International Scholars Journals

Frontiers of Agriculture and Food Technology ISSN: 2736-1624 Vol. 11 (2), pp. 001-006, February, 2021. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Evaluation of anthelmintic activity of the stem bark extract and chemical constituents of *Bridelia ferruginae* (Benth) Euphorbiaceae

Lasisi A. A.[1]* and Kareem S. O.[2]

[1]Drug Research Unit, Department of Chemistry, University of Agriculture, Abeokuta, Ogun State, Nigeria.
[2]Department of Microbiology, University of Agriculture, Abeokuta, Ogun State, Nigeria.

Accepted 15 June, 2020

Anthelmintic properties of the stem bark extract and compounds isolated from *Bridelia ferruginae* were investigated. In relation to the traditional use of *B. ferruginae* against gastro-intestinal infections, bioactivity-guided fractionations of the $CHCl_3^-$ and $CH_2Cl_2^-$ soluble fractions of the 80% MeOH extract from the stem barks of *B. ferruginae* yielded two known triterpenoids: betuline (1), glucoside of betulinic acid (2) and other two known flavonoids: quercetin (3) and kaempferol (4). Structures of compounds 1 to 4 were elucidated by spectroscopic studies and comparison with related compounds in literature. The time of paralysis and death of the parasitic worms: *Fasciola gigantical* (liver fluke), *Taenia solium* (tape worm) and *Pheritima posthuma* (earthworm, Annelid) were determined at 25, 50, 80 and 100 mg/ml. The stem barks extract of *B. ferruginae* and isolated compounds demonstrated concentration- dependent anthelmintic potencies against parasitic worms assayed. Structural-activity relationship is explained.

Key words: *Bridelia ferruginae*, anthelmintic activity, betulinic acid, betuline, quercetin, kaempferol.

INTRODUCTION

Bridelia ferruginae (Benth)-Euphorbiaceae is one of the most popular medicinal plants used in the Northern, South-Western Nigeria and other African countries for gastro-intestinal infections (Addae-Mensah, 1992; Iwu, 1986; Ayensu, 1978) . *B. ferruginae* is among the 60 species of the genus *Brindelia* (Oliver-Bever, 1960). Its morphology is well documented (Rashid et al., 2000). *B. ferruginae* is widely distributed in the guinea savannah and coastal parts of Africa, particularly Cote d'Ivoire Ghana, Togo and Nigeria (Addae-Mensah, 1992). In Nigeria, *B . ferruginae* has many vernacular names, depending on the usage and locations. *B. ferruginae* is widely used in traditional Nigerian medicine to treat a range of diseases. The barks of *B. ferruginae* is reported for wound treatment, gonorrhea infections, antimicrobial potency, gastro-infection treatment, anti-diabetic, anti-inflammatory and radical scavenging activities (Ekanem et al., 2008; Olajide et al., 1999; De- Bruyne et al., 1997;; Adeoye et al., 1988; Iwu, 1984). Previous phytochemical attention on *B. ferruginae* has led to characterization of flavonoids, triterpenoids, flavonoid glucosides, bioflavonoids, phenols and tannins from various morphological parts of *B. ferruginae* (Cimmanga, 2001; Rashid et al., 2000; Addae-Mensah, 1985; Irobi, 1994). In spite of numerous pharmacological and phytochemical reports on *B. ferruginae*, there is a dearth of literature report on the anthelmintic potency of the plant. In a preliminary screening of plants used in South-Western Nigeria for the treatment of gastroinfectional disorders, *B. ferruginae* was investigated based on positive screening results of its stem bark extract on selected parasitic worms: *Fasciola gigantical* (liver fluke), *Taenia solium* (tape worm) and *Pheritima posthuma* (earthworm, Annelid). In response to the folkloric usage of the stem barks of *B. ferruginae* in Nigeria traditional medicine and in furtherance of our search for anthelmintic phytochemicals from Nigeria medicinal plants, *B.*

*Corresponding author. E-mail: lasialiyu@yahoo.com. Tel: +2348035830153.

ferruginae was investigated. Isolated compounds (1 to 4) were elucidated on the basis of spectroscopic studies and by direct comparison of their physical data with related compounds in literature.

