

Analgesic Appraisal of *Bidens pilosa* (Asteraceae) Leaf Extracts Used in Management of Oral Lesion Pain in HIV/AIDS Patients in Rodents

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How to cite this paper: Ezeonwumelu, J.O.C., Ntale, M., Ogbonnia, S.O., Agwu, E., Tanayen, J.K., Adedeji, A.A., Okonkwo, C.O., Akunne, A.A., Ebosie, J.C. and Byarugaba, F. (2018) Analgesic Appraisal of *Bidens pilosa* (Asteraceae) Leaf Extracts Used in Management of Oral Lesion Pain in HIV/AIDS Patients in Rodents. *Pharmacology & Pharmacy*, 9, 175-192.
<https://doi.org/10.4236/pp.2018.96014>

Received: January 16, 2018

Accepted: June 26, 2018

Published: June 29, 2018

Abstract

Oral lesions, diarrhoea, *Pneumocystis carinii* pneumonia, tuberculosis and urinary tract infections are some of the opportunistic infections (OIs) which arise when the CD4 cells of the HIV/AIDS patient fall below 200 cells/mm³. HIV/AIDS infection complications include tissue damage from oral lesions accompanied with pains. Pain is a disagreeable sensory and sensitive experience associated with actual or potential tissue damage. This condition requires immediate treatment with analgesics and antibiotics. However, the inability of rural dwellers to afford readily available drugs is a consequence for



using herbs like *Bidens pilosa* whose local usefulness in the management of oral lesions of HIV/AIDS has not been proven scientifically. Therefore, the objective of this study was to provide the scientific basis in rats for the traditional healers' use of *Bidens pilosa* leaves' extracts in managing pain associated with oral lesions of HIV/AIDS patients in South Western Uganda. Assessment of the analgesic effects of *Bidens pilosa* was conducted using acetic acid in mice, formalin-induced pain and tail flick methods in rats. Both aqueous and ethanolic extracts of the leaves of *Bidens pilosa* produced statistically significant dose dependent inhibition of acetic acid induced pain, non dose dependent pain reduction in formalin induced pain, ($p < 0.05$; student t-test) and non dose dependent tail withdrawal pattern ($p < 0.05$, Multivariate ANOVA test). Hence, we conclude that extracts of *Bidens pilosa* have an analgesic basis for their local use in treatment of oral lesions associated pain in HIV/AIDS patients in South-Western Uganda.

Keywords

Local Use, *Bidens pilosa*, Pain, Oral Lesion, HIV/AIDS, Rodents

1. Introduction

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage [1]. The word “unpleasant” describes various levels of inconvenience, misery, anguish, anxiety, depression, desperation and ultimate remedy of suicide [2] [3]. In Africa, many researchers such as [4] [5] [6] [7] [8] have all reported high prevalence of oral lesions associated with HIV/AIDS, with oral candidiasis being very prevalent (50% - 80%). In Uganda, two studies [9] [10] reported a high prevalence (70% - 80%) of oral candidiasis and oral bacterial infections among HIV/AIDS patients. Some forms of oral lesion or oral tissue damage occur and may include major aphthous ulceration (most common, 2% - 3%) [11]; necrotizing stomatitis (painful ulceration which exposes underlying bone and leads to tissue destruction) and recurrent aphthous ulcerations (present on non-keratinized tissues such as labial and buccal mucosa, the floor of the mouth, ventral surface of the tongue, posterior oropharynx and the maxillary and mandibular vestibules). Pain increases upon eating salty, spicy, acidic foods or beverages and due to trauma when consuming hard or rough foods [12]. The pain as discomforting as it may be, requires immediate treatment with analgesic agents and antibiotics. But some of the available drugs are either not affordable, not accessible or socially and culturally not acceptable to the rural populace and for some who can afford to buy the drugs, adverse effects of the drugs e.g. gastric irritation of non steroidal anti-inflammatory drugs may not allow them to use the drugs as required [13] [14]. This makes room for them to resort to use of herbs. Anecdotal evidence from South Africa suggests that a number of antiretroviral therapy (ART) patients resort to traditional medicine after experiencing side effects from ART. Studies have found that HIV positive

