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Free radical scavenging activity of sesquiterpene indole and caryophyllene-dominated leaf and stem essential oils of *Secamone afzelii* (Schult) K. Schum and *Pergularia daemia* (Forsk) Chiov (Asclepiadaceae)

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Abstract

The chemical composition and free radical scavenging activities of leaf and stem essential oils of two Asclepiadaceae species, *Secamone afzelii* and *Pergularia daemia* were investigated. They are claimed to be used in ethno-medicine for the treatment of stomach problems, diabetes, colic, dysentery and kidney problems. Essential oils of the air-dried leaves and stem of the plants were extracted by hydro-distillation using Clavenger-type apparatus. The oils were characterized by Gas Chromatography and Gas Chromatography – Mass Spectrometry (GC-MS). The free radical scavenging activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical method. Two antioxidant standards, ascorbic acid and butylated hydroxy anisole (BHA) were used. GC and GC-MS analysis of the colourless essential oil of *S. afzelii* and *P. daemia* leaves and stems showed the presence of 6, 12, 15 and 13 compounds of terpene origin representing 84.34%, 32.08%, 87.08% and 40.08% of the total essential oil respectively. The most abundant compounds are 1H-Indole, 1-methyl-2-phenyl- (72.99%, *S. afzelii* leaf), α-santalol (11.03%, *S. afzelii* stem), (E)-caryophyllene, (53.83%, *P. daemia* leaf) and azulene (7.36%, *P. daemia* stem). The leaf essential oils of the two plants also showed good scavenging activity at 0.0625 mg/mL, 85.45% (*S. afzelii*) and 69.38% (*P. daemia*). *S. afzelii* leaf however showed better activity than the standards used. It was therefore concluded that the scavenging activity of the leaves' oil could be responsible for their use in ethno-medicine and that intrinsic or genetic as well as extrinsic or environmental factors may have been responsible for the variation in the oil constituents.

Keywords: 1H-Indole 1-methyl-2-phenyl-; (E)-caryophyllene; essential oil; free radical; Asclepiadaceae.

Introduction

The term essential oil also known as essence, volatile and ethereal oils, or simply as the oil of the plant from which they were extracted such as oil of clove is a contraction of the original 'quintessential oil'. They differ from fatty oils or fixed oils because of their high volatility [1]. They are located in different parts of the plant and have variable tastes, odours, but are colourless when fresh and pure. However, upon exposure to air, they acquire various colours [2]. Essential oils constituents are primarily made up of carbon, hydrogen and oxygen or sometimes nitrogen and sulphur. They are mainly monoterpenes and sesquiterpenes whose main metabolic pathway is through mevalonate. They could also be classified into alcohols, esters, aldehydes, ketones and phenols [3-5]. There are several different extraction methods, distillation, expression, maceration, solvent extraction, and pressing. Chromatographic techniques especially gas chromatography-mass spectrometry is also used to separate the oil constituents [6-8]. Essential oils of a large number of plants possess useful biological, pharmacological and therapeutic activities and are commercially important compounds [9]. Their utilization in the various industries is influenced by the nature of their constituents Common therapeutic properties of essential oils include:

antiseptic, antioxidant, antimicrobial, prophylactic and stress relieving. Industrially, it is used in soft drinks production especially those of citrus origin, toiletries industry, especially all kinds of mint, eucalyptus and some other herbal and fruity oils. Agriculture has also shown an increasing interest in terpenes [10-13].

