



Chemical Constituents and Biological Activities of *Passiflora foetida* (Linnaeus) Stem and Fruit Essential Oils

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Abstract This study determined the essential oil constituents, antioxidant and antimicrobial activities of *Passiflora foetida* (Passifloraceae). Fruit and stem of *P. foetida* were hydrodistilled by an all glass-Clevenger apparatus and chemical compositions of the essential oils (EOs) were characterized using the Gas Chromatography-Mass Spectrometry (GC/MS) technique. Antioxidant and antimicrobial activities were examined using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH*) and agar diffusion method respectively.

EOs gave good yields of 0.40% (stem) and 0.24% (fruit) (w/w). Thirteen (99.97%) and seventeen (100%) compounds with their major constituents; pentadecanal (30.11%) and oleamide (19.32%) were identified in EOs of stem and fruit respectively. The classes of compounds identified in stem oil were alkanals (48.54%), alkanols (21.43%), esters (20.30%), amide (6.65%), alkanones (1.99%) and hydrocarbons (1.06%); while fruit oil gave hydrocarbons (49.04%), amide (19.32%), alkanals (12.01%), carboxylic acids (8.10%), esters (4.94%), alcohol (3.34%) and cyclic ether (3.24%).

Fruit oil showed higher antioxidant activity than stem oil; and had higher activity than α -Tocopherol (20 - 0.0625%) for 10 and 30 min incubation periods. Stem oil exhibited strong activity against *Staphylococcus aureus* at 50 mg/mL. This further confirms the antimicrobial activity of *P. foetida* extracts, thereby justifying its use as an anti-infective in traditional medicine.

Keywords *Passiflora foetida*, Essential oil, Antioxidant, *Staphylococcus aureus*, GC/MS

Introduction

Essential oils (EOs) originate naturally from various therapeutic plants and have a distinctive aroma with a density lower than that of water [1]. EOs have been found useful as antioxidant, antiviral, antimalarial, insecticidal, anticancer and antibacterial agents, to mention a few of its uses. Phytochemicals present in EOs have been found responsible for these biological activities [2, 3].

The genus *Passiflora* (Passion fruit) belongs to the family Passifloraceae. It is the largest and the most widespread genus of tropical flora [4]. Passion fruit is commonly found in many tropical and subtropical countries as an important plant due to its edible fruit, ornamental use and medicinal properties. Some common species include: *P. edulis*, *P. flavicarpa*, *P. ligularis*, *P. mollissima*, *P. quadrangularis*, and *P. caerulea*. *Passiflora* species are used in folk medicine mainly because of their sedative and anxiolytic properties [5, 6]. However, other properties of passion fruit include hypoglycemic, anti-inflammatory, anti-spasmodic, antimicrobial, antidiabetic, antioxidant and hypotensive effects [7-10]. Alkaloids, phenols, glycosyl flavonoids and cyanogenic compounds are class of phytochemicals isolated from this genus [6].



Passiflora foetida commonly known as “stinking passion flower” is a swarming vine that produces an edible fruit. It is found in river beds, dry forest floors, and way side thickets, covering the top of thorny shrubs and also growing near hamlets [11]. In Nigeria, *P. foetida* is traditionally used for the treatment of anxiety, stress, insomnia and hysteria [12]. The ethanol extract has been reported to have anti-ulcerogenic, analgesic and anti-inflammatory activities which could be related to its antioxidant potentials [13, 14, 15]. Secondary metabolites of *P. foetida* contain alkaloids, phenols, glycoside flavonoids and cyanogenic compounds. Phytochemicals such as hydrocyninic acid, harmine, harmalol, harmine were reported present in *P. foetida* [16]. Passifloricins, which are polyketides α -pyrones, have been isolated from *P. foetida* resin [17]. The anti-bacterial tests of leaf and fruit extracts, against four human pathogenic bacteria showed that both parts were active, which may be as a result of polyacetylenic compounds [11, 18, 19]. There is little or no report on volatile oils of *P. foetida* and so this study presents for the first time the essential oil composition, antioxidant and antimicrobial activities of *P. foetida* oils (stem and fruit).

