Phytochemical screening, Antimicrobial and Antioxidant Activities of Crude Extracts of *Senecio abyssinicus* Flower

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ABSTRACT

With the emergence of more resistant pathogenic micro-organisms against synthetic antibiotic, it is pertinent to search for plant extracts with broad spectrum antimicrobial compounds from folklore medicine. The n-hexane, ethyl acetate and methanolic extract of *Senecio abyssinicus* flower were investigated for their phytochemical composition, antimicrobial and antioxidant activities. Standard methods, disk diffusion method and 2, 2-diphenyl-2-picryl-hydrazyl free radical ($DPPH^{\cdot}$) were used to determine the phytochemical composition, the antimicrobial activity and the *in-vitro* antioxidant activity respectively. The flower was found to contain alkaloids, flavonoid, saponins, tannins, anthraquinones and steroids. The antibacteria linvestigation of the n-hexane, ethyl acetate and methanolic extracts of *Senecio abyssinicus* flower inhibited the growth *of Staphyloccocus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* at concentrations between 50 and 200mg/ml. The ethyl acetate extract exhibited higher intrinsic antifungal properties *on Candida albicans*, *Aspergillus niger*, *Rhizopus stolon*, and *Pneumonae notatum*. Only the methanol extract acted as hydrogen/electrons donor or scavenger of radicals with IC_{50} of 46.24 μg/mL while that of Ascobic acid (standard) was found to be 12.24 μg/mL.

KEY WORDS: *Senecio abyssinicus*; phytochemical screening, antimicrobial, antioxidant

INTRODUCTION

Pathogenic micro-organisms have built great resistance against several synthetic antibiotics, as a result much attention is being been paid to extracts and biologically active compounds isolated from plant species used in folklore medicine. Medicinal plants offer a natural and new source of antibacterial agents for use.[1] *Senecio abyssinicus* is a useful plant of west tropical Africa.[2,3,4] The parts of the plant mostly used are leaves, stems, and flowers. *Senecio abyssinicus* (Compositae), commonly called Amùnimúyè by the Yoruba speaking tribe in Nigeria[5] is a plant believed to possess potent medicinal properties in ethno medicine. It is an annual herb that grow in open places to heights of about 50 cm and found in lowlands and mountain elevations in North and South Nigeria, and West Cameroons. It is widely distributed in central and east tropical Africa. The plant is used in the treatment of blood disorders, arthritis, rheumatism, stomach troubles, syphilis,[6] skin, mucosae, venereal diseases, yaws,[7] religion, superstitions and magic.[8]

MATERIALS AND METHODS

I. Plant Collection

The plant was collected from Oko Oba area, Tanke Oke-Odo, Ilorin, Kwara State, Nigeria. The plant materials were taxonomically identified and authenticated at the Department of Plant Biology, University of Ilorin, Ilorin, where a voucher specimen (UIH002/1187) was deposited.

II. Preparation of Plant Extracts

Fresh flowers of *Senecio abyssinicus* were collected in the month of August 2014, washed, air-dried under shade. The dried flowers of *S. abyssinicus* were successively extracted using hexane, ethyl acetate and methanol for 7 days respectively using cold extraction method. The resultant hexane, ethyl acetate and methanol

extracts were concentrated using rotary evaporator and later dried in a desicator. When dried, it was stored in vials at 4°C for further investigation.

III. Phytochemical Studies

The Preliminary phytochemical screening of the hexane, ethylacetate and methanol extracts of *S. abyssinicus* were done using standard procedures.[9,10,11]

1. Saponins: Small quantity of each extract was boiled with 5 ml of distilled water, filtered and cooled.

a). Frothing: To the filtrate (2.5 ml) about 10 ml of distilled water was added and shaken vigorously for 2 minutes. Frothing observed indicates a positive test.

b). Emulsification: To 2.5 ml of the filtrate was added 3 drops of olive oil and shaken vigorously for 2 minutes. An emulsified layer indicates a positive test.

2. Alkaloids: Small quantity of each extract was stirred with 5 mL of 1% hydrochloric acid for five minutes on a water bath and then filtered. The filtrate of each extract was divided into two portions. Mayer's reagent was added to one portion; occurrence of creamy white precipitate was taken as positive. To the second portion few drops of Dragendorff's reagent was added and appearance of orange red precipitate was regarded as positive for the presence of alkaloids.