Secamone afzelii and *Pergularia daemia* belong to the family Asclepiadaceae which consists of about 130 genera and 2,000 species distributed all over the world. Some of them are tropical and sub-tropical shrubs, often twining, or perennial herbs containing latex rich in triterpenes. Other constituents include cyanogenetic glycosides, saponins, tannins and cyclitols [14, 15]. They are creeping woody climber found on fences and on trees and grows to a very long length of about 2-3 cm. The plants occur in secondary forest and savannah thickets or abandoned fields or field boundaries. Both plants are widespread in West and Central Africa and Asia. *Secamone afzelii* is used in traditional medicine for stomach problems, diabetes, colic, dysentery and also for kidney problems [16, 17]. The decoction of the entire plant is prescribed for cough and catarrhal conditions, an excellent remedy for gynaecological problems especially for the treatment of sexually transmitted infections. Crushed-leaves of *S. afzelii* are applied as an enema to treat female sterility, to facilitate pregnancy and for easy delivery [18]. It is also taken as an aphrodisiac and to improve blood circulation. In Nigeria, the dried ground leaves of *S. afzelii* are mixed with black soap to treat measles. Phytochemical screening of the methanol extract of the leaves of *S. afzelli* revealed the presence of alkaloids, tannins, cardiac glycosides and saponins [19, 20]. *P. daemia* is used as an anthelmintic, analgesic, laxative, antipyretic, expectorant and emetic. It is useful in treating cough, toothache and infantile diarrhoea. Its extract is used for uterine and menstrual troubles and to facilitate parturition [21, 22]. Suresh and Mishra [23] reported that the flavonoid in the ethanol extract of aerial parts of *P. daemia* exhibited significant hepatoprotective effect against CCl_4 induced hepatotoxicity in rats. The lipophilic fraction of the stem bark was shown to possess antimalarial activity against *Plasmodium falciparum* while the implication of the mineral ratios of *P. daemia* in human diets has also been studied [24, 25].

Other biological studies reported include anti-implantation and abortifacient effect [26], antihyperglycemic (anti-diabetic) activity [27], anti fertility activity [28] and antimicrobial [29]. Plants secondary metabolites like flavonoids alkaloids, terpenoids, tannins, steroids and carbohydrates have also been reported [30, 31].

This study aimed at determining the chemical constituents of the essential oils of *S. afzelii* and *P. daemia* as there was no information on the essential oil of the two plants in literature. The study also seeks to investigate the free radical scavenging activity of essential oils using 2,2-diphenylpicryl hydrazyl radical method. Free radicals are known to cause various degenerative disorders, like mutagenesis, carcinogenesis, cardiovascular disturbances and ageing [32]. They are produced in biological systems and are also encountered exogenously [33]. Antioxidants are the compounds, which combat the free radicals by intervening at any one of the three major steps of the free radical mediated oxidative process, viz., initiation, propagation and termination [34]. These antioxidants are also produced by biological system and occur naturally in many foods and the balance between oxidants and antioxidants decides the health and vigour [35-39]. The search for natural antioxidants would therefore contribute to the growing campaign on the importance of antioxidants.

Materials and methods

Materials

Plant collection and identification: Fresh leaf and stem samples of *S. afzelii* were collected from vegetation around the Faculty of Pharmacy, University of Ibadan, while *P. daemia* leaf and stem samples were collected at the Botanical Garden of the same University. Both were collected in August, 2015 and duly identified at the Botany Department, University of Ibadan.

Reagents: Hexane and methanol (BDH chemical), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich). Reference standards: ascorbic acid, butylated hydroxyanisole (BHA) (BDH chemicals).

Major equipment used

The following equipment and apparatus were used: Heating mantle, electronic weighing balance (OHAUS), Oven (Carbolite), Clavenger apparatus, glass condenser, hydro-distillation flask, Gas chromatograph/GC-MS (HP 6890) and UV spectrophotometer (Unico1200 and Perkin Elmer Lambda 25 model).

Methods

Preparation of samples

The leaves and stem were air dried for two weeks,

chopped into pieces and the oil was extracted by hydro distillation.