Bioassay-guided separation of the $CHCl_3^-$ and $CH_2Cl_2^-$ soluble fractions of stem bark extracts of *B*. *ferruginae* using column chromatography afforded two known triterpenoids: betuline (1) and glucoside of betulinic acid (2) reported for the first time from *B*. *ferruginae*. Further chromatographic analysis of the CH_2Cl_2 extract of *B*. *ferruginae* yielded two known flavonoids: quercetin (3) and kaempferol (4) previously reported in this plant (Addae-Mensah, 1989). In this paper, we report the anthelmintic activities of the stem bark extracts and constituents from *B*. *ferruginae*. In developing countries like Nigeria, helminth infections are a major health concern because they predispose human to other infections such as bacterial and fungal infections (Cox, 2001). Such helminth infections can lead to serious diseases among poor people due to poor sanitation, poverty and malnutrition (Brooker et al., 2006).

MATERIALS AND METHODS

Plant material

The stem bark of *B*. *ferruginae* used in this study was collected at Olokemeji Forest Reserve in Ibarapa Local Government Area of Oyo State, Nigeria in July, 2009. The plant was botanically authenticated by Mr. Odewo, T. K., a taxonomist in the Forest Research Institute of Nigeria (FRIN) through comparison with authentic samples in the herbarium of FRIN (Accession number FHI 1234).

Extraction and isolation of plant material

The stem barks of *B*. *ferruginae* were air-dried at room temperature. Dried and powdered stem bark (1.5 kg) were defatted by soxhlet extraction with *n*-hexane (bp 68°C). The dried plant material (marc) was macerated and percolated successively with 80% MeOH. The MeOH extract was concentrated *in vacuo* at reduced pressure, yielding a residue of 560 g. 500 g of the residue was dissolved in hot water (60°C) and filtered after 24 h. The filtrate was extracted successively with $CHCl_3$ (5 × 500 ml), CH_2Cl_2 (10 × 500 ml), and *n*-butanol (10 × 500 ml) and concentrated separately by means of rotatory evaporator to afford 15, 21 and 27 g of the $CHCl_3$, CH_2Cl_2 and *n*-butanol extracts respectively. 10 g of the $CHCl_3$ extract from the stem barks of *B*. *ferruginae* was subjected to fractionation by column chromatography (CC) on silica gel (Merck 60. 70 to 230 mesh), eluted with gradient solvent systems of *n*- hexane-EtOAc (0 to 1, 1 to 0, vol/vol), collecting100 ml fraction each time. The fractions were monitored by TLC and later pooled together into 6 subfractions on the basis of similarity in TLC. Fractions were subjected to *in vitro* anthelmintic assay, active fractions were purified further. 20% hexane-EtOAc afforded betuline (1) (120 mg) as white powder, 50% hexane- EtOAc yielded 3-*O*- - D-glucopyranoside of betulinic acid (2) (150 mg) as an amorphous white solid. Compound 1: 3β , 24-dihydroxyl lup-20 (29) -ene (betuline), white powder, mp 234 to 235°C (lit mp 236°C), R_f = 0.50 (analytical TLC, silica gel, Merck 60 developed in hexane/EtOAC 3:1 vol/vol); ^{1}H-NMR [$CDCl_3$, 400 MHz, (ppm)]: 4.75 [1H, d, *J* = 1.92 Hz, H- 29a], 4.62 [1H, dd, *J* = 1.9 Hz, 2.72 Hz, H-29b], 3.02 [1 H, m, H-3], 0.76 to 1.98 [6x -CH_3]; ^{13}C - NMR [$CDCl_3$, 400 MHz, (ppm)]:150.3(C-24), 109.6 (C-29), 79.0 (C- 3), 60.8 (C-28), 50.6 (C-9), 48.8 (C-19), 47.9 (C-18), 47.8 (C-17), 42.8 (C-14), 41.0 (C-5), 38.8 (C-1), 38.3 (C-4), 37.4 (C-10), 37.0 (C-13), 34.3 (C-7), 34.0 (C-22), 29.8 (C-21), 29.3 (C-16), 28.0 (C-23), 27.4 (C-2), 27.1 (C-15), 25.6 (C-12), 20.9 (C-11), 19.4 (C-30), 18.3 (C-6), 16.1(C-25),15.3 (C-20), 14.7 (C-27) and 13.9 (C-26). Compound 2: glucoside of 3 - hydroxyl-lup-20(29)-ene-28-oic acid, amorphous white solid, mp 219 to 220°C (lit. mp 211°C); R_f 0.4 (analytical TLC, silica gel, Merck 60 developed in Hexane/EtOAC 2:1 vol/vol); ^{1}H- NMR [400MHz , $CDCl_3$, (ppm)]: 5.00 (1H, d *J* = 7.8 Hz, H-1'), 4.71 (1H,dd *J* = 1.8 Hz *J* = 2.7 Hz, H-29a), 4.59 (1H d, *J* = 1.8 Hz, H-29b), 4.46 (1H, dd, *J* = 11.2, 2.5 Hz, H- 6'a), 4.29 (1H, dd, *J* = 11,2, 5.3 Hz, H-6'b), 4.18 (1H, m, H-4'), 4.15 (1H, dd, *J* = 8.7, 8.1, H-3'), 3.97 (1H, dd, *J* = 7.6, 8.7 Hz, H-2'), 3.86 (1H, ddd, *J* = 8.7, 5.3, 2.5 Hz, H-5') 3.2 (1H, m , H-3), 0.76 to 1.98 (6x - CH_3); ^{13}C - NMR [$CDCl_3$, 400 MHz, (ppm)]: 174.9 (C-28), 150.8 (C- 20), 110.0 (C-29), 100.87 [C – 1'], 88.7 (C-3), 78.2 (C-5'), 78.1 (C - 3'), 75.32 (C - 2'), 71.0 (C - 4'), 61.1 (C - 6'), 55.9 (C-5), 50.8 (C-9), 49.8 (C-19), 47.1(C-18), 39.6 (C-4), 39.0 (C-1), 38,3 (C-13), 37.1 (C-10), 36.8 (C-22), 34.5 (C-7), 30.6 (C-21), 30.1 (C-15), 28.3 (C-23), 26.8 (C-12), 26.0 (C-2), 21.0 (C-11).