3. Glycosides: Small quantity of each extract was diluted in 10 ml of distilled water and divided into two portion. a. Keller-killiani Test: to one portion, 2 ml of glacial acetic acid containing one drop of ferric chloride solution (3.5%) was added. This was underlay with 1 ml of concentrated sulfuric acid. A radish brown ring formed at the interface and upper layer turns bluish green on standing indicates the presence of a deoxy sugar characteristic of cardiac glycosides. b) Method-2: To the second portion, few drops of chloroform were added (to enhance enzymatic activity). A sodium picrate–saturated filter paper strip was hanged at the neck of the flask with the help of the cork and warmed the flask. The filter paper strip turned brick-red or maroon indicates the presence of cyanogenetic glycosides.

4. Tannins: a) Ferric Chloride Test: Small quantity of each extract was boiled in 10 ml of water in a test tube and then filtered while hot and a few drops of 0.1% ferric chloride solution were added to the filtrate. A brownish green or a blue-black coloration indicates the presence of tannins. b) Lead Acetate Test: Small quantity of each extract was taken in a test tube and diluted with 5 ml of distilled water. few drops of a 1% solution of lead acetate was added to each. A yellow or red precipitate indicates the presence of tannins.

5. Flavonoids: Three methods were used to determine the presence of flavonoids in the extracts. a) Method-1: Dilute ammonia solution (5 ml) was added to aqueous filtrate of each extract followed by addition of concentrated H_2SO_4 (1 ml). A yellow colouration that disappears on standing indicates the presence of flavonoids. Method-2: Few drops of 1% aluminium solution were added to aqueous filtrate of the each extract. A yellow coloration indicates the presence of flavonoids.

6. Steroids: Liebermann-Burchard's Test: Small amount of each extract was dissolved in 1 ml of chloroform and 2 ml of acetic anhydride, 1 ml of concentrated $H_2SO_4$was added to each portion. A greenish color produced which turns blue on standing indicates the presence of steroids. b) Salkowski's Test: Small amount of the extract was dissolved in 2ml of chloroform. Concentrated sulphuric acid was carefully added to a lower layer. A reddish-brown colour at the interphase indicates the presence of deoxysugar characteristics of cardenolides. A violet ring may form just above the ring and gradually spread throughout the layer, indicating the presence of steroids.

7. Anthraquinones: A small portion of each extract was boiled with 10 ml of sulfuric acid, traces of ferric chloride solution was added and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was taken into another test tube and 1 ml of dilute ammonia was added to each portion. Rose-pink color in the aqueous layer indicates the presence of anthraquinones.

ANTIMICROBIAL ASSAY

Microorganisms

Microorganism's cultures of six human pathogenic bacteria made up of four Gram negative and two Gram positive were used for the antibacterial assay. They were; *Salmonella typhii* (UCH 4801), *Escherica coli* (UCH 00260), *Pseudomonas aeruginosa* (UCH 1102) and *Klebsiellae pneumonae* (UCH 2894) for Gram-negative while *Bacillus subtilis* (UCH 74230) and *Staphylococcus aureus* (UCH 2473) were Gram-positive. The Antifungal assay was tested on hod*notatum*. The microorganisms used were clinical strains from the Medical Microbiology (University College Hospital, Ibadan) and screened in the Laboratory of Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria.

Media: Nutrient agar, Sabouraud dextrose agar, nutrient broth and tryptone soya agar were used in this study. Hexane, ethylacetate and methanol were also used in solubilizing the extracts and as negative controls in the assays. Gentamycin (10 µg/mL) for antimicrobial and Tioconazole (0.7 mg/mL) for antifungal were included as standard reference drugs (positive control) in the study.

Determination of zone of inhibition

Agar diffusion method (bacteria): An overnight culture of each organism was prepared by taken two wire loop of the organism from the stock and inoculated each into the sterile nutrient broth of 5ml, each incubated for 18-24hr at 37^{o}C. From overnight culture, 0.1 mL of each organism was taken and put into the 9.9 mL of sterile distilled water to obtained 10^{-2} inoculum concentration of the organism. From the diluted organism (10^{-2}), 0.2 mL was taken into the prepared sterile nutrient agar cooled to about 40-45 ^{o}C, then poured into sterile Petri dishes and allowed to solidify for about 1 h. Using a sterile cork-borer of 8 mm diameter, 8 wells were made according to the number of the test tubes for the experiment. The graded concentrations (6.25 – 200 mg/mL) of the extracts were put into the wells accordingly including the controls. The study was done in triplicates to ascertain the results obtained. The plates were left on the bench for about 2 h allowin the extract to diffuse properly into the nutrient agar i.e. pre-diffusion. The plates were incubated for 18-24 hr at 37^{o}C.[12,13]