Extraction of essential oil

Hydro-distillation method in accordance with the European Pharmacopoeia [40] was used. Dried leaves (253 g) and stem (354 g) of *S. afzelii* and leaves (267 g) and stem (403 g) of *P. daemia* were separately transferred into a 10-Litres round bottom flask. A Clavenger type apparatus connected to a condenser was fitted to the round bottom flask and placed on a heating mantle. The heater was set at a thermo-stated temperature of 100°C. Each of the essential oil was collected after 3 hours and made to dissolve in a known quantity of n-Hexane (injected into the Clavenger to trap the oil). The oil was then collected in vials and stored below 4°C before taken for analysis, Table 1.

Table 1: Physical properties of the essential oils.*

Properties	Leaves of *S. afzelii*	Stem of *S. afzelii*	Leaves of *P. daemia*	Stem of *P. daemia*
Colour	Colourless	Colourless	Colourless	Colourless
Odour	Woody	Woody	Leafy	Spicy

*Physical characteristics of the essential oils obtained by hydro distillation.

Analysis of the essential oils

Gas chromatography: Analysis of the essential oil was carried out on an HP 6890 powered with ChemStation Rev. A09.01 [1206] Software at the following specifications: injection temperature (split injection), split ratio: (20:1), carrier gas: hydrogen, flow rate: 1.0 ml/min, inlet temperature: 150°C, column type: HP 5MS, column dimensions: 30 m × 0.25 mm × 0.25 ìm, oven program: initial at 40°C, ramped at 5°C/min to 200°C, and run at 220°C for 2 minutes. The chromatograms of the samples are presented in Figures 1-4.

Gas chromatography – mass spectrometry: HP 6890 powered with ChemStation Rev. A09.01 [1206] Software was also used for GC-MS analysis. GC oven temperature and conditions were as described above. The injector temperature was set at 220°C and mass spectra were recorded at 70 eV.

Identification of components: The various constituent and their percentage compositions were obtained from electronic integration measurement using a Flame Ionization Detector (FID) set at a temperature of 300°C. The peak numbers and relative percentages

