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Anti-mycobacterial assessment and characterization of 5-O-caffeoylquinic acid methyl ester and rutin from *Pavetta crassipes*

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ABSTRACT

Pavetta crassipes leaf (Fam. Rubiaceae) is used as part of a combination herbal remedy for the treatment of tuberculosis (TB) and other respiratory infections in Nigerian ethno medicine. However, little scientific data is available to support the use in ethnomedical therapy so the objective of the study was to assess the anti-tubercular property and to identify the bioactive components. The dried powdered leaf was sequentially extracted with solvents to obtain hexane, dichloromethane, methanol and water extracts. Following which, the extracts were then screened against Mycobacterium aurum, a rapidly growing saprophytic mycobacterium species for activity. The methanol extract exhibited inhibitory activity at an MIC value of 250 µg/mL against M. aurum and two known polyphenolic compounds were isolated as 5-O-caffeoylquinic acid methyl ester and quercetin-3-rutinoside (rutin). Reversed phase semi-preparative HPLC, mass spectrometry and 1H and 13C NMR techniques were utilized in isolating and characterizing the two components. The assignments of the structures were consistent with data from the literature. The study has shown that the methanol extract has some activity and hyphenation of LC-MS can be used for the isolation of polyphenols from the methanol fraction without a rigorous purification process.

INTRODUCTION

Pavetta crassipes K Schum (Rubiaceae) is a plant found in the West African sub - region with about 91 genera and 531 species (Gill, 1988). The leaves are used as food, as an anti-malarial remedy, therapy for respiratory disorders, hypertension, gonorrhea, mental illness, and hookworm in ethno medicine (Hutchinson and Dalziel, 1954; Amos *et al.*, 2003; Chabra *et al.*, 1991). Anti-malarial, anti-inflammatory, muscle relaxant and hypotensive effects have been reported for the aqueous and ethanol extracts of the leaves (Amos *et al.*, 1998a, b; Sanon *et al.*, 2003). Although alkaloids and polyphenols have been implicated in the biological activities reported by other investigators, from other species of *Pavetta* and other plant parts, there is no data on the structure elucidation of the bioactive constituents against tuberculosis based on literature accessed (Sanon *et al.*, 2005; Weniger *et al.*, 2004; Baldé *et al.*, 1991a, b). This study describes the anti-mycobacterial activities, isolation and structural identification of two compounds, 5-O-caffeoyl quinic acid methyl ester and rutin using reversed phase semi-preparative HPLC, 1D and 2D NMR in addition to MS techniques.

EXPERIMENTAL

Plant material

The leaves of *P. crassipes* were collected from Jos, Plateau State, in the middle belt region of Nigeria, West Africa. The plant was identified by G.S Suberu and authenticated by Prof. O. Olorede, University of Abuja, Nigeria. The plant identity was further confirmed by Dr Martin Cheek of Royal Botanic Gardens, Kew, UK (Voucher specimen number: K000411874).

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Extraction and isolation

The leaves (100 g) were dried and sequentially extracted with hexane, dichloromethane, methanol and water at room temperature. The methanol extract (13% yield) was dissolved in methanol-water (15:85, v/v) and filtered with a 0.2 µm pore size syringe filter (PTFE ACRODISC FILTER). HPLC separation was initially performed on an analytical column (ACE 5 SIL C18 column, 250 x 4.6 mm, HiChrom Ltd, UK) at 1 mL/min using a 1 mg/mL sample and scaled up to preparative levels using a semi-preparative column (ACE 5 SIL C18, 250 x 10 mm, HiChrom Ltd, UK). The methanol extract (10 mg/ml) was eluted with methanol-water (15:85, v/v) mobile phase at a flow rate of 5ml /min with injection volume of 1000 µl using the gradient profile as follows;

A-methanol (15%) Time (mins)	B-water (85%) % B
0 -5	80 - 50
5 -16	50
16 -17	50 - 80
17-20	80

The eluents were collected in eight fractions and dried with fractions 5 and 7 yielding 6.1 mg and 9.1 mg respectively after 11 injections.

Isolation and cultivation of M. aurum

M. aurum NCTC 10438 was inoculated into glucose broth and incubated at 37˚C for 72 hours. Working suspensions were prepared from dilutions of 72-hour cultures made by comparison to 0.5 McFarland turbidity standard.