persons use traditional and alternative medicines at high rates between 15% and 79%, higher than people with other serious illnesses [15] [16] [17]. A study on the use of traditional herbal medicine was conducted on 137 AIDS patients in Kabarole District, Western Uganda, for relief of pain and fever [18]. One of the most highly used herbs by traditional healers and local people in South Western Uganda in treating the pain associated with oral lesions of HIV/AIDS patients is *Bidens pilosa* (Unpublished data). The traditional healers and local users of *Bidens pilosa* in South-Western Uganda were ascertained to prepare decoctions of the leaves of the herb with water and by soaking in ethanol for some days. This observation forms the basis for our use of water and ethanol in the extraction of *Biden pilosa* leaves for this study (Unpublished data). *Bidens pilosa*, an Astera-ceae, is a cosmopolitan, annual herb which originates from the tropics especially Central America, Africa and Asia [19]. *B. pilosa* is an erect, tall branched, perenn-ial plant with green leaves, thin white or yellow flowers that develop into a cluster of tiny, barbed black seeds with short, stiff hairs which stick to feathers, fur, or socks and other such materials [3] [10] [11] [20] [21] [22] [23]. It has many common names which include black-jack, [4] [24] beggar-ticks, cobbler's pegs, farmer's friends [5] [25] and Spanish needle [2] [6] [7] [8] [9] [26] [27] [28] [29] [30]. It is called "Enyabarashana" in Runyankole language in Uganda and "Ogwumma" in Igbo language in Nigeria. It is globally distributed and widely used as a folk remedy, serving as an extraordinary source of food and medicine [20]. *Bidens pilosa* serves as a medicinal plant in areas of Africa, Asia, and tropical America. Its different parts are e.g. roots, leaves, and seeds are in-formed to be used against bacteria, dysentery, inflammation, microbes, and ma-laria; for diuretic, hepatoprotection, and hypotension. In Africa and tropical areas of Central and Southern America, tropical and subtropical areas of Asia, *B. pilosa* is reported in folk medicine for treatment of headaches, ear infections, hangovers, diarrhoea, kidney problems, malaria, jaundice, dysentery, burns, arthritis, inflammatory disorders, ulcers, abdominal symptoms and facilitation of child labour; and studies have demonstrated its anaesthetic, antibacterial, an-tidiabetic andcoagulant activities [31]-[36]. It is also used in Uganda by tradi-tional healers in the treatment of opportunistic infections of HIV/AIDS patients mostly oral lesions as reported by the local people (Unpublished data). *B. pilosa* is claimed to treat more than 40 disorders, and 201 compounds have been iden-tified from this plant. Polyynes, flavonoids, phenylpropanoids, fatty acids, and phenolics are the primary bioactive compounds of *B. pilosa*, and they have been reported by some studies to be effective in the treatment of tumours, inflamma-tion/immune modulation, oxidative processes, diabetes and hyperglycaemia, vi-ruses, bacteria, fungi, protozoans and other microbes, wounds, gastrointestinal diseases, hypertension, vasoconstriction, ulcer, and cardiovascular diseases. However, caution should be exercised in the therapeutic use of *B. pilosa* for hy-poglycaemia, hypotension, bleeding, and allergy [20]. Generally, there is need for evidence-based research on herbal medicines when quality, efficacy, safety and well-defined cause/effect relationships are on discourse [37]. In this study, we

investigated the scientific basis for ethnomedical use of extracts of *Bidens pilosa* leaves in the management of the pain involvement of oral lesions of HIV/AIDS patients in South Western Uganda.

2. Materials and Methods

2.1. Plant Extract

Extracts were obtained from the air-dried, ground *Bidens pilosa* leaves using a 1-hour hot water extraction method for aqueous extracts and a 72-hour cold ethanolic extraction method according to the procedure used by traditional and local users of the herb. It was ascertained that the local people usually boil the plant materials in water for their extraction either as decoction or infusion and few others soak plant materials in cold alcohol locally called waragi. Thus, extracts were obtained using these methods. For an aqueous extract, 200 g of plant material was soaked and boiled in 1000 mL of clean distilled water in a clean beaker for one hour. The extraction in cold ethanol was carried out with 200 g of plant material soaked in 1000 mL of absolute ethanol for 72 hours with occasional shaking using the electronic shaker (Nüve® SL 350, serial number: 02-0974, Ankara, Turkey). Thereafter, the boiled and cold soaked plant materials were each filtered using clean white cotton cloth and subsequently using No.1 Wattmann's filter papers to obtain particle free filtrate. The filtrate for the aqueous extract was dried by boiling off all water elements in a water bath at 100°C while filtrate for the ethanolic extract was dried by evaporation in a hot air oven at 40°C. The extracts were stored without solvent at freezing temperature (–20°C) with minimal exposure to freeze-thaw cycles and humidity [38]. Freshly prepared solution of aqueous extracts was always reconstituted in distilled water and suspensions of ethanolic extract was always reconstituted with little amount of Tween 80 in distilled water ready for use in the studies.

