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In vitro Antimicrobial, Antioxidant, and Antidiabetic Activities of Extracts of *Senecio Abyssinicus* Leaves

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ABSTRACT: Despite advancement in technology and healthcare delivery, infectious diseases still ravage humanity, plants-based remedies still remain our major kick back against them. The phytochemical screening, antimicrobial, antioxidant, and anti-hyperglycemic activities of n-hexane, ethyl acetate, and methanolic extracts of *Senecio abyssinicus* leaves were investigated in this study, in line with SDG 3, 9 and 12 goals, following standard methods. The *in vitro* antioxidant properties were tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis(3-ethylbenzthiazoline-6-sulphonate) ($ABTS^{\cdot+}$) radical scavenging assays. Agar diffusion-pour plate methods was used to evaluate the antimicrobial activities of the extracts. The phytochemical evaluation of the extracts unveiled the presence of polyphenols, steroids, terpenoids, alkaloids, and cardiac glycosides. The methanol extracts showed varying degrees of antibacterial activity against the tested bacterial: *Klebsiella pneumonia, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Salmonella typhi* at concentrations between 6.25 – 200 mg/mL relative to Gentamycin. Furthermore, it showed significant activity on the tested fungal strains: *Rhizopus stolons,, Aspergillus niger, Pneumonae notatum*, and *Candida albicans* between 12.5 – 200 mg/mL, with regards to Tioconazole. The methanol extract had the greatest inhibitory effect (IC_{50}, 26.59µg/mL) on α-glucosidase enzyme, with respect to Acarbose (IC_{50} 11.31 µg/mL). Similarly, it showed low $ABTS^{\cdot+}$ and DPPH antioxidant activity (IC_{50} >50 µg/mL) when compared to the standard Trolox (IC_{50} 5.91 µg/mL) and Ascorbic acid (IC_{50} 12.24 µg/mL) respectively. These findings demonstrated that *S. abyssinicus* leaves exhibits moderate to significant antimicrobial, antioxidant, and antidiabetic activities. Thus, could be considered as a good source of antioxidant, hypoglycemic, and antimicrobial agents for good health and well-being.

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Infectious diseases have continued to ravage humanity notwithstanding the rapid advancement in technology, healthcare delivery, and production of drugs and vaccines to curb the menace (Manilal *et al*., 2020). Over the years, many aetiological agents including parasites and microorganisms have become more resistant to orthodox/synthetic drugs and antibiotics of choice as such, this has necessitated the search for

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novel compounds including natural plant isolates for the treatment of many microbial and parasitic diseases (Mahady, 2005; Al-Judaibi, 2014). Besides, researches have shown that active compounds extracted from plants have antimicrobial, antidiabetic, antiparasitic, antioxidant and anticancer properties (Sissing *et al*., 2011; Egharevba *et al*., 2018; 2019; Panda *et al*., 2018; Dkhil *et al*., 2020; Manilal *et al*., 2020). Nature provides a plethora of herbs and plants that can be utilized as sources of traditional medicines to treat many types of illnesses and diseases. The usage of medicinal plants is continuously increasing by the day due to their effectiveness and minimal side effects in comparison to conventional drugs (Al-Daihan *et al*., 2013; Ashraf *et al*., 2020). Hence, screening of crude plant extracts for therapeutic purposes is imperative. Many studies have documented the antimicrobial, antiparasitic, and antioxidant properties of different plant extracts (Oluwatosin *et al*., 2014; Muthukrishnan *et al*., 2018; Egharevba *et al*., 2019; Yahia *et al*., 2019; Manilal *et al*., 2020). The genus *Senecio* belongs to the family Asteraceae and is one of the largest genera of flowering plants, and it comprises approximately 1500 species (Zhao *et al*., 2015; Romo-Asunción *et al*., 2016). *Senecio* spp. are also known for the production of compounds such as alkaloids, terpenoids, and flavonoids (Egharevba *et al*., 2018). The genus has various biological activities, including antibacterial, antioxidant, antiviral, antitumour, analgesic, and anti-inflammatory activities (González *et al*., 2013; Wang *et al*., 2013; Egharevba *et al*., 2018). Within this large group of species is *Senecio abyssinicus*.