5 g of CH_2Cl_2 stem bark extract of *B*. *ferruginae* was subjected to CC on silica gel (Merck, 60, 70 to 230 mesh), eluting progressively with gradient solvent system of hexane-EtOAc (collecting 100 ml each), starting from 100% hexane to 100% EtOAc. Fractions were monitored using TLC and bulked into five subfractions A to E on the basis of their TLC profile. Fractions A to E was assayed for *in vitro* anthelmintic activity on the parasitic worms. Active fractions were purified and recrystallised. Fraction C eluted with 40% hexane in EtOAc afforded a gummy yellow solid, which was washed severally in isopropyl alcohol, to give pure yellow powder quercetin (3, 120 mg), 60% hexane-EtOAc solvent eluted yellowish solid, recrystallised in hexane: EtOAc (7:3, vol:vol) to afford yellow solid kaempferol (4, 96 mg). Compound 3: quercetin, yellow powder, mp 189°C (lit mp 188°C); R_f 0.35 (analytical TLC, silica gel, Merck 60 $PF_{254+360}$ developed in Hexane/EtOAC 1:1 vol/vol); ^{1}H-NMR [400 MHz , $CDCl_3$, (ppm)]: 7.8 (1H, d, *J* = 2.0 Hz, H-6'),7.4 (1H, d, *J* = 2.1 Hz, H-8), 7.1 (1H, d, *J* = 2.0 Hz, H-3'), 6.7(1H, dd, *J* = 8.1, 2.0, H-2'), 6.60 (1H, dd, 7.05, 2.0, H-6), 6.50 (1H, d, 7.05 Hz, H-5), 12.5 to 12.8 (1H each, -OH); ^{13}C-NMR [400 MHz, $CDCl_3$, (ppm)]: 184.0(C-4), 168.1 (C-8), 166.1 (C-3), 165.1(C-2), 160.1(C-5), 156.1 (C-9), 148.1 (C-4'), 147.1 (C-5'), 130.0 (C-3'), 120.3 (C-1'), 112.1 (C-6'), 106 (C-10), 105.2 (C-2'), 98.9 (C-6), 93.0 (C-8). Compound 4: Kaempferol, yellow powdery solid, R_f 0.20 [analytical TLC, silica gel (Merck 60 $PF_{254+360}$) developed in hexane/EtOAC 1:1 vol/vol]; ^{1}H-NMR [400MHz, $CDCl_3$, (ppm)]: 7.6 (1H, dd, *J* = 2.0, 7.6Hz, H-6'), 7.5 (1H, dd, *J* = 7.6, 2.0 Hz, H-5'), 7.4 (1H, d, *J* = 2.1 Hz, H-8), 7.1 (1H, d, *J* = 2.0 Hz, H-3'), 6.7(1H, dd, *J* = 8.1, 2.0, H-2'), 6.60 (1H, dd, 7.05, 2.0, H-6), 6.50 (1H, d, *J* = 7.05 Hz, H-5), 12.5 to 12.8 (1H each, -OH); ^{13}C-NMR [400 MHz, $CDCl_3$, (ppm)]: 184.0 (C-4), 168.1 (C-8), 166.1 (C-3), 165.1(C-2), 160.1(C-5), 156.1 (C-9), 148.1 (C-4'), 130.0 (C-3'), 120.3 (C-1'), 112.1 (C-6'), 110.1(C-5'), 106 (C-10), 105.2 (C-2'), 98.9 (C-6) and 93.0 (C-8).

