RESEARCH ARTICLE

Analgesic effect of ethylacetate fraction of the methanol leaf extract of *Hannoa klaineana* in rats

Ibrahim Abubakar [1]
Mu'azu Abubakar Gusau [1]
Jabir Aliyu Danyaya [2]
Shehu Ibrahim [3]
Zayyanu Abdullahi [2]
Abdulhakim Zubairu [2]
Abdulsalam Umar Sahabi [2]

Authors' addresses:
[1] Department of Biochemistry, Faculty of Science, Usmanu Danfodiyo University Sokoto, Nigeria.
[2] Biochemistry Unit, Department of Science Technology, Waziri Umaru Federal Polytechnic, Birnin Kebbi, Nigeria.
[3] Department of Chemistry, Shehu Shagari University of Education, Sokoto, Nigeria

Correspondence:
Ibrahim Abubakar
Idris Koko Technical College, Farfaru, Sokoto. North-West, Nigeria.
P.M.B 2127
Tel.: +2348069352429
e-mail:
ibrahimabubakarsok@gmail.com

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ABSTRACT

Pain remains a major health, social, and economic problem worldwide. *Hannoa klaineana* Pierre & Engl. is a medicinal plant found in many African countries and used for the treatment of many diseases including pain-associated disorders. This study was conducted to evaluate the analgesic effect of the ethylacetate fraction of methanol leaf extract of *Hannoa klaineana* in rats. The analgesic effect of the ethylacetate fraction of methanol leaves extract of *Hannoa klaineana* (200, 400, and 600 mg/kg b.wt) was evaluated using acetic acid-induced writhing, tail flick (immersion), and hot plate model. In the acetic acid-induced writhing test, the extract (200, 400, and 600 mg/kg) demonstrated a significant ($p < 0.05$) decrease in the number of writhes with maximum percentage inhibition (75.61%) at 600 mg/kg dose of the extract. In tail flick and hot plate tests, the extract (200, 400, and 600 mg/kg b.wt) exhibited a significant ($p < 0.05$) increase in rats' response with a steady increase in reaction time. Findings from this study show that ethylacetate fraction of the methanol leaves extract of *Hannoa klaineana* possessed analgesic activity which provided justification for the local use of the plant in the treatment of pain.

Key words: Acetic acid, Analgesics, Diclofenac, *Hannoa klaineana*, Pain

Introduction

Pain remains a major health, social, and economic problem affecting about 80% of the world's adult population (WHO, 2012). The report showed that more than 20% of the people in the world have been suffering from chronic pain comprising about 10% and more than 11% of the world's adults and young adults population live with chronic pain, respectively (Murray et al., 2022; IASP, 2012). Pain is a symptom of various diseases associated with high morbidity and mortality rates worldwide. It is an unpleasant sensation, physical discomfort, and emotional experience associated with real or potential tissue damage or its equivalent (IASP, 2015; Hofman et al., 2017; Love-Jones, 2019; Raja et al., 2020). The pathophysiological mechanism of pain involves central nervous system (CNS) or peripheral pain mechanisms. Pain is commonly initiated by noxious stimuli and transmitted over specialized neuronal networks to the central nervous system (Tadele et al., 2009; Salzano, 2013). Untreated and persistently prolonged pain results in physical damage and psychological disorders (Henschke et al., 2015). Analgesics commonly as pain relievers are drugs used in the management and treatment of pain (Daniel & Donald, 2023). Analgesia is a Greek word derived from "an" meaning without and "alges(is)/algos/" meaning pain, hence analgesia means without pain (Helen et al., 2000; Murat, 2024). Analgesia is defined as the relief of pain without the intentional cause of any change in mental condition (Cornelis et al., 2019).