Agar diffusion-surface plate method (fungi): A sterile sabouraud dextrose agar was prepared accordingly and aseptically poured into the sterile plates in triplicates and solidified properly. 0.2 mL of the 10^{-2} inoculum concentration of the organism was spread on the surface of the agar using a sterile Petri-dish to cover all the surface of the agar. Eight wells were bored using a sterile cork-borer of 8 mm diameter. The graded concentrations (6.25 – 200 mg/mL) of the extracts were put into the wells including the controls. All the plates were left on the bench for 2 h to allow the extract diffuse properly into the agar i.e. pre-diffusion. The plates were incubated at 25 ^{o}C for 72 h.[12,13]

ANTIOXIDANT ACTIVITY

Antioxidant activities of the n-hexane, ethylacetate and methanol crude extracts of the flower of *S. abyssinicus* was determined on the basis of their scavenging potential of the stable 2,2-diphenly-1-picrylhydrazyl (DPPH) free radical in both qualitative and quantitative assay. Ascorbic acid was used as a standard.

I. Qualitative assay:

A suitably diluted extract was spotted on a pre-coated silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar). The dried developed plates were sprayed with 0.02% DPPH in ethanol. Bleaching of DPPH solution by the applied extracts from purple to yellow indicates the presence of antioxidant compounds.[14]

II. Quantitative Assay

The quantitative DPPH radical scavenging activity of the extract was determined by the standard method with suitable modifications. The stock solution of the extract was prepared in ethanol to achieve the concentration of 1mg/mL. Dilutions were made to obtain concentrations of 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.99, 0.97 μg/mL. Diluted solutions (1mL each) were mixed with 1ml of methanolic solution of DPPH in concentration of 1 µg/mL. After 30 minutes incubation in the dark at room temperature, the absorbance was recorded at 517 nm. Control sample contained all the reagents except the extract.[15] Percentage inhibition was calculated using equation below, while the IC_{50}

value were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm.

$$\% \text{ inhibition} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\textit{absorbance of control}} \times 100$$

Statistical Analysis

Statistical analysis was carried out with GraphPad Prism 6 software (Graph Pad Software, Inc.,

USA), and results are expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Phytochemical screening

The results of the phytochemical screening carried out on the n-hexane, ethylacetate and methanolic extract of *Senecio abyssinicus* flower is shown in table 1 below. The result shows the presence of alkaloids, steroids, cardiac glycosides, tannins, flavanoids and terpenoids.

***Table 1*: phytochemical composition of the n-hexane, ethyl acetate and methanolic extracts of *Senecio abyssinicus* flower.**

Secondary metabolites	Extracts		
	n-Hexane	Ethyl acetate	Methanol
Alkaloids	-	+	+
Steroids	+	-	+
Cardiac glycosides	-	-	+
Tannins	-	+	+
Saponins	-	+	–
Phlobotannins	-	+	–
Terpenoids	-	-	+
Flavanoids	-	-	+
Anthraquinones	+	-	–

KEY: - = Absent , + = Present

Table 1 shows the result of the phytochemical analysis of the n-hexane, ethylacetate and methanolic extract of *Senecio abyssinicus* flower. The ethylacetate and methanolic extracts both revealed the presence of alkaloids. The methanolic extract of *S. abyssinicus* reveals the presence of steroids, cardiac glycosides, terpenoids and flavonoids. The analysis revealed the presence of constituents which are known to exhibit medicinal activities. The result provides an empirical basis for the potential use of this plant as anthelmintic, antimicrobial and antioxidant herbs.