Animal material

The worm used for the study: *F. gigantica* (liver fluke, mean weight of (0.06 to 0.08 g), *T. solium* (tape worm, mean weight 2.5 to 2.9 g) were obtained from freshly slaughtered cows at Odo-eran abattoir, Abeokuta, Ogun State, Nigeria. Nigeria earthworm *P. posthuma* (Annelid) were collected from the water logged areas of soil in Oba river, Obantoko, Ogun State, Nigeria. The average size of earthworm was 6 to 8 cm; the worm was washed with cold water to remove dirt. All parasitic worms were authenticated at the Parasitological Research Unit, Zoology Department, University of Agriculture, Abeokuta, Ogun State, Nigeria.

Table 1. Anthelmintic activity of extracts from *B. ferruginae*

Extracts	Concentration (mg/ml)	*F. gigantica*		*T. solium*		*P. posthuma*	
		P	D	P	D	P	D
CH_2Cl_2 extract	10	52.1± 0.1	>60	25 ±0.1	38 ±0.3	100 ±0.80	175 ±0.9
	0	39.2± 0.3	57±0.2	19± 0.3	32±0.1	122 ±0.7	155 ±0.7
	50	27± 0.2	40±0.7	16±0.5	25±0.3	92 ±0.5	123 ±0.2
	100	10±0.3	35±0.5	14±0.2	12±0.1	83 ±0.5	99 ±0.6
$CHCl_3$ extract	10	38±0.3	31±0.3	17±0.3	27±0.4	75 ±0.4	86 ±0.5
	20	26±0.2	28±0.3	15±0.2	25±0.3	35 ±0.3	65 ±0.5
	50	20±0.1	19±0.2	14±0.2	26±0.2	33 ±0.3	55 ±0.5
	80	18±0.1	15±0.3	12±0.1	20±0.5	30 ±0.2	35 ±0.5
Piperazine citrate	10	25±0.1	20±0.2	17±0.04	19±0.3	60±0.07	80±0.03
Distilled water	-	-	-	-	-	-	-

P = mean paralysis time in minute, D = mean death time in minute, conc. = concentration (mg/ml). Control worms were alive after 40 h except *T. solium* which were alive after 20 h.

