British Microbiology Research Journal
8(4): 525-531, 2015, Article no.BMRJ.2015.144
ISSN: 2231-0886

SCIENCEDOMAIN *international*
www.sciencedomain.org

In vitro Antibacterial Activity of Aqueous Extracts of *Bidens pilosa* L. (Asteraceae) from Nigeria

Oladipupo A. Lawal[1*], Kehinde O. Amisu[2], Segun K. Akinyemi[2], Adetayo A. Sanni[3], Mthokozisi B. C. Simelane[4], Rebamang A. Mosa[5] and Andy R. Opoku[5]

[1]Departments of Chemistry, Lagos State University, PMB 0001 LASU Post Office, Ojo Lagos, Nigeria.
[2]Department of Microbiology, Lagos State University, PMB 0001, LASU Post Office, Ojo, Lagos, Nigeria.
[3]Department of Botany, Lagos State University, PMB 001, LASU Post Office, Ojo, Lagos, Nigeria.
[4]School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, Scottville 3209, South Africa.
[5]Department of Biochemistry and Microbiology, University of Zululand, KwaDlangezwa 3886, South Africa.

Authors' contributions

This work was carried out in collaboration between all authors. Author OAL designed the study and wrote part of the manuscript. Author AAS performed the phytochemical screening. Authors KOA and SKA carry out the antibacterial activity. Authors MBCS and RAM managed the literature searches and wrote the final draft of the manuscript. Author ARO supply the chemicals and supervised the work. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2015/17900
Editor(s):
(1) Laleh Naraghi, Plant Disease Research Department, Iranian Research Institute of Plant Protection, Tehran, Iran.
Reviewers:
(1) Anonymous, Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca, Romania.
(2) P. R. D. Perera, Department of Food Science and Technology, University of Sri Jayewardenepura, Sri Lanka.
(3) Anonymous, State University of Ponta Grossa, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1090&id=8&aid=9259

Original Research Article

Received 29th March 2015
Accepted 26th April 2015
Published 14th May 2015

ABSTRACT

Aims: The aim of this work was to investigate the *in vitro* antibacterial activity of aqueous extracts of different organs of *Bidens pilosa*.
Study Design: The design includes the extraction of crude extracts from the air-dried leaves, stems and roots samples of *B. pilosa* and the screening and determination of antibacterial activity of the extracts.
Place and Duration of Study: The leaves, stems and roots of *Bidens pilosa* were exhaustively

**Corresponding author: E-mail: jumobi.lawal@lasu.edu.ng;*

extracted separately with double distilled water. The extracts were tested for the presence of secondary metabolites. The antibacterial activity of extracts was tested against some clinical and environmental isolates using agar-disc diffusion and broth-microdilution methods.

Results: The leaf extract exhibited significant inhibition on growth of the bacteria tested than the seed and root extracts. The mean zones of inhibition of the leaf extract ranged between (9.0±0.6 - 27.3±1.2) mm compared with (6.0±0.6- 11.7±0.6) mm and (6.0±0.6 - 16.3±0.6) mm for the seed and root extracts, respectively. The minimum bactericidal concentrations (MBC), 1.3 - >10 mg/ml was found to be equal or twice the minimum inhibitory concentrations (MIC), 0.6 - >10 mg/ml for the leaf extract and twice or greater for the stem and root extracts.

Conclusion: The activity of the aqueous leaf extract of *Bidens pilosa* provides the scientific justification for the use of this plant in traditional medicinal.

Keywords: Bidens pilosa; asteraceae; aqueous extracts; antibacterial activity.

1. INTRODUCTION

Bidens pilosa L. (Asteraceae) is an annual, erect cosmopolitan weed of about 0.6-2 m high with more than three different varieties [1,2]. It is native to South America and widely distributed in tropical and subtropical regions of the world [3,4]. The leaves (*ca* 15-20) mm long are opposite with saw-like edges and divided into 3-5 lance shaped segments. The flowers are mostly yellow with terminal head (6 -12) mm in diameter and solitary arranged, but can have white or pinkish florets for the periods of the flower's development. The seeds are black or dark brown, slender and about one cm long, clustered on the end of the stalk with three tiny prongs [4]. In traditional medicines of many countries of the world, different parts of *B. pilosa* in form of juice, powder, decoction or taken orally have been reportedly used to treats various diseases and disorders [5-7]. In addition, numerous biological and pharmaceutical activities have been reported for the plant [6-14]. Furthermore, the leaves are eaten as a vegetable [5,15,16]. Previous phytochemical studies have shown that *B. pilosa* was affluent in alkaloids, saponins, tannins, aliphatic and aromatic hydrocarbons, phenylheptatriyne, cytopiloyne, phytosterols, chalcones, aurones, centaurein, centaureidin, caffeic acids, glucosides, polyacetylenes, terpenoids, porphyrins and nitrogen and sulphur-containing compounds and many flavonoids derivatives, some of which possesses antimicrobial, anthelmintic, cercaricidal, anti-inflammatory, anticonvulsant, antioxidant, antibacterial and cyctotoxic activities [6,14,17,18]. The plant also contains calcium, iron and zinc, as well as β-carotene [16,19].