S. abyssinicus is an annual herb that grows to 50 cm tall. It is widely distributed in tropical Africa (Adekunle et al., 2002; Odugbemi, 2008). Studies have shown its potency against blood and stomach disorders, rheumatoid arthritis, and venereal diseases including syphilis (Ainslie, 1937; Watt and Brandwijk, 1962). There is a dart of scientific report on the antimicrobial, antioxidant, and antidiabetic activities of extracts from the leaves of *S. abyssinicus in literature. This study therefore seeks to evaluate S. abyssinicus leaves extracts for their* antimicrobial, *antioxidant, and* antidiabetic effects.

MATERIALS AND METHODS

Collection of plant samples: Samples of *Senecio Abyssinicus* were collected from Oko Oba region, Tanke, Oke-Odo, University of Ilorin, Ilorin, Kwara State. The plant was subsequently identified and confirmed at the Department of Plant Biology, Faculty of Life Sciences, University of Ilorin. A voucher specimen (UIH002/1187) was thereafter created and deposited in the University's herbarium.

Chemicals and reagents: Ferric chloride, *sulphuric* acid, aqueous sodium hydroxide, hydrochloric acid, Dragendorff's reagent, Fehling's solution, acetic anhydride, ammonia solution, glacial acetic acid and benzene, n-hexane, ethyl acetate, methanol, acetone, ethanol and chloroform were used in this study.

Preparation of plant extract: Healthy *S. abyssinicus* leaves (1kg) collected were thoroughly washed under running tap water and shade-dried at room temperature for 7 days to maintain their green colour. The different extracts were prepared by successively macerating the dried leaves in n-hexane, ethyl acetate, and methanol for 5 days, respectively, using cold extraction method and thereafter filtered to obtain the different solvent extracts of the plant. The resultant crude extracts were concentrated under reduced pressure using a rotary evaporator and dried in a desiccator. The dried crude extracts were weighed and kept in sterile sealed sample bottles and stored at 4 ºC in the dark for further analysis. Although water is mainly employed as the extracting solvent for traditional herbal preparation, n-hexane, ethyl acetate, and methanol were used in this study, since they are amphiphilic and dissolve a diverse array of compounds than water. Besides, they evaporate readily as such, are easier to separate from the extract than water (Ogbole *et al*., 2018).

Phytochemical screening of S. abyssinicus leaves extract: The n-hexane extract (SAHL), ethyl acetate extract (SAEAL), and methanol extract (SAML) of *S. abyssinicus* leaves were each screened to determine the presence of bioactive compounds such as cardiac glycosides, saponins, anthraquinones, Phlobatannins, steroids, tannins, Terpenoids, flavanoids, Polyphenols and alkaloids using standard techniques (Van den Berge and Vlietinck, 1991; Harborne, 1998; Newman *et al*., 2002; Yadav and Agarwala, 2011; Kumar *et al*., 2013; Egharevba *et al*., 2018).

Antimicrobial assay: Microorganism's cultures of six human pathogenic bacteria made up of four Gram negative (*Escherichia coli* (UCH 00260), *Pseudomonas aeruginosa* (UCH 1102), *Klebsiellae pneumonae* (UCH 2894) and *Salmonella typhi* (UCH 4801)) and two Gram positive (*Bacillus subtilis* (UCH 74230) and *Staphylococcus aureus* (UCH 2473)) were used for the antibacterial assay. The Antifungal assay was tested on four fungi: *Candida albicans*; *Aspergillus niger*; *Rhizopus stolon*; and *Penicillum notatum*. The microorganisms were obtained from the Medical Microbiology Laboratory (University College Hospital, Ibadan) and screened in the Laboratory of the Department of Pharmaceutical Microbiology, University of Ibadan.Media: Nutrient agar, Sabouraud dextrose agar (SDA), nutrient broth, and tryptone soya

agar were used in this study. Ethyl acetate, n-hexane, and methanol were used to solubilize the extracts and as negative controls in the assays. The standard reference drugs (positive control), Gentamycin (10 µg/mL) for antibacterial and Tioconazole (0.7 mg/mL) for antifungal were also included in the assay.