Anthelmintic assay

All chemicals used were of IP/HP specifications. Parasitic worms: *F. gigantical* (liver flukes), *T. solium* (tape worms) and *P. posthuma* (earthworms) of comparable size were used for evaluating anthelmintic activity using piperizine citrate, as standard anthelmintic drug. The anthelmintic procedure followed the method described by Asuzu et al. (1999), Asuzu and Onu (1994) and Mali et al. (2005) with slight modifications. Five worms (of the same types) were placed in 9 cm petri dishes in solution of crude extracts containing four different concentrations (10, 20, 50, 100 mg/ml in distilled water) . A solution of each concentrate was prepared in distilled water. Five worms of approximately the same size were placed in each petri dish containing 50 ml of the test extracts, isolated compounds and standard drug at the concentrations mentioned above. This was done in duplicate for all the worm types. The control test having five worms in 50 ml of distilled water was equally conducted simultaneously. The average time required for the paralysis and death of worms was recorded. The mean paralysis time (minute) of the worms was recorded when the worm show no movement of any sort except when the worm was shaken vigorously or transferred into a beaker containing hot water at 50°C. The death time was recorded after ascertaining the worms neither move when shaken vigorously nor when dipped in hot water (70°C). The results are presented in Tables 1 and 2.

Statistical analysis

All data were expressed as the mean ± S.E.M., data was subjected to two-way ANOVA followed by Student's t - test, using microsoft excel* and statistical* computer software packages. Difference in mean were considered significant when P 0.05.

RESULTS AND DISCUSSION

$CHCl_3$ and CH_2Cl_2 extracts of *B. ferruginae* was subjected to silica gel CC to afford two known triterpenoids: betuline (1) and glucoside of betulinic acid (2). Compounds 1 and 2 (Figure 1), though known are reported for the first time from *B*. *ferruginae*. Compound 1 was identified as 3β, 24- dihydroxyl lup-20 (29)-ene (betuline) on the basis of ^{1}H- and ^{13}C-NMR spectral data; along side other physical data with literature values. Betuline was previously isolated from the *Polyporous pinicola* and *Bentula mandschurica*l repel plants (Dan and Dan, 1986; Patra et al., 1988). Compound 2 was eluted with 50% hexane-EtOAc and characterized as glucoside of 3 -hydroxyl-lup-20(29)- ene- 28-oic acid (a glucoside of betulinic acid) based on ^{1} H- and ^{13}C- NMR spectral data, as well as comparison of their physical data. Compound 2 has been reported from *Oplopanax nakai* plant tissue (Wang et al., 1996) and from the leaves of *Cussonia racemosa* (Liva et al., 2002). The flavonoids, quercetin (3) and kaempferol (4) were eluted with 40% hexane in EtOAc and 60% hexane in EtOAc respectively. Compounds 3 to 4 were characterized on the basis of their spectral data (^{1}H- and ^{13}C-NMR) as well as comparison with other physical data in literature. Compounds 3 and 4 have been widely reported from various plant tissues, including *B. ferruginae* (Addae-Mensah and Achenbach, 1985; Addae-Mensah and Munenge, 1989; Cimmanga et al., 1999). Anthelmintic screening of extracts and isolated compounds (1 to 4) (Figure 1) was evaluated *in-vitro* on three parasitic worms: *F. gigantica* (liver flukes), *T. solium* (tape- worms) and *P. posthuma* (earthworms). The $CHCl_3$ and CH_2Cl_2 extracts of *B*. *ferruginae* demonstrated significant anthelmintic activity against the parasitic worms assayed. CH_2Cl_2 extract exhibits higher anthelmintic activity than the $CHCl_3$ extract of *B*. *ferruginae* on the parasitic worms. It is noted that the extracts displayed concentration-related anthelmintic activity (Table 1). The time (minute) of paralysis and death compared favourably with the

Table 2 Anthelmintic activity of isolated compounds from *B. ferruginae.*

Compounds	Concentration (mg/ml)	*F. gigantica*		*T. solium*		*P. posthuma*	
		P	D	P	D	P	D
1	10	85±0.8	125±0.9	85±0.7	97±0.3	120 ±0.9	143 ±0.9
	100	70±0.4	130±0.5	78±0.5	84±0.2	12±0.8	83 ±0.8
2	10	66±0.3	92±0.3	67±0.3	77±0.4	75 ±0.7	86 ±0.6
	100	57±0.1	79±0.2	54±0.2	66±0.2	33 ±0.6	75 ±0.4
3	10	40±0.2	65±0.4	42±0.2	55±0.5	30 ±0.2	62 ±0.4
	100	30±0.1	59±0.1	34±0.1	46±0.2	33 ±0.6	55 ±0.25
4	10	28±0.05	45±0.06	22±0.2	30±0.5	30 ±0.2	50 ±0.3
	100	10±0.01	39±0.02	14±0.1	26±0.2	33 ±0.6	40 ±0.1
Piperazine citrate	10	59±0.3	80±0.7	67±0.4	79±0.6	79±0.07	88±0.03
Distilled water	-	-	-	-	-	-	-

P = mean paralysis time (minute), D = mean death time (minute), conc. = concentration (mg/ml). Control worms were alive after 40 h except *T. solium* which were alive after 20 h.