Conventional analgesic treatments are expensive and have more side effects than medicinal plants and herbs. Treatment of pain-associated diseases involves the use of nonsteroidal anti-inflammatory drugs (NSAIDs), opioids, and nonopioids. Nonsteroidal anti-inflammatory drugs (NSAIDs) are associated with many side effects including nausea, ulcers, constipation, diarrhea, hypertension, salt and water retention, headache, dizziness, depression, asthma, and hepatotoxicity (Finnerup, 2019; Ghlichloo & Gerriets, 2023). Opioids

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produce many side effects which include dysphoria constipation, nausea and vomiting, hypotension, and cardiovascular disorders (Cohen et al., 2023). The report showed that nonopioids produce many side effects including rash or hypersensitivity reactions, anemia, leukopenia, nephrotoxicity, hyperglycemia, nausea, vomiting, pruritus, constipation, and abdominal pain (Gerriets et al., 2024). Medicinal plants have been commonly used by local communities for treatment of many diseases. Plants and herbs possess medicinal properties and contain various bioactive compounds which have been used in drug discovery and development (Abubakar et al., 2022). High proportion of communities in the world relies on medicinal plants for remedies.

Hannoa klaineana Pierre & Engl. is a medicinal plant found abundantly in tropical African countries (Figure 1). The family and the genus of the plant are *Simaroubaceae* and Hannoa, respectively ((TPL, 2013). *Simaroubaceae* have a wide and diverse geographical habitat. The family is mainly distributed in tropical and subtropical countries (Saraiva et al., 2002; Biba et al., 2017). *Simaroubaceae* consists of 32 genera with more than 170 species of trees and brushes of pantropical distribution (Iasmine et al., 2014). The genus comprised many species including *Hannoa ferruginea* Engl., *Hannoa schweinfurthii* Oliv., *Hannoa kitombetombe* (Pierre) Engl., *Hannoa klaineana* Pierre & Engl., *Hannoa chlorantha* (Pierre) Engl., and *Hannoa undulata* (Guill. & Perr.) Planch (PWO, 2023; SHP, 2023). The genus of the plant includes trees, shrubs, or shrublets with non-crowded leaves at the ends of the branches. The flowers of the genus are thick and fleshy, unisexual or bisexual with one ovulate carpel, six to nine overlapping petals, and ten stamens. The seed capsule comprises one to three disruptive mericarps (FZ, 2023).

Hannoa klaineana is an evergreen plant with a typical height of about 45 meters (FZ, 2023). The tree possesses a straight, cylindrical or slightly buttressed bole. The plant has been characterized by a rough and gray-brown bark. *Hannoa klaineana* is an important source of food and habitat for various wildlife, provides stability in soil, helps in local biodiversity, and aids climate change due to its ability to sequester carbon atoms (PWO, 2023). The plant has been used by many local communities in African countries for the treatment of many diseases including malaria, fevers, pain, hypertension, cancer, and ulcers (Abubakar et al., 2020a; 2020b; 2021). The plant is abundant in many local communities in Nigeria, and is locally called "Takardar giwa" in Hausa (Northern region) and "Ofor" in Igbo (Southern region). It has been reported that *Hannoa klaineana* Pierre & Engl. demonstrated antioxidant activities (Odeghe *et al.*, 2016), anti-malarial activities (Ang et al., 1995), and anti-ulcer activities (Abubakar et al., 2020a; 2020b; 2021). There was no study reported on the analgesic effect of *Hannoa klaineana* Pierre & Engl. This study aims to evaluate the analgesic activity of the ethylacetate fraction of methanol leaves extract of *Hannoa klaineana* Pierre & Engl. This study was done to provide a scientific justification for the local use of the plant as an analgesic and to further isolate and characterize the bioactive compound(s) responsible for the plant analgesic activity. The analgesic activity of the ethylacetate fraction of methanol leaves extract of *Hannoa klaineana* Pierre & Engl. was evaluated using acetic acid-induced writhing, tail flick, and hot plate test. The models were used because the acetic acid-induced writhing test is an established model for evaluating peripheral analgesic activity while tail flick and hot plate tests are quick and prolonged response models for evaluating central analgesic activity.