Antibacterial susceptibility tests: Agar diffusion-pour plate method was employed to examine the antibacterial effect of the extracts (Afolayan and Meyer, 1997; Pandey and Madhuri, 2010). An overnight culture of each organism was prepared by taking two wire loops of the organism from the stock and inoculated each into the sterile nutrient broth of 5ml, each, incubated for 18–24 hours at room temperature. From the overnight culture, 0.1 ml of each organism was taken and put into 9.9 ml of sterile distilled water to obtain 1:100 (10^{-2}) inoculum concentration of the organism. From the diluted organism, 0.2 ml was taken from the prepared sterile nutrient agar and allowed to cool to about 45 ºC. Thereafter, it was poured into sterile Petri dishes and allowed to solidify for about 45-60 minutes. A sterile cork-borer of 8 mm diameter was used to create wells according to the number of graded concentrations of the sample. Consequently, the graded concentrations of the extracts were dispensed into the wells including the controls. This was done in duplicate to verify the results obtained. The plates were allowed to stay on the bench for 2 hours to allow pre-diffusion of the extracts into the nutrient agar. Afterwards, the plates were incubated uprightly for 18–24 hours at 37ºC.

Antifungal susceptibility tests: Agar diffusion-surface plate method was used to determine the anti-fungal activity of the extracts (Afolayan and Meyer, 1997; Pandey and Madhuri, 2010). A sterile SDA was prepared, poured into sterile plates in triplicates and left to solidify for about 45-60 minutes. 0.2 ml of the 10^{-2} inoculum concentration of the organism was spread on the surface of the agar using a sterile spreader. A cock borer (8 mm) was used to bore wells in the SDA agar. In each well, the graded concentrations of the extracts with the controls were dispensed into the wells. The plates were left on the bench for 2 hours to allow for proper diffusion of the extracts into the agar. Thereafter, the plates were incubated in an upright position at 26-28 ºC for 72 hours. The antimicrobial activity of the sample extract was evaluated after inoculation, by measuring the diameter of the zone of inhibition in millimetres.

Antioxidant activity: The antioxidant activities of the ethyl acetate, n-hexane, and methanol crude extracts of *S. abyssinicus* leaves were assessed on the basis of their scavenging ability of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) $ABTS^{\cdot+}$ cation radical. Ascorbic acid and Trolox were used as standards.

DPPH free radical scavenging assay: The DPPH free radical scavenging activity of the extracts was determined by using the documented standard method (Kumarasamy *et al*., 2007) with suitable modifications. Decolorization of DPPH solution by the extracts shows the presence of antioxidant compounds (Sadhu *et al*., 2003). The stock solution of the extract was prepared in ethanol to achieve the concentration of 1mg/mL. Serial dilutions of all extracts were prepared and analyzed (Tiwari *et al*., 2013). 25 µL of various dilutions of the extracts (methanol, n-hexane, and ethyl acetate), 100 µL of Tris HCl buffer (0.1 M, pH 7.4) and 125 µL DPPH solution (0.5 mM in methanol) were dispensed into a 96-well microtiter plate and incubated in the dark for 30 minutes at room temperature. After incubation, the absorbance was read at 517 nm. The control sample contained all the reagents except the extract. The percentage of DPPH radical scavenging ability of the extracts was calculated using the equation below:

$$\text{DPPH radical scavenging (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of extract})}{\text{Absorbance of control}} x\ 100$$

The half maximum inhibitory concentration (IC_{50}) values of the extracts were calculated from the % inhibition against a concentration plot, using a non-linear regression algorithm.

$ABTS^{\cdot+}$ scavenging assay: Scavenging of $ABTS^{\cdot+}$ cation radical was carried out following a standard procedure with slight modifications (Tiwari *et al*., 2013). A100 ml stock solution of $ABTS^{\cdot+}$ (0.5 mM) was prepared by addition of 1 ml potassium persulfate [6.89 mM in phosphate-buffered saline (PBS) (pH 8.0)]. The preparation was kept in the dark for 16 hours. 10 µL of each of the different extracts (5 µg/ml in PBS) was added to 190 µL of $ABTS^{\cdot+}$ on a 96-well microtiter plate. Percentage scavenging of $ABTS^{\cdot+}$ by test samples was obtained using the formula below:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of extract})}{\text{Absorbance of control}} x\ 100$$