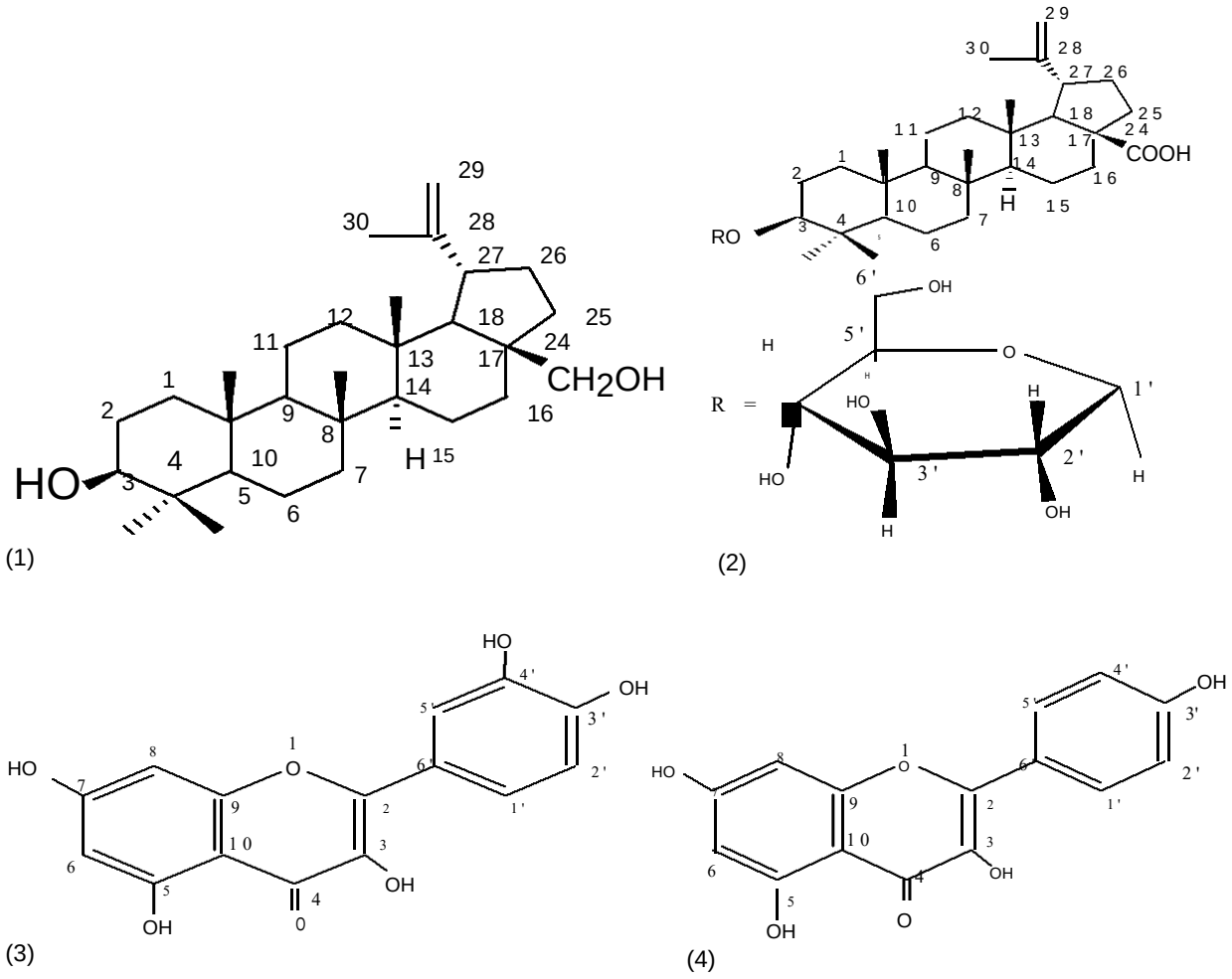

Figure 1. Structures of triterpenoids and flavonoids isolated from *Bridelia ferruginae*.