Materials and Methods

Figure 1. *Hannoa klaineana Pierre & Engl. Leaves (Source: Photographed by the researcher, 2024).*

Drugs and chemicals

The drugs and chemicals used in this study were of analytical grade. Diclofenac sodium (Salud Care (I) Pvt Ltd, India) was purchased from Rauda Pharmacy, Opposite Usmanu Danfodiyo University, City Campus, Sokoto. Glacial acetic acid, methanol, and ethylacetate were purchased from Sigma-Aldrich St. Louis, MO, USA.

Experimental animals

A total of seventy-five Wistar rats (8 – 9 weeks, 180 – 200 g) of either sex purchased from the Animal House Unit, Department of Biochemistry, Usmanu Danfodiyo University, Sokoto were used in this study. The animals were housed in standard laboratory cages, five rats per cage. The rats were fed with a standard pellet diet and water ad libitum. The animals were acclimatized to standard environmental conditions (room temperature and relative humidity 30–70%

for 12 hours of light followed by 12 hours of darkness cycle) for one week before the commencement of the experiments.

Collection and authentication of plant material

The sample (fresh leaves) of *Hannoa klaineana* Pierre & Engl. were obtained from Anka forest and Shinkafi town in Zamfara, Nigeria with the help of local vendors. The plant sample was identified and authenticated by a botanist at the Herbarium Section, Department of Biological Sciences, UDUS and the voucher specimen (UDUH/ANS/0335) was kept at the Herbarium Unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto.

Extract and fractionation

Preparation of the plant sample extract and fractionation was carried out using the method of Abubakar *et al.* (2020b). The plant sample (fresh leaves) of *Hannoa klaineana* Pierre & Engl. (5 kg) were shade-dried for fourteen days and pulverized into fine powder using a pestle and mortar (Figure 2). The plant leaves powdered (500 g) was soaked in one litre of 95% methanol for three days. The extract was filtered using Whatman filter paper No 1 and evaporated to dryness using a rotary evaporator (RV 8, 001000217+, IKA, Germany) at 40°C under reduced pressure for three hours. The dried extract was weighed (42.5 g) and the percentage yield (8.5%) was calculated. Liquid-liquid fractionation of the crude methanol leaf extract was carried out in a separation funnel. Twenty grams of the extract suspended in distilled water was partitioned three times with 300ml of ethylacetate solvent. For each partitioning, the contents were shaken vigorously and kept for 10 minutes to settle between the layers. The residue settled down and ethylacetate portion was collected and then labelled as ethylacetate fraction. The fraction was concentrated in a rotary evaporator (sRV 8, 001000217+, IKA, Germany) under reduced pressure at 40°C for three hours. The fraction obtained was stored in the refrigerator until use.

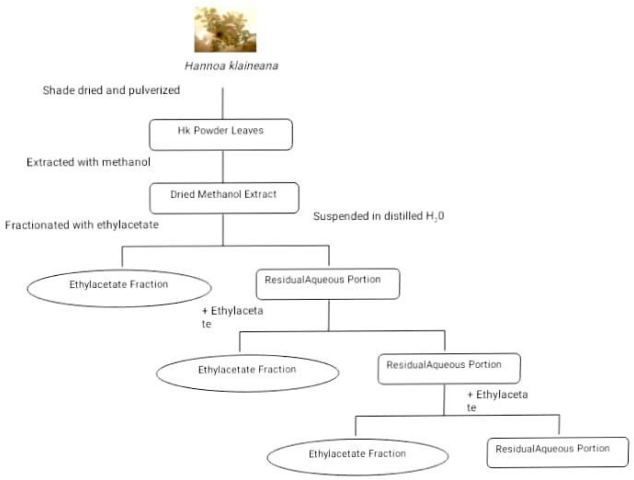

Figure 2. *Exract preparation and fractionation*.