2.2. Animals

Swiss albino mice were used for acetic acid induced pain tests while Wistar rats were used for formalin and tail-flick induced pain tests. These animals were obtained from the Animal Facility Centre (AFC) of Kampala International University, Western Campus, Ishaka. The animals were kept in a cage lined with wood shavings, at room temperature with adequate ventilation, under a naturally illuminated environment with 12 h of light and 12 h of darkness. They were fed with standard diet (Nuvita® Animal Feeds Ltd., Jinja Uganda). They were allowed access to clean drinking water *ad libitum*. The animal experiments were carried out according to the National Institute of Health Guide for the care and use of laboratory animals [39] and ethical guidelines for investigation of experimental pain in animals [40].

2.3. Acetic Acid-Induced Writhing Test (Chemical)

Each rat was placed inside a transparent observation cage (40 × 30 × 20 cm) for

an acclimatization period of 30 min. This test was carried out with the methods described by Koster *et al.* [41] and Collier [42], with slight modification. The modification applied was that one set of experiments was conducted 30 min after pretreatment with the negative, positive standard and test drugs. One set of five groups of mice ($n = 5$) were pretreated with each of the aqueous and ethanolic *Bidens pilosa* extracts (200, 400 and 800 mg/kg), distilled water (10 mL/kg) and aspirin (150 mg/kg). Each mouse was injected with 0.7% of an aqueous solution of acetic acid (10 mL/kg, i.p) after the predetermined time (30 min) and placed in transparent observation cage. The number of writhes (abdominal musculature constriction followed by extension of the hind limbs [43] exhibited by each mouse was cumulatively counted for 15 minutes after 5 minutes lag time of the aqueous acid injection [44] and the mean count of each group taken.

2.4. Formalin Test (Chemical)

This test was carried out in five groups of rats ($n = 5$) pretreated with either distilled water (10 mL/kg); the aqueous extract, ethanolic extract (200, 400 and 800 mg/kg p.o.) or acetylsalicylic acid (aspirin) (100 mg/kg) using the Dubuisson and Dennis method [45] modified by Tjolsen [46]. Formaldehyde (0.05 mL, 2.5%) was injected into the sub-plantar surface of the rat left hind paw 30 minutes after the treatment. Severity of pain was rated in two distinct phases for 1 hour; in first phase (0 - 10 minutes), readings were taken every 2 minutes and in late phase (15 - 60) minutes, readings were taken every 5 minutes using Dubuisson and Dennis method [45] of pain scoring measurements in the following manner: (0) = normal weight bearing on the injected paw; (1) = light resting of the paw on the floor; (2) = elevation of the injected paw; and (3) = licking, biting and grooming of paw. These responses were observed and recorded for a total of 60 minutes. The first 10 minutes was considered as the early phase representing aphasic (Central Nervous System) pain while the period between 15 and 60 minutes was recorded as the late phase representing tonic (peripheral/inflammatory) pain. The mean \pm SEM of the readings was recorded as the pain score.

2.5. Tail-Flick Test (Thermal)

Tail-flick test or thermal test was conducted with method of D'Armour and Smith [47], as subsequently modified for rats using hot water bath [48] [49] [50] [51] [52] with minor modifications for our local laboratory settings. The rats were initially screened for the test by immersing about 2 cm of their tails (initially measured and marked out with a marker pen from the tip of the rats' tail) into hot water maintained at $55^{\circ}\text{C} \pm 1^{\circ}\text{C}$ in a water bath. The animals that lifted their tails out of water within 5 seconds were selected for the study. A cut-off time was set at 6 seconds to prevent the rats' tail from damage by hot water. Thirty rats that showed response (withdrawing the tail within 5 seconds) were selected and grouped into 6 groups of 5 ($n = 5$). The rats in the six groups were

treated with the aqueous and ethanolic *Bidens pilosa* extracts (200, 400 and 800 mg/kg), morphine (2 mL/kg, i.p) as positive control for morphine-like activity of *Bidens pilosa* extracts and aspirin (100 mg/kg p.o) as positive control for NSAID-like activity of *Bidens pilosa* extracts and distilled water (10 mL/kg) as negative control. The tail withdrawal reflex period (latency/pain threshold) was taken at 30 and 60 minutes after treatment and recorded.