Determination of MIC by micro broth dilution assay

A concentration range of 125 -1000 µg/mL of the plant extracts was prepared directly on the multi-well plate for MIC determination. This was incubated at 37˚C for 72 hours with a bacterial suspension of *M. aurum* prepared from a fresh colony maintained on glucose agar and adjusted to a turbidity equivalent to that of a 0.5 McFarland tube. A dilution of 1 in 100 was made in broth and 100 µL transferred to wells containing 100 µL of extracts which had 2 x the final concentration. After 3-5 days of incubation, 20µL of MTT [3(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] was added to each well and the plate was re- incubated for a few hours followed by visual reading. The method is based on the reduction of a colored indicator MTT which changes from yellow to purple. The color change indicates the growth of bacteria and MIC was defined as the lowest concentration of the extract that prevented the change in color.

Chromatographic and spectroscopic procedures

HPLC (analytical and semi-preparative) was performed on HP 1090 and Agilent 1200 series equipped with a UV photo diode array detector respectively. The instruments and data processing were controlled by the Chemstation software. Ace 5 C18 columns, 250 x 4.6 mm and 250 x 10 mm (HiChrom Ltd) were used for analytical and semi-preparative HPLC respectively. HPLC grade solvents were obtained from sigma Aldrich, UK. 1D ^{1}H and ^{13}C, and 2D TOCSY, HSQC and HMBC spectra were recorded on a Bruker AVANCE 500 MHz spectrometer fitted with a BBO probe equipped with a z-gradient coil. NMR experiments were carried out at 25°C. Mass spectral data were obtained using Bruker Esquire 3000+ ion trap mass spectrometer while accurate mass measurements were recorded on a High Resolution-ESI Fourier Transform MS (Thermo 7T LTQ Ultra Hybrid Mass Spectrometer).

RESULTS AND DISCUSSION

Biological testing

The extracts of dried and powdered *P. crassipes* leaves were obtained from sequential extraction with hexane, dichloromethane, methanol and water. All the extracts were tested against *M. aurum* NCTC 10438, a preliminary model organism for investigating anti-tubercular compounds by an *in vitro* micro - plate colorimetric assay method subsequent to testing against *M. tuberculosis* H37Rv, virulent strain of tuberculosis (Chung *et al.*, 1995; Palomino *et al.*, 2002). The tests were conducted to ascertain the biological activity against tuberculosis organisms based on ethno medical information. Initial screening studies with the methanol extract of *P. crassipes* showed inhibitory activity against *M. aurum* at a minimum inhibitory concentration of 250 µg/ml using streptomycin as positive control. So attempts were made to separate and identify the constituents of the extract using LC, MS and NMR. In general, saprophytic non-pathogenic strains of mycobacteria are used in preliminary screening assays in order to obtain quick results and/or because of biosafety concerns in working with virulent *M. tuberculosis*. The non-pathogenic strains have advantages such as rapid growth and the use of Class 1 or 2 laboratory facilities which are readily available in most institutions (Franzblau *et al.*, 1998; Newton *et al.*, 2000). The methanol fraction showed inhibitory activity at 250 µg/mL against *M. aurum* and could be considered the most active fraction based on the results which led to further investigations on the fraction (Table 1). Chung *et al.*, (1995) conducted a study in which they investigated uracil uptake in *Mycobacterium aurum and M. tuberculosis* H37Rv in the bid to develop a high-through put screen for detecting anti-TB compounds. Their observations of the relative susceptibilities to four first line anti-TB drugs showed that *M. aurum* had high predictive ability for high throughput anti-TB screening though H37Ra (ATCC 25177) avirulent strain of *M. tuberculosis* and *M. bovis* BCG (ATCC 35743) are reportedly closely related to *M. tuberculosis* virulent strain in terms of drug susceptibility profile and genetic composition. The results obtained using the micro dilution method exhibited higher MIC values compared to the disc assay method not reported here (Odumosu, 2012). However, it is possible that this might result in a few false leads as suggested with some of the values obtained (Table 1).

The advantage of subjecting the false positive extracts to further testing is that the probability of missing possible active fractions or compounds would be greatly reduced since all fractions were tested (Pauli *et al.*, 2005).

