgeworthii* plant extracts and standard gallic acid obtained for Nitric Oxide scavenging assay

Conc. µg/mL	Gallic acid % Inhibition	SE- oil % Inhibition	SE-pet ether % Inhibition	SE-chloro % Inhibition	SE-alcohol % Inhibition
20	80.279±9.62	90.241±1.60	3.606±4.68	46.134±2.475	10.682±0.35
40	78.115±9.62	91.593±1.98	12.463±2.01	32.657±2.010	15.731±0.89
60	64.503±3.22	71.151±4.00	8.136±2.17	22.267±0.305	25.039±1.83
80	42.235±5.95	49.447±3.20	18.210±0.76	15.798±3.899	19.337±4.25
100	32.747±5.86	25.354±4.60	7.865±3.35	20.283±2.216	24.65±0.17
IC_{50}	68.333 µg/mL	83.736 µg/mL	21.732 µg/mL	40.454 µg/mL	40.625 µg/mL

Discussion

S. edgeworthii hook is a plant of the Asteraceae family but no work is available on this plant. From the study, significant diagnostic characters that might be helpful in formative authenticity and identifying adulteration of the crude drug are observed. It was preferred to carry out macroscopy, microscopy, phytochemical investigation and anti-oxidant activity study of this plant. All these factors were studied according to standard procedures available. These are found in the in abundant long Multicellular covering trichomes, closely packed palisade cells, spongy parenchyma, lignified vascular bundles and epidermal cells with lightly grossed walls[29].

Preliminary phytochemical analysis of alcohol, chloroform, petroleum ether, oil extracts of *S. edgeworthii* are alcohol extract showed the existence of flavonoid and glycosides. Chloroform extract showed the presence of various phytochemical constituents like tannins, flavonoid and alkaloid. Petroleum ether extract showed the presence of saponin. *S. edgeworthii* flower oil showed the existence of triterpenes[29].

In this study, the sources, composition, and antioxidant action of *S. edgeworthii* plant extracts were exploited as natural products and food, which characterizes a new frontier for therapy healthcare systems. In current years, there has been great interest in the health effects of various natural products and the *in-vivo* protective function of natural antioxidants contained in dietary food against oxidative damage caused by ROS. *In vitro* antioxidant studies are broadly carried to screen various plants containing phenolic and flavonoids constituents. They have received considerable thought because of their physiological effects like antioxidant,

anti-inflammatory, antitumor activities, and neuroprotective activity[30].

Spectrophotometric analytical methods applied for assessment of the TPC and determination of phenolic acids in the examined extracts in the present study showed that these constituents are present in a valuable amount in the *S. edgeworthii*.

The SE-Chloro and SE-Alcohol extract contains high phenolic content than SE-Oil and SE-Pet ether. This can absorb and deactivate free radicals, decompose peroxide and relative oxygen species, and help to check cell damage caused by oxidative stress[30].

Assessment of the free radical scavenging activity involves oxidizing the stable DPPH radical. The result of the DPPH scavenging activity assay in this study indicated that the *S. edgeworthii* plant extracts SE-Oil and SE-Alcohol were more potently active than SE-Pet ether and SE-Chloro. This indicated that the extracts may have contained a substance capable of donating hydrogen to a free radical in order to remove the odd electron responsible for the radical's reactivity[31].

Nitric oxide is believed to participate in the instruction of the oxidation-reduction potential of various cells and may be involved in "either the protection against or the induction of oxidative stress within various tissues, depending upon its concentration"[31]. Emerging evidence suggests that some diseases are related to either an inadequate or excessive production of NO. So the extracts SE-Oil, SE-Alcohol, and SE-Chloro might have the ability to decrease the production of NO radical.

The hydrogen peroxide scavenging activity was detected and compared with ascorbic acid. Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, generally by oxidation of essential thiol (-SH) groups. It rapidly transverses cell membrane and once within the cell interior the hydrogen peroxide can possibly react with Fe^{2+} and Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically beneficial for cells to control the quantity of hydrogen peroxide that is permissible to accumulate[32]. So, the current study provides evidence that the plant *S. edgeworthii* plant extracts SE-Oil, SE-Alcohol, and SE-Chloro show hydrogen peroxide scavenging activity.

Conclusion

The present investigation is the first report of a comprehensive study on macroscopic, microscopic characterization, phytochemical screening, estimation of total phenolics and antioxidants activities of *S. edgeworthii* plant. The secondary metabolites in plants require suitable solvents of the specific polarity index for the extraction. The identification of active extracts or fractions is helpful for the fundamental knowledge of pharmaceutical application and the isolation of active compounds. *S. edgeworthii* plant extracts SE-Oil, SE-Alcohol, and SE-Chloro exhibited an outstanding scavenging effect on DPPH, nitric oxide and H_2O_2 radical. Further studies will be carried out to perform the GCMS analysis in support of the present study especially in terms of authentication and identification of crude drugs.

Conflict of interest

The authors declare that there are no conflict of interest.

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