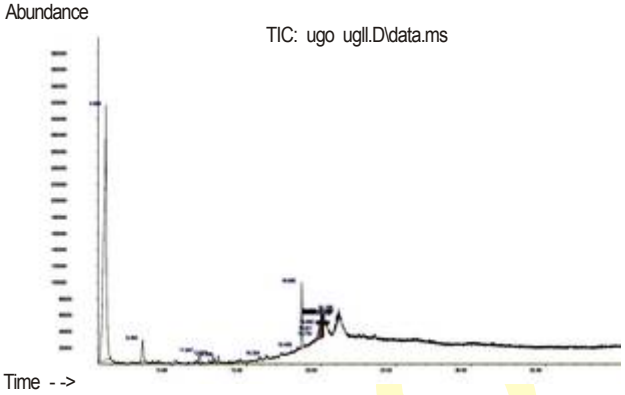

Figure 1. Gas chromatogram of the leaf essential oil of *S. afzelii.*

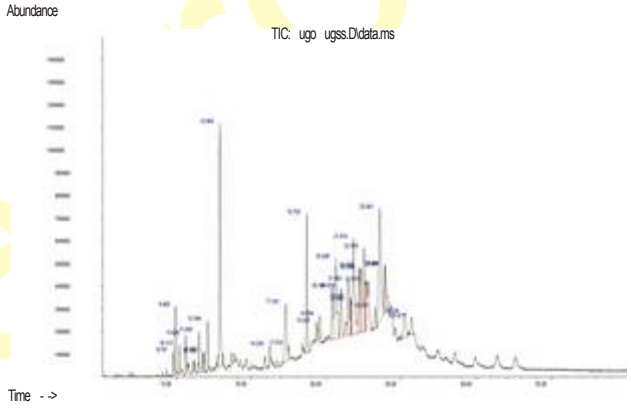

Figure 2. Gas chromatogram of stem essential oil of *S. afzelii.*

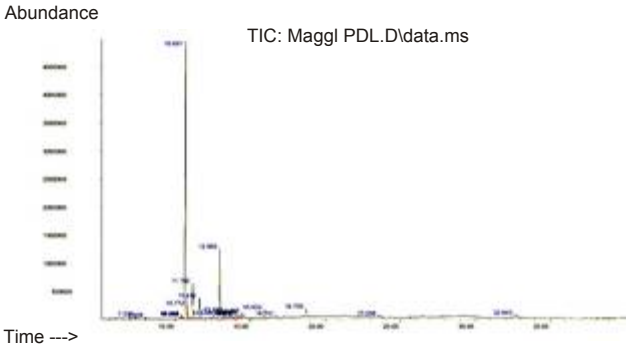

Figure 3. Gas chromatogram of the leaf essential oil of *P. daemia.*

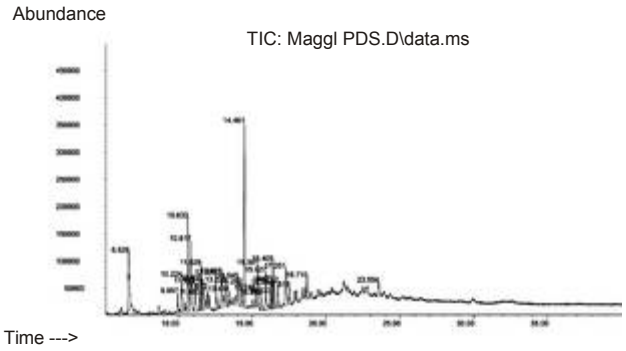

Figure 4. Gas chromatogram of the stem essential oil of *P. daemia.*

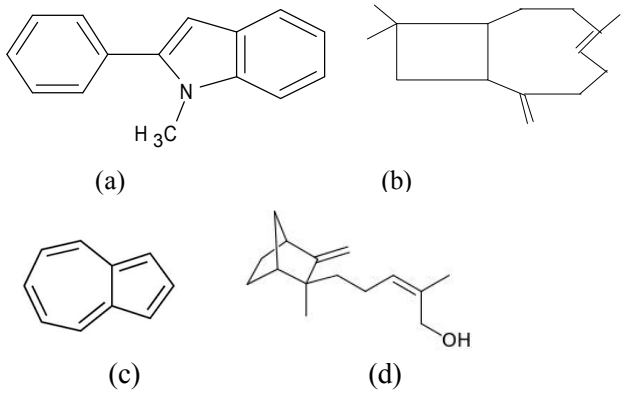

Figure 5: Structures of 1H-Indole, 1-methyl-2-phenyl- (a), (E)-caryophyllene (b), azulene (c) and α-santalol (d).

of the characterized compounds were recorded. Individual constituent were identified by their retention time identical to the compounds known from literature data and also by comparing their spectral with those stored in the NIST 0.8L/Database/chemstation data system [41-43]. The peak numbers and relative percentages of the characterized components are given in Tables 2-5.

Free radical scavenging activity

A 3.94 mg of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was dissolved in 100 mL of methanol to give a 100 µM solution. To 3.0 mL of the methanol solution of DPPH was added 0.5 mL of the oil, from stock solution of 0.1 g in 100 mL. The decrease in absorption of DPPH at 517 nm was measured in a UV spectrophotometer after 10 minutes of incubation. The tests were run in triplicates and the average results recorded. The same experiment was carried out on the standards; ascorbic acid, and BHA (Butylated Hydroxyl Anisole) with doses ranging from 0.1-0.0625 mg/mL. The percentage inhibition was also calculated [44, 45].

Results and discussion

The percentage yield of the essential oil of *S. afzelii* was 0.51% (leaf), 0.47% (stem) while leaf essential oil of *P. daemia* was 0.99% and the stem was 0.59%. The oils were colourless with characteristic odour as indicated in Table 1. Chemical constituents in the oils from the two plants varied even though they belong to the same family. The chromatograms showing the abundance and retention time of the different constituents are presented in Figures 1-4.