Table 1: *M. aurum* testing at test concentrations of 1000 – 125 µg/mL by micro plate agar dilution method.

CODE	EXTRACT SOURCE	MIC (µg/mL)
PCH	*P. crassipes* hexane	> 1000
PCD	*P. crassipes* dichloromethane	> 1000
PCM	*P. crassipes* methanol	250
PCW	*P. crassipes* water	> 1000
STM	Streptomycin	10
AMP	Ampicillin	100

Table 2: Retention time (t_R), UV (λmax) and MS data of Compounds PCM 5 and PCM 7 from *P. crassipes* methanol leaf extract.

Compound	t_R -min	UV λmax	*m/z* [identity]	
			(-) ESI-MS	molecular formula
5- O-caffeoyl quinic acid methyl ester	8.062	230, 254, 280	367.10339 $[M-H]^-$	$C_{17}H_{19}O_9$
Quercetin-3- rutinoside	9.484	230, 254, 280	609.14603 $[M-H]^-$	$C_{27}H_{29}O_{16}$

Table 3: ^1H and ^{13}C NMR spectral assignment of compound 5 from *P. crassipes* leaf isolate dissolved in CD_3OD.

Atom	5 - Me 5-CQA			
	^{13}C	^1H	^{13}C HSQC	^{13}C HMBC
1	72.14	-		
2		2.04 - 2.05 (m)	~36.9	
3		4.16 (m)	~69	
4		3.72 (m)	~71.7	
5		5.29 (m)	~71	
6		2.22 (m)	~37	
COO-	175.43			
OCH_3	52.96	3.76 (s)		
1'	122.98	6.96 (dd, 2.06 Hz, 8.25 Hz)		
2'	116.54	6.79 (d, 8.25 Hz)		
3'	146.91	-		
4'		-		~149
5'	115.09	7.06 (d, 2.06 Hz)		
6'	127.61	-		
7'	147.22	7.53 (d, 15.81 Hz)		
8'	115.01	6.22 (d, 15.81 Hz)		
9'	168.27	-		

~ estimated chemical shift values in ppm from HSQC and HMBC spectra.

Recent reports on the anti-tubercular screening of *P. crassipes* against Bacille Calmette Guerin (BCG) and *M. tuberculosis* H37Rv strains showed variable MIC values but it was considered worthy of further study (Ibekwe *et al.*, 2014). There are also reported anti-inflammatory and muscle relaxant effects of the aqueous extract of *P. crassipes* leaves which supports the 'herbal shotgun' principle where components play different functions in achieving therapy (Amos *et al.*, 1998a).

Isolation and Identification

The test of the crude methanol extract with $FeCl_3$ solution and NaOH gave positive results suggesting that tannins and flavonoids were present (Evans, 2009). Reversed phase semi-preparative LC analysis of the methanol fraction resulted in eight fractions from which fractions 5 and 7 yielded 6.1 mg and 9.1 mg respectively after 11 injections (Figure 1). The two fractions were found to be of high enough purity (approximately 85%) for analysis by NMR and mass spectrometry measurements. The same method was transferred to LC-MS in an attempt to carry out the isolation and characterization without the tedious procedures involved in preparative LC. The two fractions earlier obtained in a fairly pure state gave molecular weights of 369 (PCM 5) and 611 (PCM 7) and the accurate mass was confirmed by accurate mass and NMR.

The results obtained by mass spectrometry are presented in Table 2 while assignments of the NMR signals are presented in Tables 3 and 4 respectively.