2.6. Statistical Analysis

Data were entered into the Excel sheet and means were calculated. Student t-test, test of between-subject effects applying multivariable Analysis Of Variance (ANOVA) and multiple comparison tests applying Tuskey HSD, Scheffe, LSD or Dunnett t 2-paired tests were used to compare the means to obtain the p values from treated and control groups of rats with the help of Statistical Package for Social Sciences (SPSS 20.0) soft wares. Results were expressed as mean \pm SEM. Statistical significance was taken as $p < 0.05$ and confidence interval at 95%.

3. Results

3.1. Results of Tests of *Bidens pilosa* Extracts on Acetic Acid-Induced Writhing in Mice

In **Table 1**, for both aqueous and ethanolic extracts of *Bidens pilosa*, the acetic acid induced writhes in the mice were inhibited dose-dependently and significantly ($p < 0.05$; student t-test) in the same manner as acetylsalicylic acid. Therefore, the activities exhibited by both extracts were comparable to that of positive control (acetylsalicylic acid) though it could be seen from the results that the activity displayed by the aqueous extract was better than the activity of the ethanolic extract at the three different dose levels. Multiple dose analysis with Analysis Of Variance (ANOVA) of acetic acid induced pain inhibition indicated statistically significant relationship ($p < 0.05$) between 200 mg/kg dose ($p = 0.01$) and 400 mg/kg dose ($p = 0.001$) of the extracts of *Bidens pilosa* (test of between-subjects effects). When the effects of the various doses of aqueous extract

Table 1. Acetic acid-induced writhing test of aqueous and ethanolic extracts of *Bidens pilosa* in rats.

Dose (mg/kg)	Treatments							
	Normal saline	ASA	BPA			BPE		
	10 mL/kg	150	200	400	800	200	400	800
Mean writhes count \pm SEM 30 mins after pretreatment	36.20 \pm 5.02	0.60 \pm 0.60*	1.60 \pm 0.51*	0.60 \pm 0.40*	0.20 \pm 0.20*	2.20 \pm 0.86*	1.20 \pm 0.80*	0.60 \pm 0.60*
% Inhibition of pain (Antinociceptive effect)	-	98.3	95.6	98.3	99.5	93.6	96.5	98.3

n = 5; Data were mean \pm SEM; * = test groups varied significantly ($p < 0.05$) from normal saline group using student t-test; ASA = Acetylsalicylic acid; BPA = *Bidens pilosa* leaf Aqueous extract; BPE = *Bidens pilosa* leaf Ethanolic extract.

of *Bidens pilosa* were compared with those of the ethanolic extracts using Dunnett t 2-paired multiple comparisons tests, it showed that there was no significant difference ($p = 1.00$ and >0.05) in analgesic activity between the aqueous and ethanolic extracts.

3.2. Results of Tests of *Bidens pilosa* Extracts on Formalin-Induced Pain in Rats

Table 2 displays the results of the effects of acute administration of *Bidens pilosa* on formalin induced pain in rats. The general trend of pain reduction activity in both phases of formalin induced pain for aqueous and ethanolic extracts of *Bidens pilosa* was a significant, non dose-dependent relationship ($p < 0.05$; student t-test) when compared with negative control (distilled water). The aqueous extract showed seemingly dominant activity in the late phase of the test. But when the mean doses were tested multivariably with ANOVA, it displayed a statistically significant relationship ($p < 0.05$) between 400 mg/kg dose ($p = 0.018$) and 800 mg/kg dose ($p = 0.025$) of the extracts of *Bidens pilosa* (test of between-subjects effects). Further multiple test using Dunnett t 2-paired tests revealed there was no significant difference ($p = 1.00$ and >0.05) in both centrally and peripherally mediated analgesic activities between the aqueous and ethanolic extracts.