Materials and Methods

Sample Collection, Preparation and extraction

Passiflora foetida was collected in April, 2016 from Ojoo along Arulogun road, Idiomo, Ibadan. Sample was identified and authenticated at the Department of Taxonomy of Forestry Research Institute of Nigeria (FRIN), Ibadan and voucher number FHI 110592 was issued for it. The fresh stem and fruit of *P. foetida* were cut into smaller pieces and hydro-distilled using an all glass-Clevenger apparatus for 2 - 3 h [20]. The oils obtained were stored in the refrigerator until time for analysis.

Identification and Quantification of the Essential Oils Constituents

Essential oils were subjected to GC-MS analysis on an Agilent 7809A gas chromatograph hyphenated with an Agilent mass detector having split/splitless injector interfaced to mass selective detector operating at 70 eV. The other experimental parameters were as follows: The ion source temperature was set at 200°C with mass spectral range of m/z 50-700 at a scan rate of 1428 amu/sec. GC column was equipped with an HP-5MS column with a length of 30 m, having an internal diameter of 0.25 mm and a film thickness of 0.25 μ m. Oven temperature was programmed as follows: initial temperature 80°C for 2 min, increased at 10°C/min to temperature of 240°C for 6 min. Helium was utilized as the carrier gas at a flow rate of 1 mL/min. Injection volume, linear velocity and pressure were respectively adjusted at 1.0 μ L, 362 cm/s and 56.2 KPa respectively. Both injector and detector temperatures were fixed at 250°C. Identification of the essential oil components were based on their retention indices as determined with reference to a homologous series of normal alkane as well as by comparison of their mass spectral fragmentation patterns (NIST data/base/chemstation data system) with the data previously reported in the literature [21].

Determination of Antioxidant Activity of the Essential oils

A methanol solution of DPPH• (0.1 mM) was prepared by dissolving 3.94 mg of DPPH• in 100 mL of methanol. The stock solution of each sample was prepared by dissolving 0.5 mL of the sample in 2 mL of methanol. From this solution, 0.5 mL was taken and dispensed into 3 mL of the methanol solution of DPPH•. This gave 100% concentration of the sample. From the stock solution, 1.0 mL was taken into 1 mL of methanol and 0.5 mL of this solution was dispensed into 3.0 mL of DPPH• methanol solution to give 50% concentration. More serial dilutions were done till 3.125% concentration of each oil extract was achieved. For each of the standards, namely; ascorbic acid, butylated hydroxyl anisole (BHA), garlic, ginger and α -tocopherol, 1.0 mg was dissolved in 2 mL of methanol and serial dilutions were done to give concentrations like those of the essential oils. The DPPH solution with oil samples were allowed to incubate for 10 min and 30 min; and the absorbance of each solution was recorded using a UV Spectrophotometer (Perkin Elmer UV/Visible, Model number: LAMDA 25) at a wavelength of 517 nm. The same procedure was done for the standards. A blank DPPH• solution was analyzed as well and each test was carried out in triplicate. Radical scavenging activity (%) was calculated by the following formula (Eqn.1):

$$SA (\%) = \frac{A_c - A_s}{A_c} \times 100$$

Where; SA is scavenging activity of free radical DPPH in percent; A_c is the absorbance of control/ blank without extract at 517 nm; A_s is the absorbance of sample extract at 517 nm [22].