Antimicrobial activity

a. Antibacteria activity

The result of the antibacterial activities of the hexane, ethyl acetate and methanol extracts at concentrations between 6.25 and 200 mg/ml is presented in Table 2. The antimicrobial activities of the extract in this study varies with the solvent type used in the extraction and the concentration of the extract used. All three (3) extracts, n-hexane, ethyl acetate and methanol inhibited the growth of *S. aureus, B. subtilis* (gram positive), *E. coli, P. aeruginosa* (gram negative) at concentration between 50 and 200 mg/mL, except the n-hexane extract that showed no inhibition on *P. aeruginosa* at a concentration of 50 mg/ml. Furthermore the ethyl acetate and methanolic extract inhibited the growth of *S. aureus, B. subtilis* (gram positive), *E. coli, P. aeruginosa* (gram negative) at concentration between 25 and 50 mg/mL, except the ethyl acetate extract that shows no inhibition on *P. aeruginosa* at a concentration of 25 mg/mL. The methanol extract of the *S. abyssinicus* showed a greater antibacterial activity than the n-hexane and the ethyl acetate extract, similarly the ethyl acetate crude extract showed a better activity than the n-hexane extract.

Table 2: The results of Anti-bacteria activities of the n-hexane, ethyl acetate and methanol extract of *Senecio abyssinicus* flower

Plant Extract	Concentration (mg/mL)	ZONE OF INHIBITION OF BACTERIAL (mm) Mean ± Standard deviation					
		S. aureus	*E. coli*	*B. subtilis*	*P. aeruginosa*	*K. pneumonae*	*S. typhi*
n-Hexane	200	15±1.41	15±1.41	15±1.41	13±1.41	-	-
	100	12±0.01	13±1.41	12±0.21	10±0.11	-	-
	50	10±0.03	10±0.10	10±0.12	-	-	-
	25	-	-	-	-	-	-
	12.5	-	-	-	-	-	-
	6.25	-	-	-	-	-	-
Ethyl Acetate	200	17±1.41	17±1.41	18±0.05	14±0.10	-	-
	100	14±0.30	14±0.02	16±0.03	12±0.20	-	-
	50	12±0.11	12±0.43	14±0.20	10±0.09	-	-
	25	10±0.24	10±0.05	12±0.22	-	-	-
	12.5	-	-	-	-	-	-
	6.25	-	-	-	-	-	-
Methanol	200	19±1.41	17±1.41	17±1.41	17±1.41	-	-
	100	16±0.25	14±0.65	14±0.98	14±0.23	-	-
	50	13±1.41	12±0.32	12±0.09	12±0.65	-	-
	25	10±0.34	10±0.54	10±0.23	10±0.11	-	-
	12.5	-	-	-	-	-	-
	6.25	-	-	-	-	-	-
Control	n-hexane	-	-	-	-	-	-
	Ethyl Acetate	-	-	-	-	-	-
	Methanol	-	-	-	-	-	-
	Gentamycin (10 µg/mL)	40±0.00	39±1.41	38±0.09	39±1.41	38±0.05	39±1.41

Key: - = no inhibition

Antifungi activity

The antifungal activities of the n-hexane, ethyl acetate and methanol extracts of *S. abyssinicus flower* at concentrations between 6.25 and 200 mg/ml is presented in Table 3. Four clinical strains of fungi were used; *Candida albicans, Aspergillus niger, Rhizopus stolon, and Penicillum notatum.* The n –hexane extract of the flower of *S. abyssinicus* exhibited lower antifungi activities on only *Candida albicans, and Aspergillus niger* at concentrations between 50 and 200 mg/ml. The ethyl acetate extract exhibited higher antifungi activities on the tested organisms with reference to the standard drug, Tioconazole. It inhibited the growth of *C. albicans, A. niger, R. stolon, and P. notatum* between the concentration of 25 and 200 mg/ml. The methanol extract also showed a fairly reasonably zone of inhibition on *Aspergillus niger, Rhizopus stolon, and Penicillum notatum except Candida albicans* at concentrations of 50 and 200mg/ml. Consequently, the zone of inhibition of the test bacteria and fungi on all the extracts were concentration dependent, activity were higher as concentrations of the extracts increases.

Table 3: The results of Anti fungi activities of n-hexane, ethyl acetate and methanol extract of *Senecio abyssinicus* flower

EXTRACT	Concentration (mg/mL)	DIAMETER OF ZONE OF INHIBITION OF FUNGI (mm) MEAN±S.DEV			
		***C.** albicans*	***A.** notatum*	***R.** stolon*	***P.** notatum*
n-Hexane	200	14±0	12±0	-	-
	100	12±0	10±0	-	-
	50	10±0	-	-	-
	25	-	-	-	-
	12.5	-	-	-	-
	6.25	-	-	-	-
Ethyl Acetate	200	16±0	14±0	12±0	12±0
	100	14±0	12±0	10±0	10±0
	50	12±0	5±3.54	-	-
	25	10±0	-	-	-
	12.5	-	-	-	-
	6.25	-	-	-	-
Methanol	200	-	14±0	14±0	13±1.41
	100	-	12±0	12±0	10±0
	50	-	10±0	10±0	-
	25	-	-	-	-
	12.5	-	-	-	-
	6.25	-	-	-	-
Control	n-hexane	-	-	-	-
	Ethyl Acetate	-	-	-	-
	Methanol	-	-	-	-
	Tioconazole (70%)	28±0.00	27±1.41	27±1.41	27±1.41