Analgesic activity test

Acetic acid-induced writhing model

The acetic acid-induced writhing test was carried out using the method of Alemu et al. (2018) with some modifications. The animals (Wister rats) weighing 180 – 200 g (n=25) were randomly divided into five groups of 5 rats each. Group 1 received normal saline (10 ml/kg bwt.) and served as control. Group 2 was pre-treated with the reference standard drug, diclofenac sodium (50 mg/kg, p.o.). Groups 3, 4, and 5 received 200, 400, and 600 mg/kg of the ethylacetate fraction of methanol leaves extract of *Hannoa klaineana* Pierre & Engl. The normal saline, diclofenac sodium, and the extract were administrated to the negative, positive, and test control rats by gavage. Acetic acid (0.6%, v/v, 10 mL/kg, i.p) was injected into each rat thirty minutes after the oral administration of normal saline, diclofenac sodium, and the extract. After acetic acid injection, the animals were placed in transparent boxes and the number of writhes responses of each rat was counted and recorded for 20 minutes using a latency time of five minutes. Contractions of the animals' abdomen together with stretching of the hind limbs were counted. The percentage inhibition of writhes was calculated using the formula below:

$$\%\ Inhibition\ of\ writhes\ = \frac{MnWc - MnWt}{MnWc}\ x\ 100$$

where:
MnWc - mean number of writhes in control,
MnWt - mean number of writhes in test.

Tail flick (immersion) model

The tail flick response test was conducted using the tail flick method of Kulkarni (2002) and Ghosh (2008). Wister rats (180–200 g, n=25) were randomly divided into five groups of 5 rats each. Group 1 (control) received normal saline (10 ml/kg bwt.). Group 2 received a reference standard drug, diclofenac sodium (50 mg/kg, p.o.). Groups 3, 4, and 5 were administered 200, 400, and 600 mg/kg of the ethylacetate fraction of methanol leaf extract of Hannoa klaineana Pierre & Engl. The normal saline, test extract, and standard drug were orally administered to the respective groups. Sixty minutes after the administration of normal saline, diclofenac sodium and the extract, the tail of each rat was immersed in hot water 5 cm away from the tip of the tail using a water bath thermostatically maintained at 55 ± 0.2°C. The time taken for the rats to withdraw their tails (reaction time or tail flick latency) was recorded for 60 minutes at intervals of 15 minutes. The rats were held loosely in a suitable restrainer with the tail extended out during the test experiment. To prevent any tissue damage sixty seconds were considered the maximum time of observation.

Hot plate test

The hot plate analgesic model was carried out using the method of Schaible et al., (2011) and Abbas (2013). The rats (180 – 200 g, n=25) were randomly divided into five groups each consisting of 5 rats. Group 1 (control) received normal saline (10 ml/kg bwt.). Group 2 received a reference standard drug, diclofenac sodium (50 mg/kg, p.o.). Groups 3, 4, and 5 were administered 200, 400, and 600 mg/kg of the ethylacetate fraction of methanol leaves extract of *Hannoa klaineana* Pierre & Engl. Sixty minutes after administration of the standard drug and the extract, the rats were individually placed on the hot plate, which was heated to a constant temperature of 55°C. Responses such as jumping, withdrawal of the paws, and licking of the paws were observed. The rats were placed on the hot plate with a cut-off time of fifteen seconds to prevent lesions on the animals' paws. The time, when animals were placed and until responses occurred was noted as the latency period (reaction time) using a stopwatch. The reaction times were noted at 0 and 30, 60, 90 and 120 min after the administration of vehicle (distilled water 10 mL/kg), standard drug (morphine10 mg/kg) and 100 mg/kg, 200 mg/kg and 400 mg/kg of the extract.

Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) Statistics version 22 and GraphPad InStat version 5.5. Control groups were computed by One-way analysis of variance (ANOVA) using Mann–Whitney non-parametric and Tukey Post Hoc test for multiple comparisons to determine statistical significance among the groups at 95% ($p < 0.05$) confidence level. The data were expressed as mean ± standard error of the mean (SEM). Two-tailed p-values less than 0.05 were considered statistically significant.