Determination of Antimicrobial Activity of the Essential oils

The antimicrobial activities of the essential oils of the stem and fruit of *P. foetida* were determined by agar well diffusion method. Ten microorganisms were used; four gram-negative bacteria namely: *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Klebsiellae pneumonia*; two gram-positive bacteria namely: *Bacillus subtilis* and *Staphylococcus aureus*; and four fungi namely: *Candida albicans*, *Aspergillus niger*, *Penicillium notatum* and *Rhizopus stolonifer*. The microorganisms were obtained from the stock cultures of the Department of Pharmaceutical Microbiology, University of Ibadan, Nigeria. From the Stock that contains 1 mL of the oil, 0.5 mL was taken to 2nd tube that contains 0.5 mL of the diluents (methanol). From the 2nd tube that has the mixture of oil and methanol of 1 mL volume, 0.5 mL was taken to the 3rd tube till the 6th tube to get 50%, 25%, 12.5%, 6.25% and 3.125% concentrations. The 7th and 8th test tube were for negative and positive controls. From the diluted organism (10^{-2}), 0.2 mL was taken into the prepared sterile nutrient agar which was at 45°C, then aseptically poured into sterile petri dishes, allowed to solidify for about 45-60 min. With the aid of a sterile cork borer of 8 mm diameter, the wells were made according to the number of graded concentrations of the sample. In each well, the different graded concentrations of the sample were introduced, this was done in duplicates. The plates were allowed to stay on the bench for 2 h to allow pre-diffusion. The plates were incubated uprightly in the incubator for 18-24 h at 37°C for bacteria and 48 h at 28°C for fungi [23]. Reference standards were gentamicin (10 mg/mL) for bacteria and tioconazole (70%) for fungi. Methanol was used as negative control.

Results and Discussion

Chemical composition (GC/MS)

The yield of *P. foetida* EOs of stem and fruit gave 0.40% and 0.24% (w/w) respectively. Tables 1 and 2 showed the compounds identified in the oils while table 3 showed the common compounds in them. A total of thirteen (13) and seventeen (17) compounds were identified from stem and fruit oils of *P. foetida* respectively.

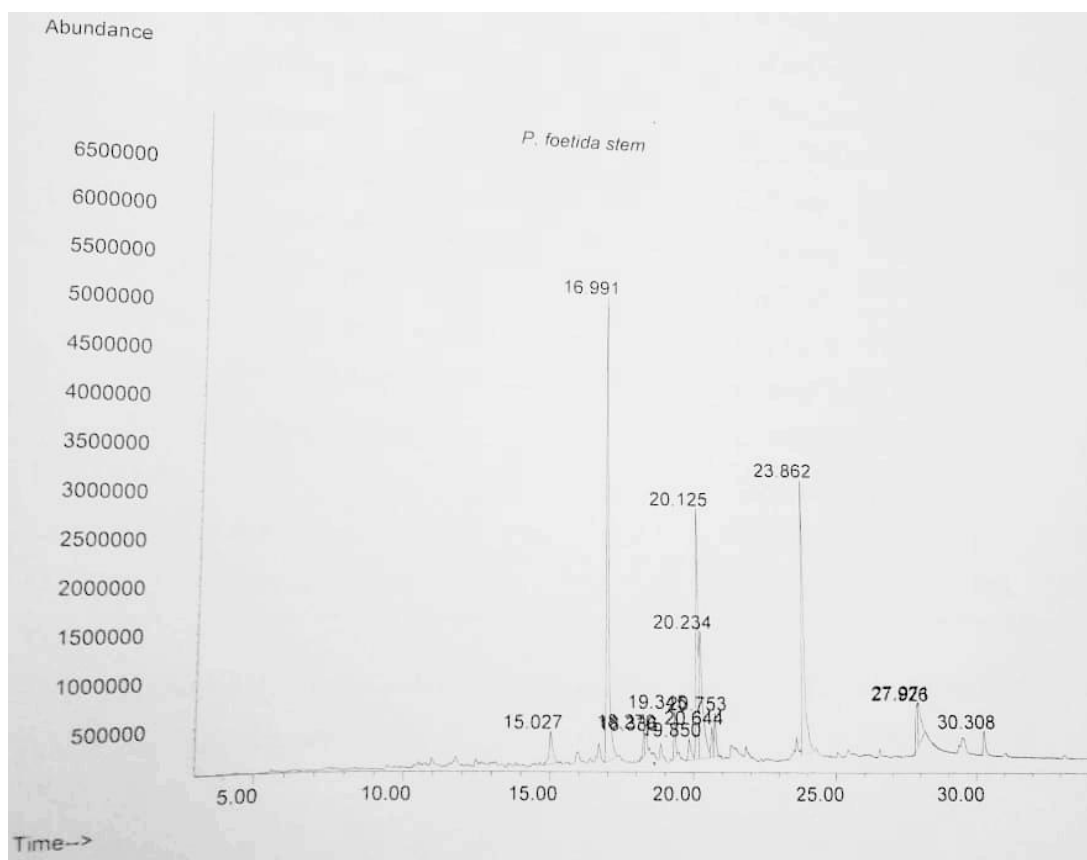
Essential oil of *P. foetida* stem (Table 1) contained alkanals (48.54%), alkanols (21.43%), esters (20.30%), amide (6.65%), alkanones (1.99%), hydrocarbons (1.06%). Pentadecanal was the most abundant (30.11%), followed by a diterpene alcohol, Phytol (19.74%) which has been reported to have high antimicrobial activity, high stability but low toxicity [22]. Fruit essential oil constituents (Table 2) were hydrocarbons (49.04%), amide (19.32%), alkanals (12.01%), carboxylic acid (8.10%), esters (4.94%), alkanol (3.34%) and cyclic ether (3.24%). Oleamide was the most abundant compound followed by trans-3-eicosene and galaxolide (ether) was the least copious. Oleamide was reported to have sleep-inducing ability in animals and this justifies the ethnomedicinal use of *Passiflora* species against insomnia in Brazil [12, 24, 25].