3.3. Results of Tests of *Bidens pilosa* Extracts on Thermally Induced Pain in Rats (Tail-Flick Response Time)

In **Table 3**, there was a general, irregular and non significant ($p < 0.05$; student t-test), non dose dependent pattern of withdrawal of tail from the 55°C hot water by rats at both 30 and 60 minutes after administration of the aqueous and ethanolic extracts of *Bidens pilosa* when the test rats were compared with negative control (distilled water) and their own values at zero (0) minute. Two positive controls were used for the tail-flick test; the acetylsalicylic acid at 100 mg/kg and morphine at 2 mL/kg of body weight. The rats administered with the positive controls showed prolonged time before withdrawal of the tail from the hot

Table 2. Effect of *Bidens pilosa* leaf extracts on formalin chemically induced pain in rats.

Dose (mg/kg)		Treatments							
		Distilled water	ASA	BPA		BPE			
		(10 mL/kg)	100	200	400	800	200	400	800
Early phase	Mean scores \pm SEM	2.52 \pm 0.23	1.96 \pm 0.29*	1.56 \pm 0.50*	1.60 \pm 0.34*	1.08 \pm 0.42*	1.60 \pm 0.42*	1.16 \pm 0.44*	1.44 \pm 0.50*
	Inhibition of pain (%)	-	22.2	38.1	36.5	57.2	36.5	54.0	42.9
Late phase	Mean scores \pm SEM	2.51 \pm 0.18	2.09 \pm 0.19*	1.87 \pm 0.34*	1.29 \pm 0.41*	1.67 \pm 0.48*	2.09 \pm 0.29	1.80 \pm 0.46*	1.90 \pm 0.38*
	Inhibition of pain (%)	-	16.7	25.5	48.6	33.5	16.7	28.3	24.3

n = 5, Data were mean \pm SEM, * = test groups varied significantly ($p < 0.05$) from normal saline group using student t-test, ASA = Acetylsalicylic Acid, *Bidens pilosa* leaf Aqueous Extract (BPA), *Bidens pilosa* leaf Ethanolic Extract (BPE).

Table 3. Effect of aqueous and ethanolic extracts of *Bidens pilosa* leaf on thermally induced pain in rats.

		Treatments and Dose (mg/kg)							
		Distilled water (10 mL/kg)	ASA (100)	Morphine (2 mL/kg)	BPA			BPE	
					200	400	800	200	400 800
MTRT (sec) ± SEM	Zero min (T ₀)	3.44 ± 0.24	3.22 ± 0.26*	3.34 ± 0.12	3.34 ± 0.26*	3.32 ± 0.26*	3.50 ± 0.14	1.39 ± 0.14	1.38 ± 0.13 1.40 ± 0.13
	30 mins (T ₁)	3.66 ± 0.28	3.84 ± 0.34	4.80 ± 0.03*	3.38 ± 0.28	3.68 ± 0.11	3.64 ± 0.30	1.47 ± 0.09	1.40 ± 0.18 1.64 ± 0.13
	60 mins (T ₂)	3.56 ± 0.28	3.86 ± 0.29	4.70 ± 0.07*	3.50 ± 0.20	3.52 ± 0.11	3.54 ± 0.14	1.40 ± 0.08	1.49 ± 0.16 1.41 ± 0.16
	T ₁ -T ₀	0.22 ± 0.37	0.62 ± 0.14	1.46 ± 0.11*	0.04 ± 0.13	0.36 ± 0.23	0.14 ± 0.31	0.08 ± 0.14	0.02 ± 0.25 0.24 ± 0.12
	T ₂ -T ₀	0.12 ± 0.37	0.64 ± 0.31	1.36 ± 0.16*	0.16 ± 0.14	0.20 ± 0.21	0.04 ± 0.16	0.01 ± 0.13	0.01 ± 0.14 0.01 ± 0.14
PI (%)	T ₁ (0 min = reference)	-	19.3	30.4	1.2	1.1	2.9	5.8	1.5 17.1
	T ₂ (0 min = reference)	-	19.9	28.9	4.8	6.0	1.2	0.7	8.0 0.7
	T ₁ (DW = reference)	-	4.9	23.6	-	0.6	-	-	- 7.9
	T ₂ (DW) = reference	-	8.4	24.3	-	-	-	-	- -

n = 5; Data were mean ± SEM; * = test groups varied significantly (p < 0.05) from normal saline group using student t-test; MTRT (sec) = Mean Tail Response Time (seconds); PI = Pain Inhibition; DW = Distilled Water; ASA = Acetylsalicylic acid; BPA = *Bidens pilosa* leaf Aqueous extract; BPE = *Bidens pilosa* leaf Ethanolic extract.

water when compared with negative control (distilled water) and their own values at 0 minute. Morphine had the highest levels of pain inhibition followed by acetylsalicylic acid and then the extracts irrespective of type and doses.