In Nigeria, the powder or ash from of the seed of *B. pilosa* have been used locally as an anaesthetic for cuts [20]. Also, different parts of *B. pilosa* in combination with *Vernonia amygdalina*, *Momordica charantia*, *Carica papaya*, *Ocimum gratissimum, Nicotiana tabacum*, *Bridelia micrantha*, *Alstonia congensis, Alchornea cordifolia*, *Securidaca longipedunculata* and *Uvaria afzelii* were used for treating diabetes in many districts of Lagos State, Nigeria [21,22]. In view of the extensive utilization of different parts of *B. pilosa* in traditional medicine of many countries of the world [6,7], there is no report to the best of our knowledge on the crude extract of *B. pilosa* growing in Nigeria. Although, the volatile constituents of essential oils from the leaves of *B. pilosa* from Nigeria had been previously reported [23]. In continuation of our growing interests on the poorly studied species of Nigerian flora [24,25], the aim of the present paper was to investigate the *in vitro* antibacterial activity of aqueous extracts of different organs of *B. pilosa* from Nigeria.

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh plant materials of *Bidens pilosa* were purchased from Iyan-iba central market, Ojo Local Government Area, Lagos State, Nigeria. Identification of the plant material was carried out at the herbarium of Department of Botany, University of Lagos, Akoka-Yaba, Lagos. A voucher specimen (LUH 2793) was deposited at Department of Botany, University of Lagos Herbarium.

2.2 Preparation of Extract

Air-dried and grounded leaves (100 g), stems (150 g) and roots (150 g) of *Bidens pilosa* were exhaustively extracted separately with double distilled water, 5:1 (v/w) solvent to dry weight of

plant sample on a Stuart Lab-Scale Orbital Shaker (Model SSL1) at 25ºC for two days. The resultant solutions were filtered and freeze-dried to produce the extracts, which were 16.88%, 12.69% and 13.07% of the starting materials, respectively. Each dried extract was weighed and re-dissolved in Dimethylsulfoxide (1mg/ml) for further experiments.

2.3 Phytochemical Screening

The phytochemical screening of *B. pilosa* extracts was carried out to detect the presence of alkaloids, flavonols saponins, tannins, flavonoids, terpenoids, reducing sugars, steroids and glycosides according to the standard procedures [26-28].

2.4 Microorganisms Tested

A total of 10 local isolates comprising of six *Salmonella* species and four other bacterial strains obtained from the Department of Microbiology, Lagos State University, Ojo, Lagos State, Nigeria were employed for this study. The microorganisms included a Gram-positive bacterium, *Staphylococcus aureus* and Gram-negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella* spp, *Salmonella arizonae*, *Salmonella choleraesuis*, *Salmonella enteritidis*, *Salmonella tyhpi*, *Salmonella paratyhpi* and *Salmonella typhimurium*. The stock cultures were maintained at 4ºC in Mueller-Hinton agar (Oxoid Ltd, England).

2.4.1 Determination of antibacterial assay

The antibacterial activity of the aqueous extracts of the leaf, stem and root of *B. pilosa* was measured by disk-diffusion method [29]. The microorganisms were grown overnight at 37ºC in 10 mL of Mueller Hinton Broth (Oxoid Ltd, England) for 24 h. The cultures were adjusted with sterile saline solution to obtain turbidity comparable to that of McFarland no. 0.5 standard (1.0×10^8 CFU/mL), according to the method [30]. Petri dishes containing Mueller Hinton agar (Oxoid Ltd, England) were inoculated with the microbial suspensions. Concentrations of 40 mg/mL of each extract were prepared. Sterile Whatman No.1 (6 mm) discs paper was placed on the surface of the seeded agar plates and 10 µL of each extract in dimethylsulfoxide was applied to the filter paper disk. The plates were incubated overnight at 37ºC for 24 h and the diameter of any resulting zones of inhibition (mm) was measured. Each experiment was carried out in triplicates. Standard antibiotic discs for ciprofloxacin (30 µg) and chloramphenicol (25 µg), and DMSO solution (positive and negative controls) were also run in parallel along with the extracts.