Results

Acetic acid-induced writhing test

Table 1 shows the effect of ethylacetate fraction of methanol leaves extract of *Hannoa klaineana* on the number of writhes in acetic acid administered in rats. The extract demonstrated a dose-dependent significant ($p<0.05$) decrease in the mean number of writhes. In comparison with the control, the extract demonstrated a significant ($p<0.05$) decrease in number of writhes at the dose 200 mg/kg, 400 mg/kg, and 600 mg/kg. However, at 600 mg/kg, the extract (22.00) exhibited a significant ($p<0.05$) decrease in the mean number of writhes higher than the standard drug, diclofenac sodium (26.20) (Table 1).

The inhibitory effect of ethylacetate fraction of methanol leaves extract of *Hannoa klaineana* on acetic acid-induced writhing in rats is shown in Figure 1. The extract exhibited a significant ($p<0.05$) increase in percentage inhibition of writhing in a dose-dependent manner. It was found that the extract (200mg/kg, 400mg/kg, and 600mg/kg) demonstrated a significant ($p<0.05$) increase in percentage inhibition of writhing compared with the control. The extract (600 mg/kg) exhibited maximum percentage inhibition (75.61%) of writhing which is higher than that demonstrated by diclofenac sodium (70.95%), the reference standard drug (Figure 1).

Table 1. *Effect of ethylacetate fraction of methanol leaves extract of Hannoa klaineana on the number of writhes in acetic acid administered in rats.*

Treatment	Mean number of writhes
Control	90.20 ± 0.86
Diclofenac (50 mg/kg)	26.20 ± 0.58*
Hk (200 mg/kg)	51.40 ± 0.92*
Hk (400 mg/kg)	31.00 ± 1.00*
Hk (600 mg/kg)	22.00 ± 0.71*

Values are expressed as mean ± SEM (n=5 rats/group)
*$p < 0.05$ statistically significant when compared with control, $^{\#}p < 0.05$ significant compared with diclofenac (One-way ANOVA) followed by Dunnett's multiple comparison test. *Hannoa klaineana* (Hk)

Tail flick (immersion) model

Table 2 shows the effect of ethylacetate fraction of methanol leaf extract of *Hannoa klaineana* on tail flick latency in rats. The extract demonstrated a dose-dependent significant ($p < 0.05$) increase in rats' tail flick response with a steady increase in reaction time (seconds) at the interval period of the extract administration (15 to 60 min) (Table 2). At all the intervals of time (15, 30, 45, and 60 min), the extract (200, 400, and 600 mg/kg) exhibited a significant

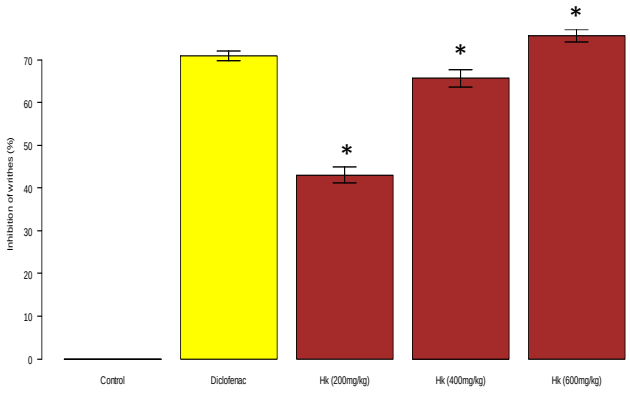

Figure 3. *Inhibitory effect of ethylacetate fraction of methanol leaves extract of Hannoa klaineana on acetic acid-induced writhing in rats.*

Data are mean ± SEM (n=5 rats/group)

**p < 0.05 statistically significant when compared with control, $^{\#}p < 0.05$ significant compared with diclofenac (One-way ANOVA) followed by Dunnett's multiple comparison test. Hannoa klaineana (Hk) stage.*

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($p < 0.05$) increase in the reaction time compared with the control (Table 2). However, the extract dose (600 mg/kg) demonstrated a significant ($p < 0.05$) increase in the reaction time at the time interval (15, 30, 45, and 60 min) compared with the reference standard drug, diclofenac sodium (Table 2).