standard anthelmintic drug, piperizine citrate at the same concentration. At concentration above 50 mg/ml, the extracts and isolated compounds demonstrated higher anthelmintic potency compared to the reference anthelmintic drug at the same concentration (Table 1). Quercetin (3) and kaempferol (4) were the most potent anthelmintic compounds, followed closely by glucoside of betulinic acid (2), and lastly betuline (1) (Table 2).

The function of worm expeller like piperazine citrate is to cause paralysis of the worms such that they are expelled in the faeces of men and animals (Lechat et al., 1978). The extracts and isolated compounds did not only paralyse the worms, but killed them at different concentrations. The intrinsic high anthelmintic potency displayed by compounds (3) and (4) (Figure 1) can be attributed to the presence of reactive 2-phenyl chromone structure (Kandarkar et al., 1998). The number and position of –OH (phenolic) groups had a marked effect on the ability of flavonoids to inhibit homovanilic acid (HVA), and to bind effectively with biological enzymes, all of which are responsible for their antioxidant and cytotoxic effects (Hillwell, 1994; Havstean et al., 1980). Previous studies have implicated flavonoids in pharmacological activities such as anthelmintic and inflammatory activities (Makkar et al., 2007). The presence of quercetin and kaempferol in *B. ferruginae* stem barks extract emphasizes its anthelmintic potentials and this justifies the use of the plant in traditional medicine as an anthelmintic plant. Triterpenoids such as betulinic acid isolated from medicinal plant has equally demonstrated high intrinsic anthelmintic potency on parasitic worms (Asuzu et al., 1993; Enwerem et al., 2001) . The reactive carbonyl in betulinic acid, coupled with the hydroxyl moiety can be use to justify its anthelmintic potency.

Conclusion

The findings in this work agree with the use of *B. ferruginae* in ethno medicinal treatment of worm infection.

ACKNOWLEDGEMENTS

Our thanks are due to Mr. Thomas Kolawole Odewo of the Forest Research Institute of Nigeria (FRIN) for the collection and authentication of the plant material used in this study. We thank Prof Benn of the Department of Chemistry, University of Calgary, Alberta, Canada for running the NMR spectral in his laboratory.

REFERENCES

Addae-Mensah I (1992). Towards a rational scientific basis for herbal medicine: A Phytochemist's two-decade contribution, Accra, Ghana University Press, pp. 305-307.

Addae-Mensah I, Achenbach H (1985). Terpenoids and flavonoids of *Bridelia ferruginae.* Phytochem., 24: 1817-1819.

Addae-Mensah I, Munenge RW (1989). Quercetin-3-neohesperidose (rutin) and other flavonoids as the active hypoglyeaemic agents in *Bridelia ferruginae.* Fitoterapia, 9(4): 359-362.

Adeoye AO, Abaeli AM, Omowumi CJ, Olukoya DK (1988). Antimicrobial Activity of *Bridelia ferruginae.* In: Book of Abstract of the symposium on drug production from natural sources, Drug Research and Production Unit, Obafemi Awolowo University, Ile-Ife, p. 24.

Asuzu IU, Gray AI, Waterman PG (1999). The anthelmintic activity of 3--*O*-methyl chiroinositol isolated from *Piliostigma thonningii* stem bark. Fitoterapia, 70: 77-79.

Asuzu IU, Onu OU (1994). Anthelmintic activity of the ethanolic extract of *Piliostigma thinningii* bark in *Ascaridia galli* infected chickens. Fitoterapia, 65: 291-294.

Asuzu IU, Nwelle OC, Anaga AO (1993). The pharmacological activities of the methanolic bark extract of *Berlinia grandiflora*. Fitoterapia, 64(6): 529-534.

Ayensu ES (1978). Medicinal plants of West Africa. Michigan Reference Publication Inc., pp. 1231-1234.

Brooker S, Alexander N, Geiger S, Moyeed R.A, Stander J, Fleming F, Hotez PJ, Oliveira R, Bethony J (2006). Human helminth infection in urban and rural environments. Int. J. Parasitol., 36: 1143-1151.