2.4.2 Determination of the minimal inhibitory and bactericidal concentrations

A broth microdilution method [31] was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts. Bacterial cultures were incubated in Muller-Hinton (MH) broth overnight at 37ºC and a 1:1 dilution of each culture in fresh MH broth was prepared prior to use in the micro dilution assay. Serial dilutions were made to obtain concentrations ranging from 10 mg/mL to 0.078 mg/mL. One hundred µL of bacterial culture of an approximate inoculum size of 1.0 x 10^8 CFU/mL was added to all well and incubated at 37ºC for 24 H. After incubation, 40 µL of 0.2 mg/mL *p*-iodonitotetrazolium violet (INT) solution was added to each well and incubated at 37ºC. Plates were examined after about 30 min of incubation. Microbial growth is indicated by the presence of a reddish colour, which is produced when INT, a dehydrogenase activity-detecting reagent is reduced by metabolically active microorganisms to the corresponding intensely coloured formazan. MIC is defined as the lowest concentration that produces an almost complete inhibition of visible microorganism growth in liquid medium. Solvent control (DMSO solution) and standard antibiotics ciprofloxacin and chloramphenicol were included in the assay. The MBC was determined by transferring 10 µL aliquot from each well at the concentration corresponding to the MIC and those concentrations above into 190 µL of appropriate broth in a sterile 96-well plate. The plates were incubated under the same conditions as in the MIC experiment. The presence or absence of bacterial growth was determined by visual inspection. The MBC was considered the lowest concentration of the extract at which no growth occurred.

2.5 Statistical Analysis

The mean and standard deviation of three experiments were determined for zones of inhibition. Statistical analysis of the differences between mean values obtained for experimental groups were calculated using Microsoft excel program, 2003. Data were subjected to one way

analysis of variance (ANOVA). *P* values ≤ 0.05 were regarded as significant and *P* values ≤ 0.01 as very significant.

3. RESULTS AND DISCUSSION

The preliminary phytochemical screening of *B. pilosa* aqueous extracts revealed the presence of some secondary metabolites (Table 1). Alkaloids, glycosides, saponins and sterols were present in all the extracts. Flavonoids, tannins and terpenoids were detected in the leaf and root extracts, while, only the leaf extract showed the presence of flavonols.

The results of the antibacterial activity (Zones of inhibition, minimal inhibitory and bactericidal concentrations) of *B. pilosa* extracts against the bacterial tested were summarized in Tables 2 and 3. The inhibitory action (Table 2) of the aqueous leaf extract (9.0-27.3) mm was high for all the bacterial strains tested than the aqueous seed (6.0-11.7) mm and root (6.0-16.3) mm extracts. In addition, the leaf extract showed very good activity against Gram-negative bacterial inhibiting *S. tyhpi* at 27.3 mm, *E. coli* 22.7 mm, *S. paratyhpi* 21.4 mm, *S. typhimurium* 21.3 mm and *P. aeruginosa* 17.7 mm than the seed and root extracts. Furthermore, a Gram-positive bacterium, *S. aureus* was also inhibited at 20.7 mm. However, all the extracts were susceptible to *S. enteritidis*, *Shigella spp*, *S. choleraesuis* and *S. arizonae* ranging from (9.0-11.0), (6.3-9.3) and (6.0-9.0) mm for the leaf, seed and root extracts respectively.

The noteworthy antibacterial activity of *B. pilosa* aqueous leaf extract was showed by the values of the MIC (0.6-10) mg/ml and MBC (1.3-10) mg/ml against MIC (5- >10) mg/ml and MBC (10 - >10) mg/ml for both the seed and root extracts. In all the bacterial tested, MBC values for the leaf extract are mostly the same or twice the MIC values, which shows the MBC/MIC ratio to be ≤ 2 against ≥ 2 for the seed and root extracts (Table 3).