The structures of the compounds, 5-caffeoyl quinic acid methyl ester (PCM 5) and quercetin-3-rutinoside (PCM 7) were confirmed by comparison of 1H and ^{13}C NMR data of the isolated compound to a standard sample of rutin (Sigma-Aldrich, S2424, Lot # 129K2568V) and with literature values for 5-O-caffeoyl quinic acid methyl ester (Weniger *et al.*, 2004; Baldé *et al.*, 1991a, b). Compound 5 (Me 5-CQA) has a molecular formula $C_{17}H_{19}O_9$ established by HR-ESI-MS (m/z: 367.10 $[M-H]^-$, calculated as 367.10, δ - 0.18 ppm (Figure 3, Table 2). Comparison of the ^1H and ^{13}C NMR data of Me 5-CQA (Table 3) with literature values showed similar signals (Chan *et al.*, 2009; Clifford *et al.*, 2003). Compound 7 (rutin) was compared to NMR data of an authentic sample and both compounds showed similar signals (Table 4). The molecular formula was established by HR-ESI-MS as $C_{27}H_{29}O_{16}$ (m/z: 609.14603 $[M-H]^-$, calculated as 609.14, δ – 0.13 ppm (Figure 4, Table 2). The ^1H NMR data showed proton peaks at δ3.00- δ4.00 with an integral of ~ 9 which were suggestive of carbon atoms bearing hydroxyl groups. The proton signals at δ5.10 and at δ3.52 show correlation in the TOCSY spectrum which is consistent with an anomeric proton coupled to the ring system of a sugar molecule.

Table 4: ^{1}H and ^{13}C NMR spectral assignment of compound 7 from *P. crassipes* methanol leaf isolate dissolved in CD_3OD.

Atom	7- Rutin		Rutin reference standard	
	^{13}C	^{1}H	^{13}C	^{1}H
1	179.38	-	179.41	-
2	135.6	-	135.64	-
3	159.29	-	159.33	-
4	158.56	-	158.50	-
5	94.95	6.39 (d, 1.83 Hz)	94.87	6.42 (d, 2.07 Hz)
6	166.48	-	165.98	-
7	100.09	6.20 (d, 1.83 Hz)	99.95	6.24 (d, 2.07 Hz)
8	162.98	-	162.96	-
9	105.51	-	105.65	-
10	123.54	-	123.57	-
11	123.12	7.62 (dd, 1.83 Hz, 8.25 Hz)	123.15	7.64 (dd, 2.17, 8.48 Hz)
12	116.06	6.87 (d, 8.48 Hz)	116.07	6.92 (d, 8.48 Hz)
13	145.86	-	145.82	-
14	149.84	-	149.78	-
15	117.66	7.66 (d, 1.83 Hz)	117.71	7.69 (d, 2.17 Hz)
1'	104.75	5.10 (d, 7.56 Hz)	104.74	5.15 (d, 7.44 Hz)
2'	78.20	3.40 (m)	78.20	3.40 (m)
3'	75.73	3.46 (m)	75.73	3.47 (m)
4'	69.71	3.44 (m)	69.70	3.45 (m)
5'	73.93	3.27 (m)	73.95	3.27 (m)
6'	68.55	3.36 (m), 3.81 (dd, 1.15,11 Hz)	68.57	3.35, 3.82 (d, 10.27 Hz)
7'	102.43	4.51 (s)	102.41	4.55 (d, 1.41 Hz)
8'	77.23	3.32 (m)	77.23	3.32 (m)
9'	72.24	3.52 (dd, 3.44, 9.39 Hz)	72.26	3.53 (dd, 3.49, 9.42 Hz)
10'	71.4	3.25 (m)	71.41	3.26 (m)
11'	72.11	3.62 (m)	72.10	3.66 (dd, 1.60 Hz)
12'	17.88	1.12 (d, 6.19 Hz)	17.87	1.16 (d, 6.22 Hz)

Values of ^{1}H and ^{13}C chemical shifts are in ppm (δ), and those of coupling constants, J in Hz