The leaf essential oil of *S.afzelii* contained 6 identified components totalling 84.34%, while 12 compounds were detected in the stem essential oil with a total percentage of 32.08%. A sesquiterpene indole. 1H-Indole, 1-methyl-2-phenyl- was the most abundant constituent in the leaf essential oil of *S. afzelii* (72.99%). The major compound in the stem essential oil of *S. afzelii* was a terpenol, α-Santalol (11.03%). The diterpenoid phytol was found in both the leaf (6.58%) and stem (5.18%) essential oils. α-Farnesene was also detected in minute quantity in both the leaves and stem. The leaf essential oil of *P. daemia* contained 15 components representing 87.08% of the whole essential oil. Compounds detected include (E)-caryophyllene (53.83%), caryophyllene oxide (15.61%) and β-Ionone (4.11%), while 13 components were however detected in the stem essential oil representing 40.38%. Azulene (7.36%), (E)-caryophyllene (7.24%), carene (6.71%), and caryophyllene oxide (4.01%) were the main components. The results of GC-MS analysis of the colourless essential oils of *S. afzelii* and *P. daemia* showed that the oils were rich in sesquiterpene hydrocarbons, oxygenated sesquiterpenes and sesquiterpene indole (leaf essential oil of *S. afzelii* only). The presence of 1H-Indole, 1-methyl-2-phenyl- and (E)-Caryophyllene as the most abundant constituents in the leaves of *S. afzelii* and *P. daemia* plant oils respectively even though they belong to the same family shows that intrinsic or genetic as well as extrinsic or environmental factors may have been responsible for the variation in the oil yield and constituents. Factors such as cultivation location, harvesting-time and conditions, also affects the composition of the oils. 1H-Indole, 1-methyl-2-phenyl- is used as a pigment dye, dye intermediate, or flourescent brightener and a synthetic intermediate for many compounds while (E)-caryophyllene, a bicyclic sesquiterpene found in a number of plant essential oil has been reported to be an effective anti-inflammatory, antioxidant, antibiotic and anticancer agent [46, 47].

Table 2. Chemical constituents of the leaves and stem essential oils of *S. afzelii* and *P. daemia.**