Table 1. Phytochemical constituents from *B. pilosa* extracts

Constituents	Test	Inference		
		Leaf	Stem	Root
Alkaloids	Dragendorff's reagent	+	+	+
	Mayer's reagent	+	+	+
Flavonoids	Sodium hydroxide test	+	-	-
	Lead acetate test	+	-	+
	Ferric chloride test	+	-	+
Flavonols	Shinoda reduction test	+	-	-
Glycosides	Keller-Killiani	+	+	+
Saponins	Frothing	+	+	+
Tannins	Ferric chloride	+	-	+
Terpenoids	Liebermann-Buchard test	+	-	+
Steroids and sterols	Salkowski test	+	+	+

+ = present; − = absent

Table 2. Antibacterial activity of *Bidens pilosa* extract[a]

Microorganisms	Zones of inhibition (mm)				
	Leaves	Stems	Roots	Cip[b]	Chl[c]
S. aureus	20.7±1.5	11.7±0.6	16.0±1.0	23.7±1.5	29.7±1.5
E. coli	22.7±1.5	11.3±1.5	16.3±0.6	24.3±1.5	28.7±1.5
P. aeruginosa	17.7±1.2	10.3±0.6	11.3±1.5	20.3±0.6	27.3±1.2
Shigella spp	11.0±0.0	9.3±0.6	10.0±0.0	15.7±1.2	19.0±2.0
S. arizonae	9.3±1.0	6.3±0.6	7.7±0.6	13.7±2.1	14.3±1.2
S. choleraesuis	9.0±0.6	6.0±0.0	6.0±0.0	15.0±0.0	17.0±0.0
S. enteritidis	11.0±0.0	9.7±0.6	10.7±0.6	18.3±0.6	18.0±1.0
S. paratyhpi	21.7±1.5	10.3±0.6	14.0±0.0	20.3±1.2	25.7±0.6
S. tyhpi	27.3±1.2	11.7±0.6	14.7±1.2	25.7±1.5	29.6±0.6
S. typhimurium	23.3±1.2	10.3±1.2	12.3±0.6	20.7±1.2	32.3±1.5

[a](n = 3, mean ± S.D); [b]Cip -Ciprofloxacin; [c]Chl- chloramphenicol

Table 3. Minimal inhibitory and bactericidal concentrations of *Bidens pilosa* extracts

Microorganisms	Leaves		Stems		Roots		Cip[c]		Chl[d]	
	MIC[a]	MBC[b]	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	2.5	2.5	10	> 10	10	10	0.4	0.8	1.1	1.1
E. coli	2.5	5	10	> 10	5	10	0.4	1.6	0.5	2.1
P. aeruginosa	5	10	5	> 10	10	> 10	1.6	1.6	1.1	2.1
Shigella spp	10	10	> 10	ND	> 10	ND	3.1	3.1	2.1	4.3
S. arizonae	10	10	> 10	ND	> 10	ND	6.3	12.5	4.3	8.5
S. choleraesuis	10	10	> 10	ND	> 10	ND	12.5	12.5	2.1	8.5
S. enteritidis	5	5	5	10	5	10	3.1	6.2	1.1	2.1
S. paratyhpi	1.3	1.3	10	10	5	10	0.4	0.8	0.3	1.1
S. tyhpi	0.6	1.3	10	10	5	10	0.1	0.1	0.1	0.1
S. typhimurium	2.5	5	10	ND	10	10	1.6	3.1	0.5	2.1

[a]MIC and [b]MBC- minimum inhibitory and bactericidal concentrations (mg/ml)
[c]Cip -Ciprofloxacin; [d]Chl- chloramphenicol; ND- not determine

To our knowledge, the antibacterial activity of *B. pilosa* from Nigeria is been reported for the first time. Although, the antibacterial activity of *B. pilosa* and different cultivars from different countries had been previously reported [32-38]. The reports showed that the activity of the leaf extract might be attributable to the presence of phenylpropanoids, terpenoids, and many flavonoids and glycosides derivatives with well-known pharmaceutical and biological activities [6,7].