Antidiabetic study: The antidiabetic study was carried out using Alpha-Glucosidase Inhibition (AGI) assay (Tiwari *et al*., 2011) and acarbose as standard. In a 96-well microtiter plate, 20 µL of each extract was incubated with 50 µL of crude intestinal α glucosidase for 5 minutes and 50 µL of substrate (5 mM, p-nitrophenyl-α-glucopyranoside, prepared in 100 mM phosphate buffer, pH 6.8) was added. The activity of α-glucosidase was determined using a spectrophotometer (BioTek Instruments Inc, Winooski, VT, USA) at 405 nm by measuring the amount of pale yellow p-nitrophenol released from the colourless4-nitrophenyl-β-D- glucopyranoside after incubation for 10 min. Negative control had 10 µL of buffer solution in place of the test entity and Acarbose was used as positive control. An individual blank for each extract was prepared to counterbalance the absorbance due to the colour of samples. Percentage enzyme inhibition was calculated using the formula below:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of extract})}{\text{Absorbance of control}} x\ 100$$

Statistical analysis: This was performed using GraphPad Prism 6 software (Graph Pad Software, Inc., USA). Results are presented as mean ± Standard Deviation (Mean ± SD).

RESULTS AND DISCUSSION

Phytochemical screening: Table 1 shows the results of the phytochemical screening carried out on the extracts of SaEAL, SaML, and SaHL.

Table 1: Qualitative phytochemical composition of the ethyl acetate, methanol, and n-hexane extracts of *S. abyssinicus* leaves

Organic compounds	Extracts		
	ethyl acetate	methanol	n-hexane
Alkaloids	+	+	+
Anthraquinones	-	-	-
Cardiac glycosides	+	+	+
Flavonoids	-	-	-
Phlobotannins	-	-	-
Polyphenols	-	-	+
Saponins	-	+	-
Steroids	+	-	+
Tannins	-	-	-
Terpenoids	+	+	+

Key: '+' for Present; -'for Absent

The result reveals the presence of secondary metabolites including alkaloids, cardiac glycosides, steroids, polyphenols, and terpenoids. All solvent extracts showed that the leaves contain bioactive phytochemical constituents. These bioactive compounds have also been reported in other plant species (Aumeeruddy-Elalfi *et al*., 2015; Egharevba *et al*., 2019; Mehwish *et al*., 2019; Malar *et al*., 2020). The phytochemical analysis revealed the presence of essential bioactive compounds which have been shown to possess medicinal properties singly, synergistically, and with antibiotics (Wagner and Ulrich-Merzenich, 2009; Sharma *et al*., 2014). The results therefore provide an empirical basis for the potential use of *S. abyssinicus* leaves as antimicrobials, antioxidant, and antidiabetic herbs.

Antimicrobial activities: Antibacterial Susceptibility The antibacterial activity of ethyl acetate, methanol, and n-hexane extracts at concentrations between 6.25 and 200 mg/mL was detected with the Agar diffusion-pour plate method and the rate of growth inhibition is presented in Table 2. The maximum inhibition zones were recorded in millimeters with the maximum (31 mm) observed at the concentration of 200 mg/ml. Although all three extracts showed some degrees of antibacterial activity, the methanolic extract was more efficacious, inhibiting the growth of both Gram-positive and Gram-negative bacteria even at the lowest concentration (6.25 mg/ml), followed by ethyl acetate and n-hexane extracts respectively, thereby showing that methanolic extracts of *S. abyssinicus* leaves is more potent and effective against representative bacteria. This was similar to the results documented by Egharevba *et al*. (2018). Methanol being a highly polar solvent probably ensured the extraction of more bioactive compounds from the leaves which have polar compounds that are soluble in highly polar solvents (Kuppusamy *et al*., 2015). The growth of the six bacteria studied was inhibited by the methanolic extract of *S. abyssinicus* leaves at all concentrations (6.25-200 mg/mL) over a range of zones of inhibition (10 – 31 mm) in comparison to the standard, Gentamycin. The methanolic extract showed maximum activity against both Gram-positive and Gram-negative bacteria, respectively, with the highest inhibition zones against *S. aureus* and *E. coli*, however, it had no effect on *S. typhi* at the lowest concentration of 6.25 mg/mL. Ethyl acetate extract inhibited the growth of all six bacteria at higher concentrations (50-200 mg/mL) but showed no inhibitory effect on any of the bacteria species under

investigation at 6.25 mg/mL in contrast to methanolic extract. Besides, *P. aeruginosa* was insensitive to the extract at 6.25-25 mg/mL and *S. typhi* at 12.5 mg/mL, respectively.