Table 1: Chemical composition of the essential oil of *P. foetida* stem

No.	RT (Min)	Compounds	MF	TIC (%)
1	15.03	Tetradecanal	C ₁₄ H ₂₈ O	3.38
2	16.99	Pentadecanal	C ₁₅ H ₃₀ O	30.11
3	18.27	Cyclotetradecane	C ₁₄ H ₂₈	1.06
4	18.34	Bromoacetic acid, tetradecylester	C ₁₆ H ₃₁ BrO ₂	1.29
5	19.34	Hexahydrofarnesylacetone	C ₁₈ H ₃₆ O	1.99
6	19.85	Phthalic acid, 8 chlorooctylisobutylester	C ₂₀ H ₂₉ O ₄ Cl	1.64
7	20.13	Z,Z-7,10-Hexadecadienal	C ₁₆ H ₂₈ O	15.05
8	20.24	E,E-6,11-Tridecadien-1-ol acetate	C ₁₅ H ₂₆ O ₂	13.26
9	20.64	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	C ₁₇ H ₃₂ O	1.69
10	20.75	Methylpalmitate	C ₁₇ H ₃₄ O ₂	2.50
11	23.86	Phytol	C ₂₀ H ₄₀ O	19.74
12	27.94	Oleamide	C ₁₈ H ₃₅ NO	6.65
13	30.31	Diethylphthalate	C ₂₄ H ₃₈ O ₄	1.61
Total				99.97

RT: Retention time in minutes; MF: Molecular formula; TIC(%): Total ion concentration in percentage