4. CONCLUSION

In conclusion, the study authenticate the therapeutic potency of *B. pilosa* leaf extract in traditional medicine and also delineate a good basis for the selection of the leaf extract for further investigations, particularly against food-borne pathogens (*Salmonella tyhpi, Salmonella paratyhpi* and *Salmonella typhimurium*), which are known to be responsible for infectious invasive enteritis and enterocolitis (typhoid fever).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sahu TR, Dakwale RN. Taximetrics study of varieties of *Bidens pilosa* species. Acta Phytotax. Sin. 1982;20(2):249-251.
2. Ballard R. *Bidens pilosa* complex (Asteraceae) in North and Central America. Amer. J. Bot. 1986;73(10):1452-1465.
3. Geissberger P, Séquin U. Constituents of *Bidens pilosa* L. Do the components found so far explain the use of this plant in traditional medicine. Acta Trop. 1991; 48:251-261.
4. Connelly P. Horrible weed or miracle herb? A review of *Bidens pilosa*. J. Aus. Trad. Med. Soc. 2009;15:77-79.
5. Hutchings A, Scott AH, Lewis G, Cunningham AB. Zulu medicinal plants. An inventory. University of Natal Press, Scottsville. 1996;324-325.
6. Silva FL, Fischer DCH, Tavares JF, Silva MS, de Athayde-Filho PF, Barbosa-Filho JM. Compilation of secondary metabolites from *Bidens pilosa* L. Molecules. 2011;16:1070-1102.
7. Bartolome AP, Villaseñor IM, Yang W. *Bidens pilosa* L. (Asteraceae): Botanical properties, traditional uses, phytochemistry, and pharmacology. Evid Based Complement Alternat Med. 2013;2013: 340215.
8. Pereira RL, Ibrahim T, Lucchetti L, da Silva AJ, de Moraes VL. Immunosuppressive and anti-inflammatory effects of methanolic extract and the polyacetylene isolated from *Bidens pilosa* L. Immunopharmacol. 1999;43:31-37.
9. Ubillas RP, Mendez CD, Jolad SD, Luo J, King SR, Carlson T, Fort DM. Antihyperglycemic acetylenic glucosides from *Bidens pilosa*. Planta Med. 2000; 66:82-83.
10. Abajo C, Boffill MA, Campo JD, Mendez MA, Gonzalez Y, Mitjans M, Vinardell MP. *In vitro* study of the antioxidant and immunomodulatory activity of aqueous infusion of *Bidens pilosa*. J. Ethno-pharmacol. 2004;93:319-323.