S/N	AI	Compound	Molecular formula	T.P *S .afzelii* Leaves	T.P *S .afzelii* Stem	T.P *P. daemia* Leaves	T.P *P. daemia* Stem
1.	969	(-)-trans-pinane	$C_{10}H_{18}$	-	-	1.84	2.28
2.	1,004	3- Carene	$C_{10}H_{16}$	-	-	-	3.23
3.	1,014	α-Terpinene	$C_{10}H_{16}$	-	-	2.10	-
4.	1,054	γ-Terpinene	$C_{10}H_{16}$	-	-	-	0.88
5.	1,171	3-methylveratrole	$C_9H_{12}O_2$	-	-	0.74	-
6.	1,274	3,5-Di-tert-butyl phenol	$C_{14}H_{22}O$	-	-	0.56	-
7.	1,289	Azulene	$C_{10}H_8$	-	-	-	7.36
8.	1,345	α – Cubebene	$C_{15}H_{24}$	-	2.15	-	1.44
9.	1,350	α – Longipinene	$C_{15}H_{24}$	-	0.83	1.18	-
10.	1,387	β – Cubebene	$C_{15}H_{24}$	-	1.67	-	-
11.	1,389	β – Elemene	$C_{15}H_{24}$	-	1.09	-	-
12.	1,393	1H-Indole, 1-methyl-2-phenyl-	$C_{15}H_{13}N$	72.99	-	-	-
13.	1,399	Longipinocarvone	$C_{15}H_{22}O$	-	-	-	2.26
14.	1,408	(E)- Caryophyllene	$C_{15}H_{24}$	-	1.27	53.83	7.24
15.	1,410	α-Cedrene	$C_{15}H_{24}$	-	-	0.80	-
16.	1,429	Thujospene (cis)	$C_{15}H_{24}$	-	-	-	1.31
17.	1,439	Aromadendrene	$C_{15}H_{24}$	-	-	0.76	3.01
18.	1,472	B-Cadinene	$C_{15}H_{24}$	-	0.70	-	-
19.	1,487	β-Ionone	$C_{13}H_{20}O$	-	-	4.11	2.37
20.	1,495	Cis-Z-α-bisabolene epoxide	$C_{15}H_{24}O$	-	-	0.54	-
21.	1,505	α – Farnesene	$C_{15}H_{24}$	0.84	0.60	-	-
22.	1,512	4-methylene-1-2-(2-methyl-1-propen-1-yl)-1vinylcycloheptane	$C_{15}H_{24}$	-	-	-	3.28
23.	1,522	Δ-Cadinene	$C_{15}H_{24}$	-	1.65	-	-
24.	1,582	Caryophyllene oxide	$C_{15}H_{24}O$	-	-	15.60	4.01
25.	1,600	Hexadecane	$C_{16}H_{34}$	1.05	-	-	-
26.	1,674	A-Santalol	$C_{15}H_{24}O$	-	11.03	-	-
27.	1,682	(E)-6,7-dihydrofarnesol	$C_{15}H_{28}O$	-	-	0.62	-
28.	1,742	Farnesol (2E, 6E)	$C_{15}H_{26}O$	-	-	0.64	-
29.	1,833	Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	-	-	1.69	-
30.	1,895	1-Nonadecene	$C_{19}H_{38}$	-	-	-	1.41
31.	1,942	Phytol	$C_{20}H_{40}O$	6.58	5.18	-	-
32.	1,959	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	-	4.98	-	-
33.	2,000	Eicosane	$C_{20}H_{42}$	2.21	-	-	-
34.	2,300	Tricosane	$C_{23}H_{48}$	-	0.93	-	-
35.	2,900	Nonacosane	$C_{29}H_{60}$	-	-	2.07	-
36.	3,500	17-Pentatriacontene	$C_{35}H_{72}$	0.67	-	-	-
				84.34%	32.08%	87.08%	40.38%

*Percentages calculated from the flame ionization detection data. RT = Retention Time; AI = Arithmetric Retention Index on DB-5 (=SE54) column using alkanes; T.P = Total Percentage.

Azulene the major constituent in the stem essential oil of *P. daemia* is a natural pigment in many plants, fungi and invertebrates. It is used as an herbal remedy, in skin care products as a natural colouring agent. It is claimed to be safe as it does not pose any risk of genetic mutations or cellular damage that can lead to cancer. It is known to reduce inflammation in the skin tissue or reduce the redness of swellings on the skin [47, 48]. Essential oils isolated from *S. afzelii* and *P. daemia* are therefore important component of cosmetic and agricultural products.

Free radical scavenging activity of the essential oils DPPH assay is an accurate means of screening plants for antioxidant activity. It is based on the measurement

Table 3. Absorbance values obtained in the free radical scavenging effect of the leaves and stem essential oil of *S. afzelii* and *P. daemia* on DPPH.*

Conc *(mg/ml)*	SAL	SAS	PDL	PDS	Ascorbic acid	BHA
1.0	0.267±0.003	0.973±0.005	0.646±0.002	0.903±0.003	0.372±0.003	0.261±0.005
0.50	0.237±0.003	0.967±0.003	0.548±0.001	0.923±0.006	0.316±0.004	0.255±0.007
0.25	0.174±0.001	0.951±0.010	0.496±0.001	0.929±0.003	0.289±0.007	0.251±0.000
0.125	0.155±0.002	0.934±0.005	0.408±0.002	0.934±0.001	0.268±0.005	0.250±0.007
0.0625	0.143±0.002	0.919±0.0007	0.301±0.004	0.938±0.001	0.253±0.003	0.249±0.007

*Absorbance measurement of leaves and stem essential oil of *S. afzelii* (SAL and SAS), leaves and stem essential oil of *P. daemia* (PDL and PDS), standards: ascorbic and butylated hydroxylanisole (BHA) at 517 nm. Absorbance of DPPH at 517 nm = 0.983.

of the scavenging capacity of antioxidants towards it. The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to form the corresponding hydrazine [12, 42]. DPPH is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerise, like most other free radicals. The delocalization also gives rise to the deep violet colour, with an absorption in methanol solution at around 517-520 nm. On mixing DPPH solution with a substance that can donate a hydrogen atom, it gives rise to the reduced form with the loss of violet colour [37, 44, 45].