Cimmanga K, Ying L, Apers S, Helmans A, Cos P, Hermans H (2001). Radical scavenging and xanthine oxidase inhibitory activity of phenolic compounds from *Bridelia ferruginae* stem bark. J. Pharm. Pharmacol., 53: 5, 757-761.

Cimmanga K, Ying L, De-Bruyne T, Apers S, Pieters L, Totte J (1999). Complement-inhibiting constituents of *Bridelia ferruginae* stem bark. Planta Med., 65: 213-217.

Cox FEG (2001). Concomitant infections, parasites and immune responses. Parasitol., 122: 23-38.

Dan S, Dan SS (1986). Isolation of betuline from *Bentula Mandschurica*l repels. Fitoterapia, 57: 45-47.

De Bruyne T, Cimmanga K, Pieters L, Claeyer M, Dominise R, Vlletinck A (1997). Gallocathechin (4', 7-epigallocathechin, a new bioflavonoid isolated from *Bridelia ferriginea.* Nat. Prod. Lett., 11: 47-52.

Ekanem JT, Kolawole OM, Abbah OC (2008). Trypanocidal potential of methanolic extracts of *Bridelia ferruginae* benth bark in *Rattus novergicus.* Afr. J. Biochem. Res., 2(2): 45-50.

Enwerem NM, Okogun JI, Wambebe CO, Okorie DA, Akah PA (2001). Anthelminthic activity of the stem bark extracts of *Berlinia grandiflora* and one of its active principles, Betulinic acid. Phytomed., 8(2): 112-114.

Havsteen BH, Lebensmitee LZ, Foresck U (1980). The Flavonoids. Scientia, 10: 36-41.

Hillwell B (1994). Antioxidant property of quercetin. Lancet, 344: 421-423.

Irobi ON, Mao-Young, Anderson WA, Daramola SA (1994). Antimicrobial activity of bark extracts of *Bridelia ferruginae.* J. Ethnopharmacol., 43(3): 185-190.

Iwu MM (1984). States of medicinal plant research in Nigeria. 4th Annual Conference, Nigeria Society of Pharmcognosy, University of Nigeria, Nsukka, pp. 57-59.

Iwu MM (1986). African Ethnomedicine. USP Press, Enugu, Nigeria, pp. 134-136.

Kandarkar SV, Sawant SS, Ingle AD, Deshpande SS, Mann GB (1998). Flavonoids: Their bioactivities. Ind. J. Exp. Biol., 36: 675-677.

Lechat P, Bisseliches F, Dechy H (1978). Pharmacologie Medicale, 3rd ed. Masson, Paris, pp. 120-122.

Liva H, Ryoji K, Kazuo Y (2002). Cussosaponins A-E, Triterpene Saponins from the Leaves of *Cussonia raceme*, a Malagasy Endemic plant. Chem. Pharm. Bull., 50(9): 1290-1293.

Makkar HP, Sidhuraju P, Becker R (2007). Plant secondary metabolites: Humana Press Inc. New Jersey, USA.

Mali RG, Shailaja M, Patil, KS. (2005). Anthelmintic activities of the root bark of *Capparis spinosa.* Ind. J. Nat. Prod., 21: 50-51.

Olajide OA, Makinde JM, Awe SO (1999). Effects of aqueous extract of *Bridelia ferruginae* stem bark on corragoenan-induced oedema and granuloma tissue formation in mice. J. Ethnopharmacol., 66(6): 113-117.

Oliver-Bever P (1960). Medicinal plants of the arid zones. Cambridge University Press, London. J. Sci. Ind. Res. (India), 8B: 234- 236.

Patra SK, Chaudhuri S, Panda SK (1988). Isolation of betuline from

Polyporous pinicola. J. Nat. Prod., 51: 2.
Rashid MA, Gustafson KR, Cardellina JH, Boyd MR (2000). A new podophyllotoxin derivative from *Bridelia ferruginae.* Nat. Prod. Lett., 14(4): 285-292.

Wang G, Chen X, Xu J, Murayama T, Shoji J (1996). *Yaoxue Xuebau.* 31: 940-944.