11. Andrade-Neto VF, Brandão MGL, Oliveira FQ, Casali VWD, Njaine B, Zalis MG, Luciana AO, Krettli AU. Antimalarial activity of *Bidens pilosa* L., (Asteraceae) ethanol extracts from wild plants collected in various localities or plants cultivated in humus soil. Phytother Res. 2004;18:634-639.
12. Kviecinski MR, Felipe KB, Schoenfelder T, Wiese LPL, Rossi MH, Goncalez E, Felicio JD, Filho DW, Pedrosa RC. Study of the antitumor potential of *Bidens pilosa* (Asteraceae) used in Brazilian folk medicine. J. Ethnopharmacol. 2008; 117:69-75.
13. Tobinaga S, Sharma MK, Aalbersberg WGL, Watanabe K, Iguchi K, Narui K, Sasatsu M, Waki, S Isolation and Identification of a potent antimalarial and antibacterial polyacetylene from *Bidens pilosa*. Planta Med. 2009;75:624–628.
14. Adedapo A, Jimoh F and Afolayan A. Comparison of the nutritive value and biological activities of the acetone, methanol and water extracts of the leaves of *Bidens pilosa* and *Chenopodium album*. Acta Pol. Pharm. Drug Res. 2011;68:83-92.
15. Odhav B, Beekrum S, Akula US, Baijnath H. Preliminary assessment of nutritional value of traditional leafy vegetables in KwaZulu-Natal, South Africa. J. Food Compost. Anal. 2007;20:430-435.
16. Orech FO, Aagaard-Hansen J, Friis H. Ethnoecology of traditional leafy vegetables of the Luo people of Bondo district, western Kenya. Int. J. Food Sci. Tech. 2007;58:522-530.
17. Ganders FR, Bohm BA, McCormick SP. Flavonoid variation in Hawaiian *Bidens*. Syst Botany. 1990;15:231-239.
18. Chang CL-T, Chang S-L, Lee Y-M, Chiang Y-M, Chuang D-Y, Kuo H-K, Yang W-C. Cytopiloyne, a polyacetylenic glucoside, prevents Type 1 diabetes in non-obese diabetic mice. J. Immunol. 2007;178:6984-6993.
19. Benhura MAN, Chitsiku IC. The extractable beta-carotene content of Guku (*Bidens pilosa*) leaves after cooking, drying and storage. Int. J Food Sci. Tech. 1997;32:495-500.
20. Mvere B. (2004). *Bidens pilosa* L. In: Grubben, GJH and OA Denton (Eds.), PROTA 2: Vegetables/Legumes. PROTA, Wageningen, Netherlands.
21. Gbolade AA Inventory of antidiabetic plants in selected districts of Lagos State, Nigeria. J. Ethnopharmacol. 2009;121:135-139.
22. Ezuruike UF, Prieto JM. The use of plants in the traditional management of diabetes in Nigeria: Pharmacological and toxicological considerations. J. Ethnopharmacol. 2014;155:857-924.
23. Ogunbinu AO, Flamini G, Cioni PL, Adebayo MA, Ogunwande IA. Constituents of *Cajanus cajan* (L.) Millsp., *Moringa oleifera* Lam., *Heliotropium indicum* L. and *Bidens pilosa* L. from Nigeria. Nat. Prod. Commun. 2009;4:573-578.
24. Lawal OA, Ogunwande IA, Salvador AF, Sanni AA., Opoku AR. *Pachira glabra* Pasq. Essential oil: Chemical constituents, antimicrobial and insecticidal activities. J. Oleo Sci. 2014;63(6):629-635.
25. Lawal OA, Ogunwande IA, Bullem CA, Taiwo OT, Opoku AR. Essential oil compositions and *in vitro* biological activities of three *Syzygium* species from Nigeria. *In* New developments in terpenes research (Chemistry research and applications). 1st edition, Edited by Hu, Jinnau. Nova Science Publisher, Inc. New York, America. 2014;93-112.
26. Sofowora A. Medicinal Plants and Traditional Medicines in Africa. Spectrum Books Ltd. Ibadan, Nigeria. 1993;150-156.
27. Harborne JB. Phytochemical Methods: A guide to modern techniques of plant analysis. 3rd edition, Chapman and Hall Ltd, London, UK. 1984;49-150.
28. Edeoga HO, Okwu DE, Mbaeble BO. Phytochemical constituents of some Nigerian medicinal plants. Afri. J. Biotechnol. 2005;4:685-688.
29. Vijoen AM, van Vuuren SF, Gwebu T, Demirci B, Baser KHC. The geographical variation and antimicrobial activity of African wormwood *(Artemisia afra* Jacq.) essential oil. J. Essent. Oil Res. 2006;18:19-25.
30. Lennette HE, Balows A, Hausler JW, Shadomy HJ. Manual de microbiología clínica. Medica Panamericana: Argentina. 1987;336-352.
31. Eloff JNA. sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 1998;64:711–713.
32. Marchant YY, Ganders FR, Wat CK, Towers GHN. Polyacetylenes in Hawaiian

Bidens. Biochem. Syst. Ecol. 1984;12:167-178.
33. Wong-Leung YL. Antibacterial activities of some Hong Kong plants used in Chinese medicine. Fitoterapia. 1988;59:11-16.
34. Horiuchi M, Seyama Y. Anti-inflammatory and antiallergic activities of *Bidens pilosa* L. var. radiate Scherff. J. Health Sci. 2006;52:711-717.
35. Brandao MG, Krettli AU, Soares LS, Nery CG, Marinuzzi HC. Antimalarial activity of extracts and fractions from *Bidens pilosa* and other *Bidens* species (Asteraceae) correlated with the presence of acetylene. J Ethnopharmacol. 1997;57(2):131-138.
36. Chang JS, Chiang LC, Chen CC, Liu LT, Wang KC, Lin CC. Antileukemic Activity of *Bidens pilosa* L. var. *minor* (Blume) Sherff and *Houttuynia cordata* Thunb. Am. J. Chin. Med. 2001;29:303-312.
37. Chiang YM, Chuang DY, Wang SY, Kuo YH, Tsai PW, Shyur LF. Metabolite profiling and chemopreventive bioactivity of plant extracts from *Bidens pilosa*. J. Ethnopharmacol. 2004;95:409-419.
38. Ashafa AOT, Afolayan AJ. Screening the root extracts from *Bidens pilosa* L. var. *radiata* (Asteraceae) for antimicrobial potentials. J. Med. Plants Res. 2009; 3(8):568-572.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=1090&id=8&aid=9259