The above antimicrobial activity results for the flowers of *S. abyssinicus* extracts further confirms the effectiveness of its flower as a rich source of antimicrobial agent for the food and pharmaceutical industries. Uzun *et al.*[16] investigated the antimicrobial activity of *Senecio vulgaris*. They carried out antimicrobial activity against *Staphylococcus aureus* ATCC 65538, *Escherichia coli* ATCC 8739, *Klebsiella pneumonia* ATCC 4352, *Pseudomonas aeruginosa* ATCC 1539, *Salmonella typhi*, and *Candida albicans* ATCC 10231, using microbroth dilution technique according to National Committee for Clinical Laboratory Standards (NCCLS) on *Senecio vulgaris* Pet ether (n-hexane) and ethanol crude plant extract, and found out that the Specie *Senecio vulgaris* showed significant anti microbial activities, with the ethanol crude extract showing a higher activity. Loizzo *et al.*[17] reported the results of the test for antibacterial and antifungal activities of *Senecio inaequidens DC*. and *Senecio vulgaris L*. The methanol extract of *S. vulgaris* showed antimicrobial activity against the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* while that of *S. inaequidens* showed no antimicrobial activity against those organisms. Gram-negative bacteria were unaffected by the methanol extracts and any of the fractions of both Senecio species. Albayrak *et al.*[18] reported the antimicrobial activity of *Senecio species* extracts that was tested against 15 microorganisms using agar diffusion and broth micro dilution assays. The extracts were found to have weak to

moderate antimicrobial activity against 8 out of the 15 microorganisms tested with minimal inhibitory concentration (MIC) values ranging from 1.5 to 12.5 mg/ml. Flavonoids have been reported to inhibit nucleic acid biosynthesis, spore germination of plant pathogens and other metabolic processes.[19,20] Thus the high antimicrobial activity of the methanolic extract of the flower may be attributed to the flavonoid content.

Antioxidant activity

DPPH qualitative assay of the n-hexane, ethyl acetate and methanolic crude extract of *Senecio abyssinicus* flower

a. DPPH qualitative assay

The DPPH qualitative assay of the n-hexane and ethylacatate extracts gave an insignificant antioxidant activities as observed on the colour change on the TLC plates, hence no further quantitative assay was carried out on them. But the activity of the methanolic extract as observed by the colour change of the DPPH purple colour in ethanol solution to a conspicuously yellow band on the purple background of the TLC plate.

b. DPPH quantitative assay

The result obtained for the quantitative DPPH analysis is presented in figure 1.

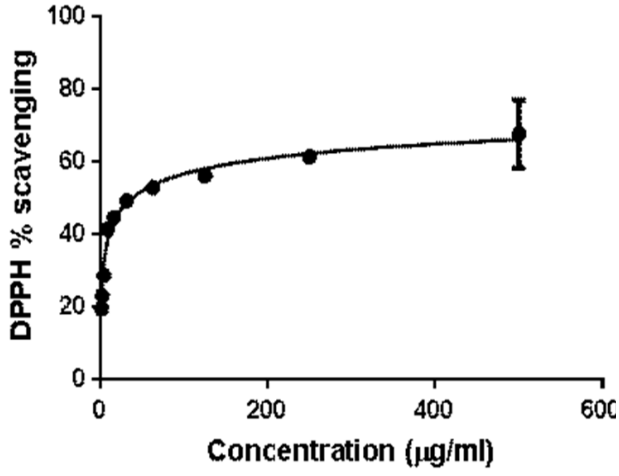

Figure 1: DPPH scavenging activity of methanolic extract of Senecio abyssinicus flower

The $\mathbf{EC_{50}}$ of the methanolic extract was found to be 64.59 ± 0.9 µg/mL which was significantly different ($P<0.05$) from that of ascorbic acid (10.02 ±0.1 µg/mL). Natural products electron donating ability of can be measured by 2,2'-diphenyl-1- picrylhydrazyl radical (DPPH) purple-coloured solution bleaching.[21] It is based on scavenging of DPPH through the addition of antioxidant that decolourizes the DPPH solution in which the degree of colour change is proportional to the potency and concentration of the antioxidants. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test.[22]

In the present study only the methanol fraction showed inhibition which increases with increase in concentration. This suggest that the methanol extract contain secondary metabolites capable of donating hydrogen to a free radical to scavenge the potential damage.

Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules,[23] implicated in several diseases. Comparable with the findings in the literature for other extracts of plant products,[24] this present results suggest that phenolic acids and flavonoids may be the major contributors for the antioxidant activity since from the phytochemical studies this extract contains flavonoids.

The result obtained for the DPPH scavenging radical test also strengthens the antioxidant activities present in the *Senecio* species as reported by Conforti *et al.*[25,26], who reported that the ethyl acetate extracts of *S. inaequidens* and *S. vulgaris* possessed the highest radical scavenging activity with 61.60% and 44.57% of inhibition, respectively, at a concentration of 0.312 mg/ml. The aqueous extracts of *S. scandens* exhibited the high potency in inhibiting lipid peroxidation.[27] Albayrak *et al.*[28] reported that amongst nine *Senecio* species growing in Turkey. *S. salsuginea* showed the strongest free radical scavenging activity with IC_{50} = 26.23 µg/ ml and *S. mollis* showed the highest antioxidant capacity in the phosphomolybdenum method (434.48 mg AAE/ g).

CONCLUSION

The results reported in the present study can be considered as the first information on the phytochemical screening, antimicrobial and antioxidant activities of the n-hexane, ethylacetate and methanolic extracts obtained from *Senecio abyssinicus* flower belonging of the *Senecio* species. The phytochemical screening of the various extracts of *Senecio abyssinicus* flowers, especially the methanoloic extract reveals the presence of Alkaloids, Steroids, Cardiac glycosides, Anthraquinones, Tannins, Saponins, Phlobotannins, Flavanoids, and Terpenoids. The EC_{50} (64.59 ± 0.9 µg/mL) of the methanolic extract implies that the flower part has moderate antioxidant activity. The ethyl acetate extract also have good antimicrobial activities. All these observations could be the reason(s) for the use of this plant in folklore medicine for the treatment of Syphilis, yaws, rheumatism, cuts, skin diseases. Further research work is on in our research group to validate and determine the bioactive compounds responsible for these reported observed activities. It can be concluded that *Senecio flower* extracts may be considered as natural sources of antimicrobial and antioxidant agent in many industry such as food and pharmacy.

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REFERENCES

1. Samuelson, G. (1999). Drugs of natural origin: a textbook of pharmacognosy. 4th edition, Stockholm, Swedish Pharmaceutical, pp 95.
2. Adegoke, E.A., Akisanya, A., Naqvi, S.H.Z. (1969). Studies of Nigerian medicinal plants. I. A preliminary survey of plant alkaloids. *Jl. W. Afr. Sci. Ass*, *13*, pp.13-33.
3. Adekunle, M.F., Oluwalana, S.A., Aklilu, N. and Mengisteab, H. (2002). Exploratory Survey of Forest Plants in Traditional Treatment of Guinea Worm Infections (GWI) (*Dracunculus medinensis* Linn): Experiences from Nigeria and Ethiopia. *African Research Review*, *1*(3), pp.108-124.
4. Odugbemi, T. (2008). A textbook of medicinal plants from Nigeria. University of Lagos Press, Lagos, Nigeria, pp. 20-323..
5. Gbile, Z.O. (1984). Vernacular names of Nigerian plants (Yoruba). 1st edn. Forestry Research Institute of Nigeria, Ibadan *p. 101*..
6. Ainslie, J.R. (1937). The list of plants used in native medicine in Nigeria, Imp. Forest. Inst. Oxford Inst. Paper, 7.
7. Watt, J.M., Breyer Brandwijk, M.G. (1962). Medicinal and poisonous plants of southern and eastern Africa. 2nd edn., E. and S. Livingstone Ltd., Edinburgh. pp1457
8. Dalziel, J.M. (1937). The useful plants of West Tropical Africa. 1st edn. Crown Agents for the Colonies, London, *p. 413*
9. Harborne, J.P. (1998). Phytochemical Methods: A guide to modern technique of plant analysis, 2nd ed. Chapman and Hall, London. P.16-30.
10. Trease, G.E., Evans, W.C. (1983). Drugs of Biological Origin. In Pharmacognosy 12th ed. United Kingdom: Balliere Tindall. 309-540.
11. Van den Berge, A.D., Vlietinck, A.J. (1991). Methods in plants biochemistry. Vol. 6. Assays for bioactivity. Hostettmann K. Ed. Academic Press, London.47-69.
12. Pandey, G., Madhuri, S. (2010). Pharmacological activities of Ocimum sanctum (tulsi): a review. *International Journal of Pharmaceutical Science Rev and Researc*, *5*, 61-66.
13. Afolayan, A.J., Meyer, J.J.M. (1997). The antimicrobial activity of 3,5,7-trihydroxyflavone isolated from the shoots