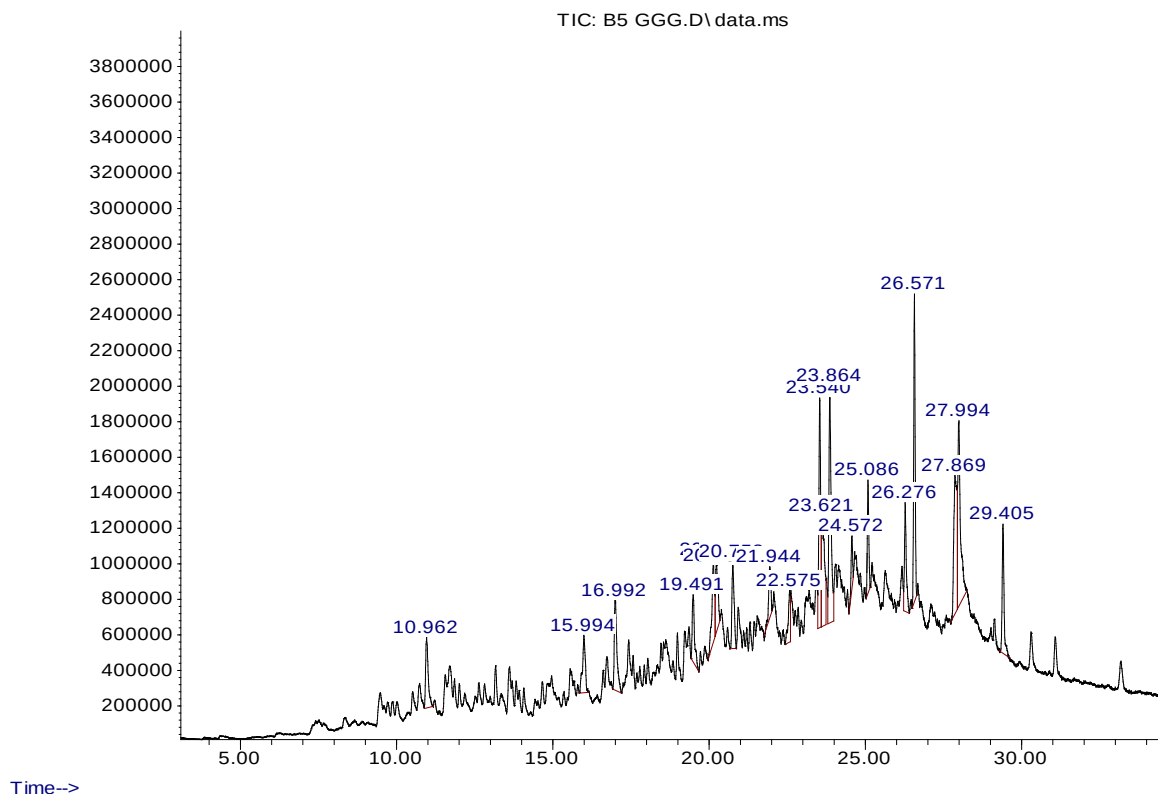
Figure 1: Chromatogram of essential oil of *P. foetida* stemTable 2: Chemical composition of the essential oil of *P. foetida* fruit

No.	RT (min)	Compounds	MF	TIC (%)
1	10.96	Caryophyllene	C ₁₈ H ₂₄	3.74
2	16.00	7a-isopropenyl-4,5-dimethyloctahydroniden-4-ylmethanol	C ₁₅ H ₂₆ O	3.34
3	16.99	Tetradecanal	C ₁₄ H ₂₈ O	4.92
4	19.49	Galaxolide	C ₁₈ H ₂₆ O	3.24
5	20.13	Linoleic acid	C ₁₈ H ₃₂ O ₂	4.24
6	20.24	Cyclotetradecane	C ₁₄ H ₂₈	3.64
7	20.76	Palmitic acid	C ₁₆ H ₃₂ O ₂	3.86
8	22.26	Octadecane	C ₁₈ H ₃₈	4.43
9	23.54	Heneicosane	C ₂₁ H ₄₄	8.22
10	23.62	Trogodermal	C ₁₇ H ₃₂ O	7.09
11	23.86	Trans-3-eicosane	C ₂₀ H ₄₀	11.01
12	24.57	Ethyllinoleate	C ₂₀ H ₃₆ O ₂	1.58
13	25.09	Carbonic acid octadecylvinylester	C ₂₁ H ₄₀ O ₃	3.36
14	26.28	9-Tricosene	C ₂₃ H ₄₆	4.11
15	26.57	Tricosane	C ₂₃ H ₄₈	9.06
16	27.93	Oleamide	C ₁₈ H ₃₅ NO	19.32
17	29.40	5-butylidocosane	C ₂₆ H ₅₄	4.84
Total				100

RT: Retention time in minutes; MF: Molecular formula; TIC(%): Total ion concentration in percentage



Abundance

Figure 2: Chromatogram of essential oil of *P. foetida* fruit**Table 3:** Common compounds identified in *P. foetida* stem and fruit oils