Table 2: Antibacterial activities of ethyl acetate, methanol, and n-hexane extracts of *S. abyssinicus* leaves at concentrations between 6.25 and 200 mg/mL

Extraction solvent	Concentration (mg/mL)	Zone of inhibition of bacterial (mm) Mean ± SD					
		Bs	*Ec*	*Kp*	*Pa*	*Sa*	*St*
Ethyl acetate	200	20±0*[a]	19±1.41*[a]	19±1.41*[a]	22±2.83*[a]	27±1.41*[a]	17±1.41*[a]
	100	18±0*[a]	17±1.41*[a]	17±1.41*[a]	19±1.41*[a]	24±0*[a]	14±0*[a]
	50	16±0*[a]	14±0*[a]	14±0*[a]	15±1.41*	20±0*[a]	12±0*[a]
	25	13±1.41*[a]	12±0*[a]	12±0*[a]	-	18±0*[a]	10±0*[a]
	12.5	10±0*[a]	10±0*[a]	10±0*[a]	-	14±0*[a]	-
	6.25	-	-	-	-	-	-
Methanol	200	25±1.41*[a]	30±0*[a]	20±0*[a]	27±1.41*[a]	31±1.41*[a]	19±1.41*[a]
	100	22±2.83*[a]	27±1.40*[a]	18±0*[a]	23±1.41*[a]	28±0*[a]	17±1.41*[a]
	50	19±1.41*[a]	23±1.41*[a]	16±0*[a]	19±1.41*[a]	24±0*[a]	14±0*[a]
	25	16±2.83*[a]	19±1.41*[a]	14±0*[a]	16±2.83*[a]	18±0*[a]	12±0*[a]
	12.5	14±2.83*[a]	16±2.83*[a]	12±0*[a]	13±1.41*[a]	15±1.41*[a]	10±0*[a]
	6.25	12±2.83*[a]	12±2.83*[a]	10±0*[a]	10±0*[a]	11±1.41*[a]	-
n-hexane	200	17±1.41*[a]	17±1.41*[a]	18±0*[a]	14±0*[a]	20±0*[a]	18±0*[a]
	100	14±0*[a]	14±0*[a]	14±0*[a]	12±0*[a]	18±0*[a]	14±0*[a]
	50	12±0*[a]	12±0*[a]	10±0*[a]	10±0*[a]	13±1.41*[a]	10±0*
	25	10±0*[a]	10±0*[a]	-	-	10±0*[a]	-
	12.5	-	-	-	-	-	-
	6.25	-	-	-	-	-	-
Control	*Ethyl acetate*	-	-	-	-	-	-
	Methanol	-	-	-	-	-	-
	n-hexane	-	-	-	-	-	-
	Gentamycin (10µg/mL)	38±0.00	39±1.41	38±0.00	39±1.41	40±0.00	39±1.41

Key: '-' = no inhibition, '*' = values significantly different when compared to negative control using t test; alphabetic superscript means significantly different ($P< 0.05$) when compared to positive control. *Bs*: *Bacillus subtilis; Ec: Escherichia coli; Kp: Klebsiella pneumonae; Pa: Pseudomonas aeruginosa; Sa: Staphylococcus aureus; St: Salmonella typhi*

The n-hexane extract showed some antibacterial effect on all six bacteria at 50-200 mg/mL, but none was observed at 6.25-12.5 mg/mL, a marked deviation from that of the methanolic extract. Generally, the concentration of the extracts was directly proportional to the inhibitory effect of all extracts. This trend was also observed by Egharevba *et al*. (2018). The antibacterial properties of several *Senecio* spp. have been documented. Lopez *et al*. (2018) evaluated the antibacterial potentials of *S. pogonias* and *S. oreophyton*, a species dominant in Argentina. They documented that essential oils from these plants showed antibacterial activity against the enterobacterium, *E. coli* clinical isolates with minimum inhibition concentration values of 2000 µg/mL. Employing the agar-well diffusion method, Kahriman *et al*. (2011) showed the essential oils from the flowers, leaves, and stems of *Senecio pandurifolius* to have antibacterial activity against *Mycobacteriumsmegmatis*, a Gram-positive bacterium, and slight activity against *E. coli* by flower and leaf essential oils. In a study by Uzun*et al*. (2004), they reported that the n-hexane and ethanol crude extract of *Senecio vulgaris* had significant antimicrobial effect against the investigated bacteria. Albayrak *et al*. (2015) reported the antimicrobial activity of extracts from four *Senecio* species extracts against tested bacteria employing agar diffusion and broth micro-dilution assays. The extracts were reported to have weak to moderate antimicrobial activity against some of the bacteria species such as *Proteusmirabilis, B. subtilis, P. aeruginosa, Mycobacteriumsmegmatis* and *S. aureus* with MIC values between 1.5 - 12.5 mg/mL but had no effect on *E. coli, K. pneumonia, Morganella morganii, Salmonella typhimurium* and *Yersinia enterocolitica.*