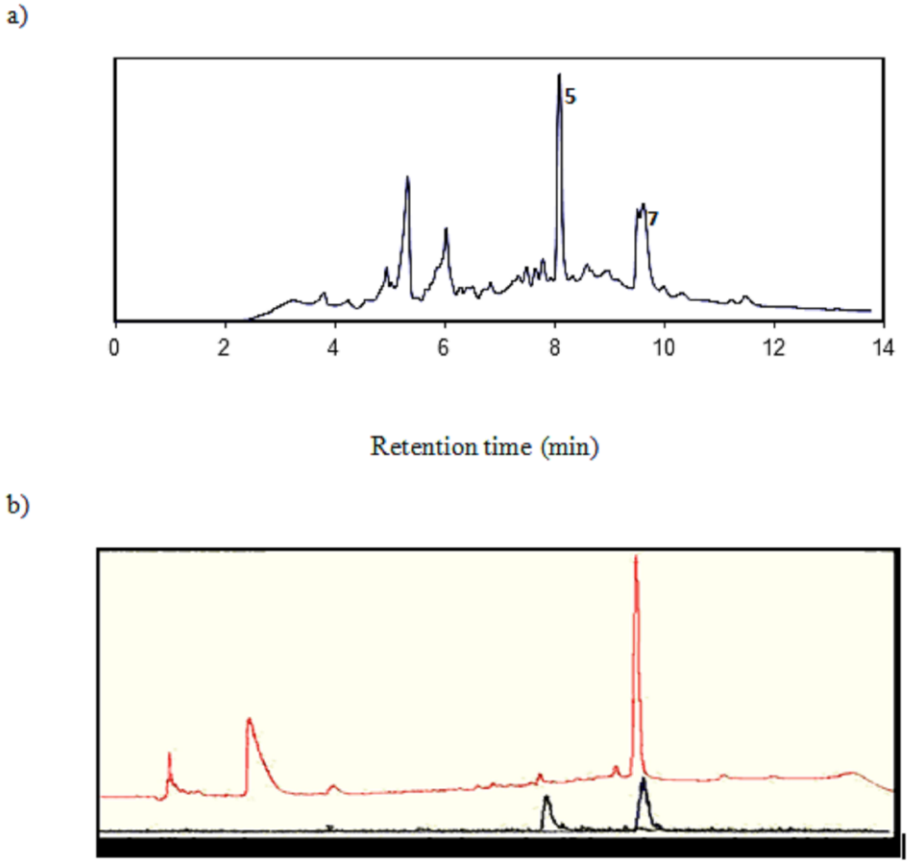

Fig. 1: a) HPLC/UV chromatogram of *Pavetta crassipes* methanol extract on ACE C18 column at 230 nm (250 x 10 mm, 5 µm). b) LC-MS UV trace at 225 nm (top trace), first peak is the extract ion chromatogram for 369 ion (PCM 5) while second peak is extract ion chromatogram for 611 ion (PCM 7). Data was acquired in positive mode.

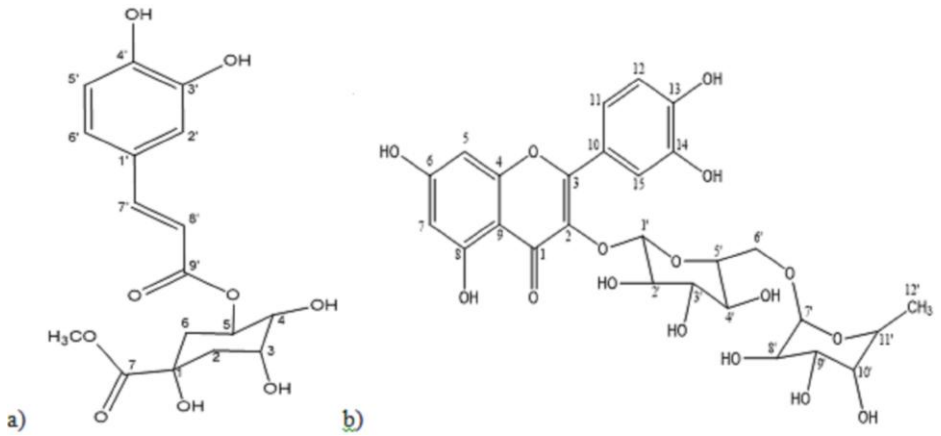

Fig. 2: Structures of 5-O-caffeoyl quinic acid methyl ester (a) and quercetin-3-rutinoside (rutin) (b).