Statistically significant relationship (p < 0.05) was found between 400 mg/kg dose (p = 0.018) and 800 mg/kg dose (p = 0.025) of the extracts of *Bidens pilosa* ((test of between-subjects effects) when the inhibitory activities of different doses of both the aqueous and ethanolic extracts of *Bidens pilosa* on thermally induced pain were tested with ANOVA. Dunnett t 2-paired multiple comparison tests exhibited no significant difference (p = 1.00 > 0.05) in thermally mediated analgesic activity between the aqueous and ethanolic extracts.

4. Discussion

The use of traditional medicines among HIV positive persons for the treatment of HIV and HIV related diseases has been reported globally in the USA, Canada, India and Uganda [15] [16] [17] [18]. Pain emerges from oral lesions due to damage done to oral tissues by HIV and AIDS [12] and hence requires immediate treatment to encourage the patients to feed well. Reports reveal that HIV infected or AIDS patients take up the option of traditional medicine or use of herbs because of freedom to choose mode of treatment, culture, cost, as well as inaccessible, unavailable and sometimes, ineffective western medicines as a consequence of adverse drug events, delayed healing and recurrence of opportunistic infections among HIV/AIDS patients [15] [16] [17]. Globally, studies show that HIV positive patients use herbal remedies for pain relief, immune boosting, relief of stress, fear, anxiety and depression, neuropathy, relaxation, supplementing dietary intake, increasing energy level, and healing to advance the gen-

eral wellbeing and quality of life of the patient [15] [53] [54]. A study of 137 AIDS patients on use of traditional herbal medicine in Kabarole District of Western Uganda by Langlois-Klassen *et al.* [18] revealed that patients use herbal medicine for relief of pain and fever. Nevertheless, 70% of these patients who use the herbal remedies for various purposes do not tell their doctors about these herbs [55]. Equally, very few researchers and people are privy to the limited data about these patients on prevalence, patterns of use, indications, effectiveness and safety of the herbs they use. However, some of these herbal remedies appear to be effective in relief of symptoms e.g. IGM-1 and no antiviral and immune effects [56]. In addition, most of the herbal remedies used by different patients have not been identified, qualified, quantified and analysed for their active and inactive constituents [18]. Results from larger studies are important to support this evidence as expressed by Liu [56] and therefore, *Bidens pilosa* was investigated in this study to substantiate the traditional healers' claims as the traditional basis for the use of *Bidens pilosa* leaf aqueous and ethanolic extracts in the treatment of pain involvement of oral lesions of HIV/AIDS patients.

The acetic acid induced writhing pain test method was used to study the anti-nociceptive effects of the aqueous and ethanolic extracts of *Bidens pilosa* in mice (Table 1). It is through these test methods that the anti-nociceptive mechanisms of the test samples are often identified and determined. In the acetic acid induced (chemical pain) test, 10 mL/kg of 0.7% of acetic acid was administered intraperitoneally to induce writhing-like pain in the mice [43] [44]. The acetic acid produces writhes through stimulation of the release of various peripheral endogenous pain mediators which in turn animate the nociceptors [57] [58] thereby increasing the level of prostaglandins (PGE2 and PGF2 α) and lipooxygenase in the fluid in the peritoneum [59]. The C fibres are responsible for mediating the resulting slow and poorly localized pain [60]. Data resulting from the current study showed significant dose-dependent ($p < 0.05$, student t-test) inhibition of acetic acid induced pain by acetylsalicylic acid, aqueous and ethanolic extracts of *Bidens pilosa*, when compared with the negative control (normal saline). Statistical test with ANOVA revealed more analgesic activity with 200 and 400 mg/kg doses than 800 mg/kg dose. However, with Dunnett t 2-paired tests, the analgesic activities of aqueous and ethanolic extracts of *Bidens pilosa* were confirmed to be statistically similar. This analgesic activity presumably could be attributable to the presence of flavonoids as revealed by our previous study [61]. This result therefore holds the opinion that the realized anti-nociceptive activity of the extracts could be derived from the previously stated two broad groups of phytochemical components in the *Bidens pilosa* extracts such as flavonoids and polyacetylene groups [35] [62] [63] [64]. The basis of this reported analgesic activity originates from the ability of these constituents in *Bidens pilosa* to reduce pain due to inflammation and this report was corroborated by Yoshida [65] on the competence of these constituents to relax muscle and relieve pain. Our results and the subsequent confirmation from previous works highly indicate the reasons and scientific basis for a region wide use of *Bidens*

pilosa by traditional healers and locals in the treatment of oral lesions of HIV/AIDS in South-Western Uganda. This conceivably suggests that the reported analgesic and anti-inflammatory effects of both aqueous and ethanolic extracts of *Bidens pilosa* may attest to the extracts' pain and inflammation reducing capability and hence can offer relief to HIV/AIDS patients with pain and inflammation due to oral lesions.