Antifungal Susceptibility: The antifungal activity of the ethyl acetate, methanol, and n-hexane extracts of *S.abyssinicus* leaves at concentrations between 6.25 and 200 mg/mL, is represented, in Table 3. The table shows that the extracts of *S. abyssinicus* leaves have some activity against the investigated species at varying concentrations. The maximum zone of inhibition (20 mm) was recorded at the concentration of 200 mg/mL. Ethyl acetate extract had varying levels of antifungal activity for all tested fungi at concentrations 50-200 mg/mL.At concentration 6.25 mg/mL, the ethyl acetate extract showed no activity. Methanolic extract showed the highest inhibitory activity on all investigated fungi at all concentrations revealed by the values of zone of inhibition regarding the standard antibiotic (Ticonazole) except for *C. albicans*, *R. stolon* and *P. notatum* at the lowest

concentration (6.25 mg/mL). The n-hexane extract showed the least antifungal activity, with reference to the standard Ticonazole. It had no sign of activity against the fungi at concentrations of 6.25-25 mg/mL.The inhibitory activity of all extracts against the investigated fungi was recorded to increase with an increase in concentration agreeing with Egharevba *et al*. (2018).

Table 3: Antifungal activities of ethyl acetate, methanol, and n-hexane extracts of *S. abyssinicus* leaves at concentrations 6.25 and 200 mg/mL

Extraction solvent	Concentration (mg/mL)	Zone of inhibition of fungi (mm) Mean ± SD			
		C. albicans	*A. niger*	*R. stolon*	*P. notatum*
Ethylacetate	200	18±0*	17±1.41*	18±0*	14±0*
	100	14±0*	14±0*	16±0*	12±0*
	50	12±0*	12±0*	14±0*	10±0*
	25	10±1.41*	10±0*	12±0*	-
	12.5	-	-	10±0*	-
	6.25	-	-	-	-
Methanol	200	20±0*	20±0*	18±0*	18±0*
	100	18±0*	18±0*	16±0*	16±0*
	50	14±0*	16±0*	14±0*	14±0*
	25	12±0*	14±0*	12±0*	12±0*
	12.5	10±0*	12±0*	10±0*	10±0*
	6.25	-	10±0*	-	-
n-hexane	200	14±0*	15±1.41*	14±0*	12±0*
	100	12±0*	12±0*	10±0*	10±0*
	50	10±0*	10±0*	-	-
	25	-	-	-	-
	12.5	-	-	-	-
	6.25	-	-	-	-
Control	*Ethyl acetate*	-	-	-	-
	Methanol	-	-	-	-
	n-hexane	-	-	-	-
	TIOCONAZOLE (70%)	28±0.00	27±1.41	27±1.41	27±1.41

*Key: - = no inhibition; * = values significantly different when compared to negative control using t test; alphabetic superscript other than that of Ticonazole means significantly different (P<0.05) when compared to positive control.*