of Helichrysum aureonitens. *Journal of ethnopharmacology*, *57*(3), 177-181.

14. Sadhu SK., Okuyama E., Fujimoto H., Ishibashi M. (2003). Separation of Leucas aspera, a medicinal plant of Bangladesh, guided by prostaglandin inhibitory and antioxidant activities. Chem Pharm Bull; 51: 595-598.
15. Kumarasamy Y., Byres M., Cox PJ., Jaspars M., Nahar L., Sarker SD. (2007). Screening seeds of some Scottish plants for free-radical scavenging activity. Phytother. Res. 21: 615-621.
16. Uzun, E., Sariyar, G., Adsersen, A., Karakoc, B., Ötük, G., Oktayoglu, E., Pirildar, S. (2004). Traditional medicine in Sakarya province (Turkey) and antimicrobial activities of selected species. Journal of Ethnopharmacology, 95(2), pp.287-296.
17. Loizzo, M.R., Statti, G.A., Tundis, R., Conforti, F., Bonesi, M., Autelitano, G., Houghton, P.J., Miljkovic B.A., Menichini, F. (2004). Antibacterial and antifungal activity of Senecio inaequidens DC. and Senecio vulgaris L. Phytotherapy research, 18(9), pp.777-779.
18. Albayrak, S., Aksoy, A., Yurtseven, L. and Yaşar, A. (2015). A Comparative Study on Antioxidant and Antibacterial Activities of Four Senecio Species From Turkey. *International Journal of Secondary Metabolite*, *2*(2):26-36.
19. Cushnie, T.P.T., Lamb, A.J. (2005). Antimicrobial activity of flavonoid. Int. J. Antimicrob. Agents. Volume 26, Pp 343-355
20. Harborne, J.B., Williams, C.A. (2000). Advances in flavonoid research since 1992. Phytochemistry, 55(6), pp.481-504.
21. Nunes, X.P., Silva, F.S., Almeida, J.R.G.D.S., Barbosa Filho, J.M., de Lima, J.T., de Araújo Ribeiro, L.A., Júnior, L.J.Q. (2012). Biological oxidations and antioxidant activity of natural products. INTECH Open Access Publisher.
22. Krishnaiah, D., Sarbatly, R., Nithyanandam, R. (2011). A review of the antioxidant potential of medicinal plant species. *Food and Bioproducts Processing*, 89(3):217-233.
23. Bravo, L. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutrition reviews, 56(11), pp.317-333.
24. Sahreen, S., Khan, MR., Khan, RA. (2011). Phenolic compounds and antioxidant activities of Rumex hastatus D. Don. Leaves. J Med Plants Res, 5:2755–2765.
25. Conforti, F., Loizzo, M. R., Statti, G. A., Houghton, P.J., Menichini, F. (2006b). Biological properties of different extracts of two *Senecio* species. *International Journal of Food Sciences and Nutrition*, 57(1/2),1 - 8.
26. Conforti, F., Marrelli, M., Statti, G., Meninchini, F. (2006a). Antioxidant and cytotoxic activities of methanolic extract and fractions from *Senecio gibbosus* subsp. *gibbosus* (GUSS) DC. *Natural Product Research*, 20(9), 805-812.
27. Liu, F., Ng, T. B. (2000). Antioxidative and Free Radical Scavenging Activities of Selected Medicinal Herbs. *Life Sciences*, 66(8), 725-735.
28. Albayrak, S., Aksoy, A., Yurtseven, L., Yaşar, A. (2014). A comparative study on phenolic components and biological activity of some *Senecio* species in Turkey. *Journal*

of Pharmacy and Pharmacology, 66, 1631-1640.