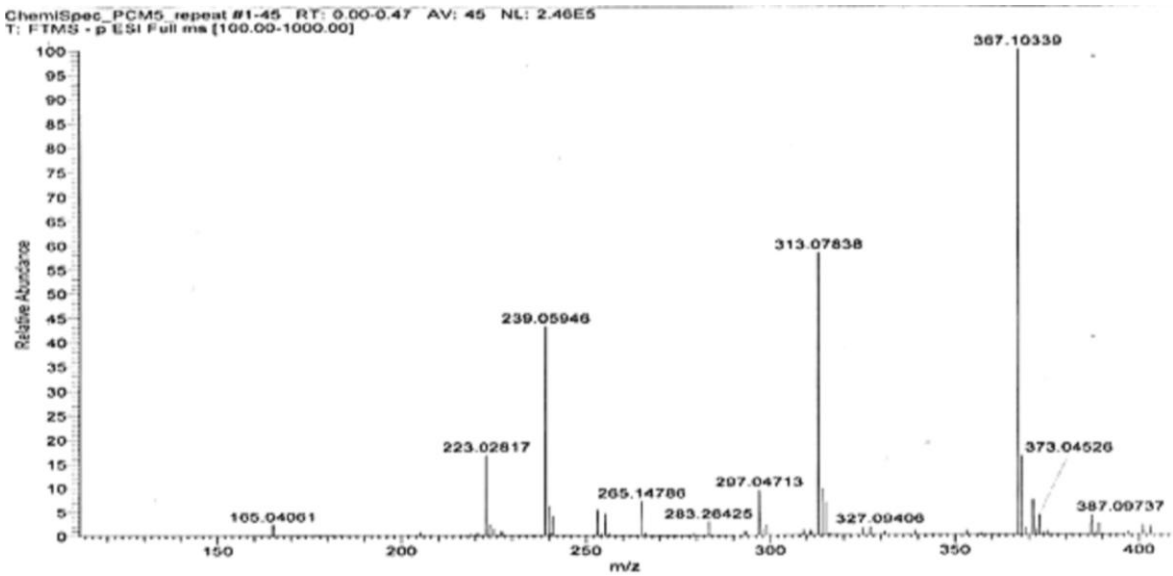

Fig. 3: Accurate mass spectrum of PCM 5 from P. crassipes leaf by ESI-MS (negative ion mode).

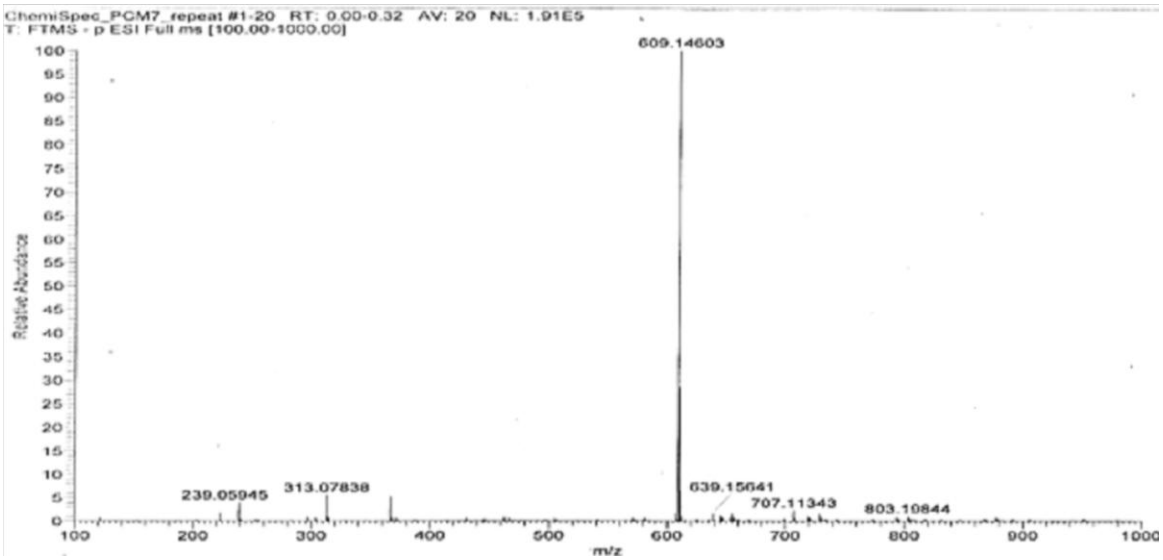

Fig. 4: Accurate mass measurement for PCM 7 from P. crassipes leaf by ESI- MS (negative ion mode).