Singh *et al*. (2017) demonstrated the antifungal properties of *Seneciochrysanthemoides.* Their study showed a positive effect of methanolic and petroleum extracts of *S. chrysanthemoides* against species of fungi tested including *C. albicans* and *A. niger* but not against *A. flavus.* A marked deviation from the result documented in this study, Albayrak *et al*. (2015) reported that all *Senecio* spp. used in their research showed no activity against the fungi tested, including *C. albicans.* The antimicrobial effects of the extracts in this study vary across the different extraction solvents and their concentrations. Terpenoids were recorded in the species of *Senecio* studied. This may be responsible for the antimicrobial activity of the extract. This activity was previously described for these metabolites (Pérez *et al*., 1999; Elsharkawy and Aljohar, 2016). Terpenoids often exert their antimicrobial effects on the cytoplasmic membrane by altering its structure and function (Cowan, 1999). They also do this by interfering with the energy (ATP) generation system in the cell by inhibiting enzymatic activities (Holley and Patel, 2005). Furthermore, they induce membrane swelling and increase membrane permeability of the cell (Cox *et al*., 2000). Following the antibacterial and antifungal activities of the extract of *S. abyssinicus* leaves documented in this study, it is safe to assert that this extract is a good source of antimicrobial agent.

Antidiabetic activity: The results of the in vitro α-glucosidase enzyme inhibitory assay of ethyl acetate, methanolic, and n-henaxe extract of *S. abyssinicus* leaves showed varied antidiabetic activities. Significant activity was observed by n-hexane (76.55%) and methanolic (75.13%) extracts, respectively, while that of ethyl acetate was moderate (54.23%) in comparison to the standard, Acarbose (92.95%) (Figure 1). N-hexane and methanolic extracts of *S. abyssinicus* exhibited substantial inhibitory activities (IC_{50} 29.16µg/ml and IC_{50} 26.59µg/ml respectively) as against Acarbose (IC_{50} 11.31 µg/ml) which is statistically significant ($p<$ 0.05), but the ethyl acetate extracts had a high $IC_{50}>$ 50(Figure 2). Okoro *et al*. (2014) reported that the petroleum ether extract of *Senecio biafrae* administered orally to streptozotocin-induced diabetic rats showed some significant antidiabetic activity comparable to the standard drug glibenclamide (Okoro *et al*. 2018). In a similar vein, Ayoola *et al*. (2019) demonstrated the methanolic extract of the whole plant of *Senecio biafrae* to have significant antidiabetic activity. Tundis *et al*. (2007) also showed that several

extracts of *Senecio leucanthemifolius* had some anti-hyperglycaemic activity. These previous reports support the suggestion that aqueous leaf extract of *S. abyssinicus* possesses hypoglycaemic properties as such could be further investigated for its as antidiabetics activity.

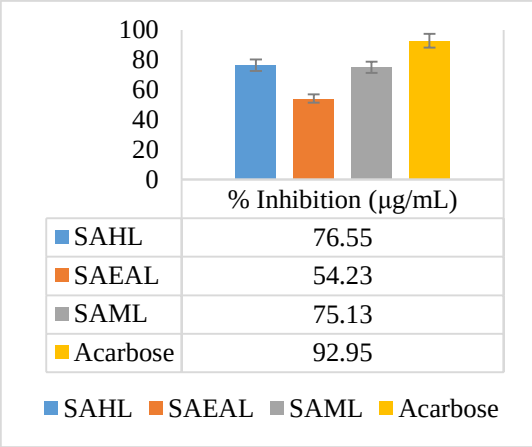

Fig 1: α - Glucosidase % Inhibition of SAHL, SAEAL, SAML, and Acarbose

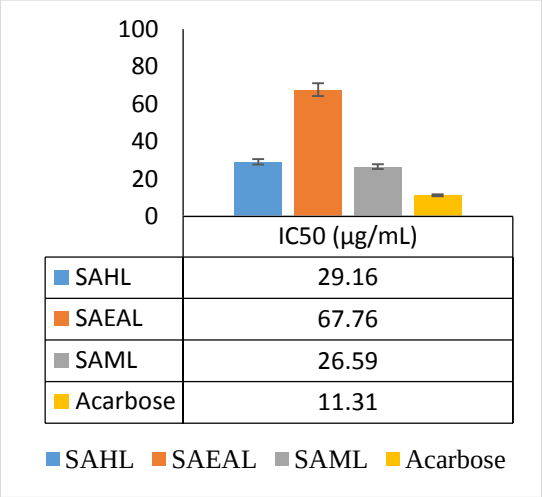

Fig 2: IC_{50} Values of SAHL, SAEAL, SAML, and Acarbose Extracts from α – Glucosidase Inhibitory Assay