S/N	Compound	MF	Samples (TIC %)	
			PFF	PFS
1	Oleamide	C ₁₈ H ₃₅ NO	19.32	6.65
2	Cyclotetradecane	C ₁₄ H ₂₈	3.64	1.06
3	Tetradecanal	C ₁₄ H ₂₈ O	4.92	3.38

RT: Retention time in minutes; MF: Molecular formula; TIC (%): Total ion concentration in percentage, PFS: *Passiflora foetida* stem; PFF: *Passiflora foetida* fruit

Antioxidant activity

DPPH free radical scavenging method offers the first approach for evaluating the antioxidant potential of a compound, an extract or other biological sources. This is the simplest method, wherein the prospective compound or extract is mixed with DPPH solution and absorbance is recorded after a defined period [26]. The number of DPPH molecules that are reduced seems to be correlated with the number of electron-donating atoms or hydroxyl groups in the antioxidant molecule [22, 27].

The antioxidant activity of *P. foetida* stem and fruit EOs were compared with ascorbic acid (ASA), butylated hydroxyl anisole (BHA), α -tocopherol (α -T), ginger (GNR) and garlic (GLC) used as standards. The decrease in antioxidant activity of all test samples are as shown; ASA > BHA > GNR > GLC > PFF > PFS > α -T (Table 4 and 5). Ascorbic acid showed the highest activity and the least activity was observed in α -tocopherol at 10 and 30 min of incubation, the stem and fruit EOs of *P. foetida* showed better activity as compared to α -tocopherol at all concentrations (20 - 0.625%) and incubation periods (10 and 30 min). However, the fruit oil showed more activity than stem oil at both incubation periods. Presence of fatty acids such as palmitic and linoleic acids in fruit oil may be responsible for the stronger antioxidant activity [28].



Table 4: Percentage inhibition of samples at 10 min incubation period

Samples	(Concentration %)					
	20	10	5	2.5	1.25	0.625
PFS	18.10±0.0015	18.31±0.0010	19.25±0.0000	19.54±0.0006	17.53±0.0010	17.34±0.0006
PFF	20.06±0.0000	18.99±0.0010	18.11±0.0000	16.75±0.0006	17.62±0.0000	18.01±0.0010
ASA	97.51±0.0006	97.63±0.0006	97.74±0.0006	97.97±0.0006	98.19±0.0006	97.97±0.0000
BHA	96.56±0.0000	96.91±0.0000	96.91±0.0006	96.91±0.0000	92.90±0.0012	97.15±0.0012
GNR	66.58±0.0012	81.53±0.0010	81.16±0.0020	67.09±0.0010	51.38±0.0006	56.28±0.0006
GLC	26.92±0.0015	27.17±0.0015	7.42±0.0010	5.28±0.0015	13.83±0.0010	16.23±0.0006
α-T	14.57±0.0006	14.45±0.0006	15.61±0.0000	16.30±0.0012	15.95±0.0010	15.84±0.0006

Percentage inhibition expressed as mean ± Standard deviation. PFS: *Passiflora foetida* stem; PFF: *Passiflora foetida* fruit; ASA: Ascorbic Acid; BHA: Butylatedhydroxyanisole; GNR: Ginger; GLC: Garlic; α-T: α-Tocopherol

Table 5: Percentage inhibition of samples at 30 min incubation period

Samples	(Concentration %)					
	20	10	5	2.5	1.25	0.625
PFS	18.22±0.0006	18.12±0.0000	18.60±0.0006	19.27±0.0006	16.87±0.0006	16.68±0.0006
PFF	21.07±0.0015	19.92±0.0006	19.44±0.0026	18.01±0.0010	18.77±0.0000	18.10±0.0006
ASA	96.81±0.0006	96.81±0.0000	97.42±0.0006	97.91±0.0012	98.03±0.0010	97.67±0.0000
BHA	96.53±0.0006	96.99±0.0006	96.88±0.0000	96.99±0.0000	96.88±0.0006	87.50±0.0010
GNR	69.63±0.0015	84.50±0.0006	87.88±0.0006	76.13±0.0006	95.75±0.0006	87.50±0.0010
GLC	29.86±0.0112	40.02±0.0010	23.30±0.0021	12.27±0.0010	18.96±0.0006	19.70±0.0010
α-T	14.29±0.0006	14.06±0.0010	15.55±0.0006	16.01±0.0006	15.55±0.0006	17.83±0.0006