The elucidation of the structure was completed with the aid of HSQC and HMBC spectra. From the report of a review on plant-derived anti-mycobacterial natural products, bioactivity spans a wide range of phytochemicals such as lipids/fatty acids and simple aromatics, phenolics and acetogenic quinones, peptides, alkaloids, terpenes (monoterpenoids, diterpenes, sesquiterpenes, sesterterpenes) and steroids. Highly active compounds from these plants included; the lactone - containing tubelactomicin A, phenolics drummondins and ferulenol, xanthone, brasiliquinones A and B, the alkaloids celastramycin A, sampagine and the manzamines and steroids fusidic acid, saringosterol and ergosterol-5,8-endoperoxide which all exhibited antimycobacterial activity with MIC values ≤ 4 µg/mL (Copp, 2003).

These compounds show the chemical diversity and complexity displayed by plants compared to first-line and second – line drugs such as rifampicin or amikacin used in therapy. Caffeoylquinic acid derivatives have been investigated and shown to have properties such as peroxynitrite-scavenging, hepato-protective, anti-viral, anti-obese, and antidiabetic activities (Park, 2010). From this study, *P. crassipes* leaf extracts were not as active against the saprophytic TB organism used when compared to the reference antibiotics however, the inclusion in the herbal preparation could be justified by the principle of synergy where an extract is added to enhance the activity of the actives, such as stabilizing the actives through anti-oxidant action or enhancing bioavailability rather than anti-bacterial action (Williamson, 2001; Olthof *et al.*, 2003). Further studies could be conducted to find out if the plant extracts have efflux properties or synergistic properties with the other components of the herbal preparation. In summary, *M. aurum* was easy to grow on glucose agar and useful for initial screening studies as well as for monitoring fractions during isolation of bioactive components. The compounds, 5-caffeoyl quinic acid methyl ester and rutin isolated from the leaf of *Pavetta crassipes* Fam. Rubiaceae using reversed phase semi- preparative LC have been reported to have other useful activities such as anti-oxidant properties. The inclusion of *Pavetta* leaves in herbal remedy used in treatment of respiratory infections possibly serves to enhance the activity of other plant component. The identification of compounds by their 1D/2D NMR & mass spectrum was achieved without any need for further purification.

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REFERENCES

Amos S, Akah PA, Enwerem NM, Ogundaini A, Wambebe C, Hussaini IM, Gamaniel KS. Hypotensive activity of the ethanol extract of *Pavetta crassipes* leaves. Biol Pharm Bull, 2003; 26 (12):1674 -1680.

Amos S, Gamaniel K, Akah P, Wambebe C, Okwuasaba FK. Anti-inflammatory and muscle relaxant effects of the aqueous extract of *Pavetta crassipes* leaves. Fitoterapia, 1998a; 69 (5): 425 – 429.

Amos S, Okwuasaba FK, Gamaniel K, Akah P, Wambebe C. Inhibitory effects of the aqueous extracts of *Pavetta crassipes* leaves on gastrointestinal and uterine smooth muscle preparations isolated from rabbits, guinea pigs and rats. J Ethnopharmacol, 1998b; 61: 209-213.

Baldé A M, Pieters LA, Gergely A, Kolodziej H, Berghe DAV, Claeys M, Vlietinck, AJ. A-type proanthocyanidins from stem-bark of Pavetta owariensis. Phytochem, 1991; 30 (1): 337-342.

Baldé AM, Pieters TLA, Wray V, Kolodziej SH, Vanden Berghe SDA, Claeys M, Vlietinck AJ. Dimeric and trimeric proanthocyanidins possessing a doubly linked structure from Pavetta owariensis. Phytochem, 1991; 30 (12): 4129- 4135.

Chabra SC, Mahunna RL, Mshiu EN. Plants used in Traditional Medicine in Eastern Tanzania V. Angiosperms (Passifloraceae to Sapindaceae). J Ethnopharmacol, 1991; 33 (1-2): 143-157.

Chan EWC, Lim YY, Ling SK, Tan SP, Lim KK, Khoo MGH. Caffeoylquinic acids from leaves of Etlingera species (Zingiberaceae). Food Sci and Tech, 2009; 42: 1026 -1030.

Chung GAC, Akhtar Z, Jackson S, Duncan K. High-throughput screen for detecting antimycobacterial agents. Antimicrob Agents Chemother, 1995; 39: 2235–2238.