In vitro antioxidant capacity assays: The DPPH assay of the ethyl acetate and n-hexane extracts showed very little or no antioxidant properties, whereas moderate activity of the methanolic extract was observed as shown (Figure 3). The percentage inhibition of methanolic extract (33.74 ± 0.29 with IC_{50}> 50 µg/mL) was statistically low with respect to Ascorbic acid (94.23 ± 0.00with IC_{50}12.24 µg/mL) lower than 64.59 ± 0.9 µg/ml reported by Egharevba *et al*. (2018) for the extract of *S. abyssinicus* flower. The three extracts showed some activities as revealed by $ABTS^{\cdot+}$ free radical scavenging ability. Ethyl acetate (12.15 ± 0.36) and methanolic (25.06 ± 2.86) extracts showed little inhibition as compared to the standard compound, Trolox (98.73 ± 0.30) whereas that of n-hexane extract was substantial (99.49 ± 0.00); it was not significantly different from that of the standard compound, Trolox (98.73 ± 0.30). In a similar manner, the IC_{50} of n-hexane extract (4.59µg/mL) was not statistically different from the standard compound, Trolox (5.91 µg/mL). The results above therefore show that methanolic and n-hexane extracts showed moderate and significant DPPH and $ABTS^{\cdot+}$ antioxidant activities in comparison to the standard compounds-Ascorbic acid and Trolox respectively.

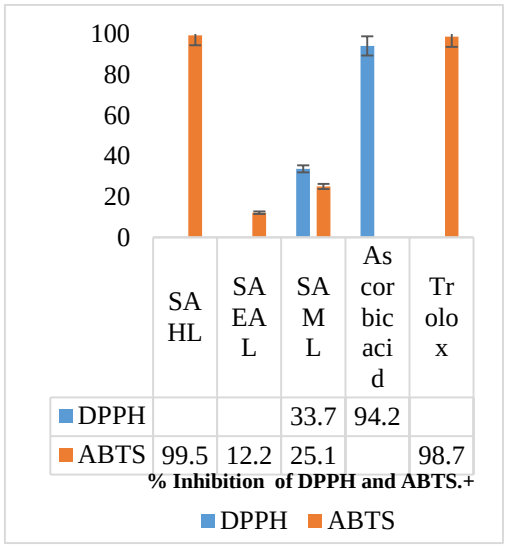

Fig 3: % Inhibition of DPPH and ABTS+ Assay, Respectively.

The antioxidant scavenging activities of the extracts of *S. abyssinicus* leaves documented in this study support previous reports on the antioxidant properties of extracts of various parts of *Senecio* spp. (Hariprasath *et al*., 2015; Egharevba *et al*, 2018; Faraone *et al*., 2018). In a survey by Albayrak *et al*. (2014) in Turkey, the methanolic extract of *S. salsuginea* was shown to have the strongest free radical scavenging activity with IC_{50} value of 26.23 µg/ml while *S. mollis* showed the highest antioxidant activity using the phosphomolybdenum method (434.48 mg AAE/g) amongst the different *Senecio* spp. Used in the study. In addition, Egharevba *et al*. (2018) reported that the methanolic extract of *S. abyssinicus* flower showed great activity as well. Tundis *et al*. (2012) demonstrated that ethyl acetate extract of *Senecio stabianus* showed moderate antioxidant activity with IC_{50} values of 35.5 and 32.7 mg/ml on DPPH and ABTS tests, respectively.

Conclusion: The results of the current survey showed that the extract of *S. abyssinicus* leaves has some important phytochemicals, namely, alkaloids, cardiac

glycosides, polyphenols, saponins, steroids and terpenoids and hence may be the evidential reason for its medicinal value. The plant has substantial anti-hyperglycaemic potential; it exhibited significant α-glucosidase enzymatic inhibitory activity making it a good source of antidiabetics agents. Furthermore, it exhibited varied antioxidant activity. The antimicrobial activity of the extracts of *S. abyssinicus* leaves documented in this survey justified the ethnomedicinal use of *Senecio* spp. as a possible source of antimicrobial agents. The pharmacological potential exhibited by the aqueous extract of *S. abyssinicus* is considered the outcome of the synergistic effect of the phytochemicals. Overall, this study showed that *S. abyssinicus* leaves could be considered as a potential natural source to mitigate some human diseases. However, extensive research is recommended to further understand the impact of *S. abyssinicus* phytochemical constituents on health.

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