Hot Plate Method

The effect of ethylacetate fraction of methanol leaves extract of *Hannoa klaineana* on hot plate response in rats is shown in Table 3. The result showed that the extract exhibited a dose-dependent significant ($p < 0.05$) increase in rats' response with a progressive increase in reaction time (seconds) at interval times (0, 30, 60, 90, and 120 min) of the extract administration (Table 3). The extract (400 and 600 mg/kg) at all the intervals of time (0, 30, 60, 90, and 120 min), exhibited a significant ($p < 0.05$) increase in the reaction time compared with the control (Table 3). However, the extract (600 mg/kg) demonstrated a significant ($p < 0.05$) increase in the reaction time at a 120-minute time interval compared with the reference standard drug, diclofenac sodium (Table 3).

Discussion

The acetic acid-induced writhing test is an established model for evaluating the peripheral analgesic activity of substances (Subedi et al., 2016). Acetic acid induced writhing by irritating and stimulating the peritoneal cavity that triggers the synthesis and release of various endogenous inflammatory mediators such as histamine, serotonin, bradykinin substance P, and prostaglandins (Konaté et al., 2012). The endogenous inflammatory mediators elicit chemical-induced visceral pain characterized by constriction of abdominal muscles, extension of the forelimbs, and elongation of the body part (Tadiwos et al., 2017). Acetic acid administration causes the release of prostaglandins (PGE2 and PGF2α) in peritoneal fluid by the activity of lipooxygenase (Demsie et al., 2019). The PGE2 and placental PGF released by intraperitoneal administration of acetic acid at the peritoneal receptors induce irritation leading to the stretching of limbs and elongation of the body together with constriction of abdominal muscles (Yimer *et al.*, 2020).In the present study, the ethylacetate fraction of methanol leaves extract of *Hannoa klaineana* exhibited a significant decrease in the mean number of writhes with a significant increase in percentage inhibition of writhing.

Tail flick is an acute thermic and phasic pain test used to evaluate the central analgesic activity of a substance (Vogel, 2007). The tail flick test is a response at the spinal level and mediates a spinal reflex to a nociceptive stimulus (Chapman et al., 1985). The tail flick test has two variants; the first variant uses dipping the tail into water that is maintained at a constant temperature, while the second variation uses the application of radiant heat stimulus to a part of the tail (D'Amour & Smith, 1941; Deuis et al., 2017). In this study, the ethylacetate fraction of methanol leaves extract of *Hannoa klaineana* demonstrated a significant increase in rats' tail flick response with a steady increase in reaction time at interval periods of administration.

In the hot plate model, the result of the present study indicated that the ethylacetate fraction of methanol leaves extract of *Hannoa klaineana* exhibited a significant increase

Table 2. *Effect of ethylacetate fraction of methanol leaves extract of Hannoa klaineana on tail flick response in rats.*

Treatment groups	Reaction time (sec)			
	15 Minutes	**30 Minutes**	**45 Minutes**	**60 Minutes**
Control	5.40 ± 0.51	7.40 ± 0.51	12.00 ± 0.71	15.80 ± 0.37
Diclofenac (50 mg/kg)	42.20 ± 0.86*	49.00 ± 0.71*	56.80 ± 0.58*	63.00 ± 0.71*
Hk (200 mg/kg)	15.20 ± 0.66*	20.20 ± 0.66*	29.60 ± 0.50*	38.20 ± 0.58*
Hk (400 mg/kg)	37.60 ± 0.93*	40.80 ± 0.97*	47.60 ± 0.51*	54.00 ± 0.71*
Hk (600 mg/kg)	49.00 ± 0.71*#	60.00 ± 0.71*#	68.20 ± 0.58*#	74.00 ± 0.51*#