Clifford MN, Johnston KL, Knight S, Kuhnert N. Hierarchical scheme for LC-MS^n identification of chlorogenic acids. J Agric Food Chem, 2003; 51: 2900-2911.

Copp BR. Antimycobacterial natural products. Nat Prods Rep, 2003; 20: 535 – 557.

Evans WC. 2009. Phenols and phenolic glycosides. In: Trease and Evans Pharmacognosy 16th ed. Saunders Elsevier Ltd 225- 252.

Franzblau SG, Witzig, RS, Mclaughlin, JC, Torres P, Madico G, Hernandez A, Degnan MT, Cook MB., Quenzer VK, Ferguson RM, Gilman RH. Rapid, low technology MIC determination with clinical Mycobacterium tuberculosis isolates by using the microplate alamar blue assay. J of Clin Microbiol, 1998; 36: 362-366.

Gill LS. 1998. Taxonomy of flowering plants. Africana FEP Publishers: Onitsha, Nigeria;

Hutchinson J, Dalziel JM. 1954. Flora of West Tropical Africa, Vol 1. Crown Agents for Overseas Government and Administrations: London.

Ibekwe NN, Nvau JB, Oladosu PO, Usman AM, Ibrahim K, Boshoff HI, Dowd CS, Orisadipe, AT, Aiyelaagbe O, Adesomoju AA, Barry, CE III, Okogun JI and in collaboration with 73 Visited Herbalists. Some Nigerian anti-tuberculosis ethnomedicines: A preliminary efficacy assessment. J Ethnopharmacol, 2014; 155(1): 524–532.

Newton SM, Lau C, Wright CW. A review of anti-mycobacterial natural products. Phytother Res, 2000; 14: 302-322.

Odumosu PO. 2012. Evaluation of the plant extracts of an anti-tubercular herbal remedy. Doctoral thesis. University of Sunderland, UK. [Online] Available at: http://ethos.bl.uk/OrderDetails.do?uin=uk.bl.ethos.569064 [Accessed 12 August 2016].

Olthof MR, Hollman PCH, Buijsman MNCP, van Amelsvoort JMM, Katan MB. Chlorogenic acid, quercetin-3-rutinoside and black tea phenols are extensively metabolized in humans. J Nutr, 2003; 133**:** 1806-1814.

Palomino JC, Martin A, Camacho M, Guerra H, Swings J, Portaels F. Resazurin microtiter assay plate: simple and inexpensive method for detection of drug resistance in Mycobacterium tuberculosis. Antimicrob Agents and Chemother, 2002; 46: 2720–2722.

Park H-J. Chemistry and pharmacological action of caffeoylquinic acid derivatives and pharmaceutical utilization of Chwinamul (Korean mountainous vegetable). Arch of Pharmacol Res, 2010; 33 (11): 1703-1720.

Pauli GF, Case RJ, Inui T, Wang Y, Cho S, Fischer NH, Franzblau SG. New perspectives on natural products in TB drug research. Life Sci, 2005; 78: 485 – 494.

Sanon S, Azas N, Gasquet E, Ollivier V, Barro N, Cuzin-Ouattara N, Mahiou V, Traore SA, Esposito F, Balansard G, Timon-

David P. Antiplasmodial activity of alkaloid extracts from *Pavetta crassipes* (K. Schum) and Acanthospermum hispidum (DC), two plants used in traditional medicine in Burkina Faso. Parasit Res, 2003; 90: 314 – 317.

Sanon S, Mahiou V, Nebie I, Azas N, Ollivier E, Timon-David P, Balansard G, Traore A. In-vitro antimalarial properties of two plants used by traditional herbal practitioners of Burkina Faso: *Pavetta crassipes* and Mithragyna inermis [MIM-SS-81397]. Parallel sessions-abstracts of presentations. Acta Trop, 2005; 95 (1): 1-506.

Weniger B, Lagnika L, Vonthron-Sénécheau C, Adjobimey T, Gbenoud J, Moudachirou M, Brun R, Anton R, Sanni A. Evaluation of ethnobotanically selected Benin medicinal plants for their in-vitro antiplasmodial activity. J Ethnopharmacol, 2004; 90: 279 - 284.

Williamson EM. Synergy and other interactions in phytomedicines. Phytomed, 2001; 8 (5): 401- 409.

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