Percentage inhibition expressed as mean ± Standard deviation. PFS: *Passiflora foetida* stem; PFF: *Passiflora foetida* fruit; ASA: Ascorbic Acid; BHA: Butylatedhydroxyanisole; GNR: Ginger; GLC: Garlic; α-T: α-Tocopherol

Antimicrobial activity

Antimicrobial assay (Table 6) showed that *P. foetida* EOs have broad spectrum activity against all the test microorganisms between 12.5 – 50 mg/mL, except fruit oil which was inactive at 12.5 mg/mL against *Klebsiellae pneumonia* and *Aspergillus niger*.

Table 6: Antimicrobial activities of stem and fruit of *P. foetida* oils

Sample	Conc. (mg/mL)	Diameter of Inhibition Zone in mm									
		Gram negative					Gram positive		Fungi		
		E.c	S.t	P.a	K.p	B.s	S.a	C.a	A.n	P.n	R.s
PFS	50	16	18	18	14	18	20	14	14	16	14
	25	14	14	16	12	14	18	12	12	14	12
	12.5	12	12	14	10	12	14	10	10	10	10
	6.25	10	10	12	-	10	10	-	-	-	-
	3.125	-	-	-	-	-	-	-	-	-	-
PFF	50	18	16	14	14	16	16	14	12	16	14
	25	16	14	12	10	14	14	12	10	14	12
	12.5	14	12	10	-	12	12	10	-	10	10
	6.25	12	10	-	-	10	10	-	-	-	-
	3.125	10	-	-	-	-	-	-	-	-	-
Gentamicin		24	26	24	24	24	26				
Tioconazole								20	22	20	22
Methanol		-	-	-	-	-	-	-	-	-	-

PFS: *Passiflora foetida* stem; PFF: *Passiflora foetida* fruit; E.c = *Escherichia coli*; S.t = *Salmonella typhi*; P.a = *Pseudomonas aeruginosa*; K.b = *Klebsiellae pneumonia*; B.s = *Bacillus subtilis*; S.a = *Staphylococcus aureus*; C.a = *Candida albicans*; A.n = *Aspergillus niger*; P.n = *Penicillium notatum*; R.s = *Rhizopus stolonifer*; (-) = No activity.

The highest antimicrobial activity was observed in stem EO against *S. aureus* at 50 mg/mL. Fruit and stem oils were active against *E. coli* and *P. aeruginosa* respectively at 3.125 mg/mL. However, the two EOs were inactive against *K. pneumonia* at 3.125 and 6.25 mg/mL. Fruit and stem essential oils were inactive at 6.25 mg/mL and 3.125 mg/mL against all the test fungi. From previous works, the leaf and fruit (ethanol and acetone) extracts of *P. foetida* had



remarkable antibacterial activity against *Pseudomonas putida*, *Vibrio cholerae*, *Shigella flexneri* and *Streptococcus pyogenes* [19, 29]. The antimicrobial result is in agreement with previous reports on the antimicrobial activity of *P. foetida*.

Conclusion

In conclusion, we have characterized the EOs from the stem and fruit of *P. foetida*; which to the best of our knowledge has been sparsely reported in literature. Most of the dominant compounds in the essential oils justify the ethno medicinal uses of *P. foetida*; although it is also true that the action of essential oils is due to the major component which might be activated by synergistic action of minor components. *P. foetida* displayed appreciable antioxidant and antimicrobial activities and can be used against infections caused by microbes as an alternative medicine as well as a potential source of antioxidant agent.

Acknowledgements

Authors acknowledge the use of J-laboratory facilities, Department of Chemistry, University of Ibadan, in essential oil isolation and are greatly thankful to Mr. Festus, of the Department of Pharmaceutical Microbiology, University of Ibadan, Nigeria, for his assistance on antimicrobial assay.

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