The results of the free radical scavenging activity of the two Asclepiadaceae species, *S. afzelii* and *P. daemia* on 2,2-diphenylpicrylhydrazyl radical (DPPH) are presented in Table 3 and Figure 6. There was reduction in absorbance values of the oil samples after incubation in DPPH. *S. afzelii* leaf essential oil (SAL) with absorbance value of 0.143±0.002 at 0.625 mg/mL was the most active and better in activity than the standards ascorbic acid and BHA at the same concentration (Table 3). Figure 6 also showed that SAL gave the highest inhibition percent having 85.45%.

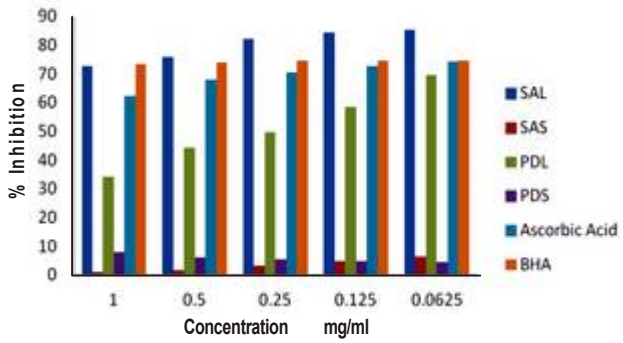

Figure 6. Percentage inhibition of DPPH free radical scavenging activities of the leaves and stem essential oil of *S. afzelii* (SAL and SAS), leaves and stem essential oil of *P. daemia* (PDL and PDS) and standards ascorbic and butylated hydroxyl hydroxylanisole (BHA).

The scavenging activities of the leaf essential oils of both plants were better at lower concentrations which is also true of the standards. At 0.0625 mg/ml, SAL with 85.45% inhibition, was the most active and better than PDL (69.38%), Ascorbic acid (74.26%) and BHA (74.67%). The stem essential oils on the other hand exhibited very low inhibition property having 6.51% and 4.58% for SAS and PDS at 0.0625 mg/ml respectively (Figure 6).

Conclusion

GC and GC-MS analysis of the essential oils obtained from *S. afzelii* and *P. daemia* revealed that sesquiterpene hydrocarbons, oxygenated sesquiterpenes and sesquiterpene indole (leaf essential oil of *S. afzelii* only) were the main components. The leaf and stem essential oils of *S. afzelii* contained 6 and 12 components totalling 84.34% and 32.08%. A sesquiterpene indole. 1H-Indole, 1-methyl-2-phenyl- (72.99%) is the most abundant constituent in the leaf essential oil while α-santalol (11.03%) is the major component in the stem essential oil. *P. daemia* leaf and stem essential oil contain 15 and 13 components respectively with a total percentage of 87.08% and 40.38%. (E)-caryophyllene (53.83%) and caryophyllene oxide (15.61%) were the prominent compounds in the leaf while azulene (7.26%) and (E)-caryophyllene (7.24%) were the major components in the stem essential oil. The presence of 1H-Indole, 1-methyl-2-phenyl- and azulene confirm the use of these plants as pigment and other herbal application. The leaf essential oil of the two plants also showed better free radical scavenging activity at the lowest

concentrations (0.0625 mg/mL) using the 2,2-diphenylpicrylhydrazyl radical (DPPH) method. The leaf essential oil at low concentrations can be utilized in antioxidant deficient drugs especially in the treatment of cardiovascular diseases.

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