Formalin test was used to study the scientific basis for the traditional use of aqueous and ethanolic extracts of *Bidens pilosa* against inflammation and pain in the oral lesions of HIV/AIDS patients (**Table 2**). Formalin test is usually used to investigate the effects of drugs on chemically induced analgesia. Formalin is an agent with the potential to induce oedema which in turn induces inflammation by releasing several inflammatory mediators including prostaglandins, histamine, serotonin, bradykinin [46] [60]. There are two phases in formalin test; one, is neurogenic pain in the first phase (0 - 10 minutes) in which case the nociceptors are directly stimulated, without any activity on these nociceptors by anti-inflammatory agents and two, is inflammatory pain in the second phase (15 - 60 minutes) which is directly related to peripheral inflammation and centrally mediated changes resulting from chemical mediators from damaged cells. Centrally acting agents have equal inhibition on both phases. Peripherally acting agents have inhibition only on the second phase. This test, in essence, assesses the response to an enduring nociceptive stimulus comparable to clinical pain and is recommended as a tool in basic pain research for studying the mechanisms of analgesic agents due to its association to tissue injury [46]. The formalin test with aqueous and ethanolic extract of *Bidens pilosa* leaf demonstrated a statistically significant, non dose dependent pain inhibition in both neurogenic and inflammatory phases when compared with the negative control (distilled water) [$p \leq 0.05$ for aqueous extract (200 mg/kg early phase $p = 0.016$, late phase $p = 0.01482$; 400 mg/kg early $p = 0.0704$, late phase $p = 0.0002$; 800 mg/kg early $p = 0.0090$, late phase $p = 0.0002$)] and for ethanolic extract (400 mg/kg early $p = 0.0125$, late phase $p = 0.0194$; 800 mg/kg early $p = 0.0058$, late phase $p = 0.0047$), student t-test). Further multiple testing with Dunnett t 2-paired tests revealed no significant difference in centrally and peripherally mediated analgesic activities shown by both the aqueous and ethanolic extracts. Our observation was supported by display of anti-inflammatory properties of *Bidens pilosa* reported by Geissberger and Sequin [66]; its anti-inflammatory, muscle relaxant and pain-relieving effects by Yoshida [65]; and immunomodulatory, antioxidant and cellular protective properties by Chiang [67]; treatment of headache in Zulu land by inhibiting cyclooxygenase, an activity associated with the flavonoid components reported by Jager [63]. These results therefore suggest that the anti-nociceptive activity of the extracts of *Bidens pilosa* is as a result of the phytochemical components of the extracts which include flavonoids and polyacetylene groups [61] or polyynes, flavonoids, phenylpropanoids, fatty acids, and phenolics [20] such as alkaloids, tannins, terpenoids, saponins, phenolic compounds and steroids; thereby proving its efficacy in the traditional medicine system in

management of pain involvement of oral lesions of HIV/AIDS patients in South Western Uganda.

The tail immersion test is a standard model for selective investigation of centrally but not peripherally mediated nociception and analgesia utilising thermal painful stimuli [47]. This method measures a response to a brief, noxious stimulus seemingly emanating from reflexes of the spinal cord modulated by supraspinal inhibitory mechanisms, indicating morphine-like effect implicating the activity of selective centrally acting analgesics [68] [69]. Pain stimulus is initiated through the A δ fibres and inhibited centrally or through interaction with the widely distributed opioid receptors at peripheral sites [57]. Our findings on tail-flick test with *Bidens pilosa* extracts in **Table 3** showed the presence of a central analgesic activity. I did *et al.* [70] stated that hot plate tail flick technique has bias for centrally acting agents but not sufficiently sensitive to distinguish between opiates and non-opiates with central activity. Conversely, in the work of Pizziketti *et al.* [71], they revealed that the cold tail flick test has sufficiently demonstrated differentiation of opiates from non-opiates with central activity. However, Domer [72] in his study emphasised that the type of analgesic activities exhibited by *Bidens pilosa* indicates morphine-like activity and that corroborates the results of our present study. This formed the basis for the use of morphine as one of the positive controls in this study. In another study, Fields in his work [73] explained that this type of analgesic activity shown by *Bidens pilosa* can be referred to as NSAIDs-like occurring through inhibition of cyclooxygenase in peripheral tissues thereby interfering with the mechanism of transduction in primary afferent nociceptors. In another situation, this was the reason for using aspirin as another positive control for NSAID-like phytochemicals in *Bidens pilosa*. The contributory factor for these activities can be associated with presence of phytochemicals in the plants such as tannins, flavonoids, phlobatanins, terpenoids and cardiac glycosides [61]. In this present study, hot aqueous extract of *Bidens pilosa* was found to contain saponins, flavonoids, terpenoids and phenolic compounds while its cold ethanolic extract contained alkaloids, tannins, steroids, terpenoids and cardiac glycosides (Unpublished data). Any of these phytochemical components or their combinations could be the effective phytochemicals responsible for the observed analgesic effects of aqueous and ethanolic extracts of *Bidens pilosa*. Other preceding studies demonstrated the anti-inflammatory properties of *Bidens pilosa* by Geissberger and Sequin [66]; anti-inflammatory and muscle relaxant and pain-relieving effects [65]. All the stated results of tail flick test go further to accentuate the capability of *Bidens pilosa* extract to control both centrally and peripherally mediated pain, confirming the scientific basis of its traditional use in treatment of painful and inflammatory conditions and oral lesions of HIV/AIDS patients in South Western Uganda. The implication is that *Bidens pilosa* extracts have found use in treatment of pain and inflammation involvement of oral lesions of HIV/AIDS patients.

5. Conclusion and Recommendations

In conclusion, *Bidens pilosa* possesses significant analgesic effects based on acetic acid, formalin and tail-flick induced pain tests in rats and can be used in the treatment of pain involvement of oral lesions of HIV/AIDS patients to justify its use for this purpose by traditional healers and other local users of the herb. We hereby recommend that further studies be conducted on the anticonvulsant property and mechanisms of action of the analgesic activities of *Bidens pilosa* with identified drug entities responsible for the activities to conclude the reason for the analgesic effects of *Bidens pilosa*.

Acknowledgements

We thank Mr. Ronald Kizza for his technical assistance in the School of Pharmacy Laboratories of Kampala International University and School of Pharmacy of Kampala International University Western Campus Ishaka Bushenyi Uganda for providing the laboratory space and some materials for the tests.

Ethical Approval

Ethical approval was sought and given by Uganda National Council for Science and Technology (UNCST) and Mbarara University of Science and Technology Institution's Research and Ethics Committees. These clearances enabled us to use the plants and the animals for this research.

Competing Interests

There is no conflict of interest among the authors.

Authors' Contributions

The authors worked together in conducting the tests involved as follows: JOCE initiated the study. Authors JOCE, EA, AAA, JCE and FB collected the data. Authors JOCE, MN, SOO, EA, COO and FB designed the study, wrote and corrected the protocol. Authors JKT and JOCE wrote the protocol and the first draft of manuscript, searched for literature, analyzed the data, read through the data and made corrections. Authors MN, SOO, EA and FB managed the experimental processes, read through and made corrections to the manuscript draft. Authors COO, JCE and AAA read through and made corrections to the manuscript draft. All authors read and approved the manuscript for publication.

Consent

Written consent was sought and secured from the Kampala International University School of Pharmacy Research Laboratories, Ishaka, Bushenyi where the animals were kept.

Consent to Publish

The authors consented to publishing of this article.

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Abbreviations

OIs: Opportunistic Infections.

ANOVA: Analysis Of Variance.

ART: Antiretroviral Therapy.

AFC: Animal Facility Centre.

NIH: National Institute of Health.

i.p: Intraperitoneally.

p.o: per oral.

SEM: Standard Error of the Mean.

BPA: *Bidens pilosa* Leaf Aqueous Extract.

BPE: *Bidens pilosa* Leaf Ethanolic Extract.

MTRT: Mean Tail Response Time.

PI: Pain Inhibition.

DW = Distilled Water.

sec: seconds.

NSAIDs: Non Steroidal Anti-inflammatory Drugs.