Values are mean ± SEM (n=5 rats/group)
*$p < 0.05$ statistically significant when compared with control, #$p < 0.05$ significant compared with diclofenac (One-way ANOVA) followed by Dunnett's multiple comparison test. *Hannoa klaineana* (Hk)

Table 3. *Effect of ethylacetate fraction of methanol leaves extract of Hannoa klaineana on hot plate response in rats*.

Treatment groups	Reaction time (sec)				
	0 Minutes	**15 Minutes**	**30 Minutes**	**45 Minutes**	**60 Minutes**
Control	3.40 ± 0.50	4.00 ± 0.31	4.40 ± 0.24	4.80 ± 0.37	5.20 ± 0.37
Diclofenac (50 mg/kg)	5.80 ± 0.37*	7.40 ± 0.51*	8.20 ± 0.37*	10.60 ± 0.50*	11.80 ± 0.86*
Hk (200 mg/kg)	3.80 ± 0.36	4.60 ± 0.24	5.40 ± 0.25*	6.40 ± 0.24*	7.40 ± 0.50*
Hk (400 mg/kg)	4.40 ± 0.24*	5.20 ± 0.37*	7.20 ± 0.38*	8.80 ± 0.38*	10.40 ± 0.51*
Hk (600 mg/kg)	6.40 ± 0.51*	8.00 ± 0.31*	9.20 ± 0.37*	11.20 ± 0.39*	13.00 ± 0.71*#

Data are expressed as mean ± SEM (n=5 rats/group)
*$p < 0.05$ statistically significant when compared with control, #$p < 0.05$ significant compared with diclofenac (One-way ANOVA) followed by Dunnett's multiple comparison test. *Hannoa klaineana* (Hk)

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in rats' responses (jumping, withdrawal of the paws, and licking of the paws) with a progressive increase in reaction time (seconds) at the interval time of the extract administration. The hot plate test is a model used to evaluate the central analgesic effect of a substance (Tadiwos et al., 2017). The mechanism of the model involves detecting supra-spinal nociception and the central antinociceptive (Tadiwos et al., 2017). The model is sensitive to the most effective analgesics and has precision outcomes, less tissue injury, and limited time consumption (Sharma et al., 2007).

The results of this were in agreement with the studies by Alemu et al., (2018) and Yimer et al., (2020), who reported the analgesic activity of medicinal plants in a dose-dependent manner. The analgesic activities of medicinal plants might be due to the presence of various phytochemicals in the plants (Nigatu et al., 2021). Studies showed that glycosides (Khan et al., 2020), saponins (Passos et al., 2021), flavolignans (Shah et al., 2021), tannins (Soyocak et al., 2019), flavones (Taheri et al., 2021; Aboulaghras et al., 2022), and flavonoids (Komakech et al., 2019; Mondal et al., 2020; Karrat et al., 2022) demonstrated analgesic activity. Abubakar et al. (2020a) reported that methanol leaves extract of *Hannoa klaineana* Pierre & Engl. contains various phytochemicals including alkaloids, flavonoids, tannins, and steroids which have been documented to possess a significant analgesic activity (Çadirci et al., 2012). Therefore, the analgesic effects of the ethylacetate fraction of methanol leaves extract of *Hannoa klaineana* Pierre & Engl. recorded in this study could be due to the presence of various phytochemicals in the extract.

CONCLUSION

The ethylacetate fraction of the methanol leaf extract of *Hannoa klaineana* possesses peripheral and central analgesic activity which might be attributed to its inhibitory effect on the synthesis and release of endogenous pain mediators and suppression of prostaglandin synthesis through inhibition of lipoxoygenase and cyclooxygenase. This study provided information to justify the local use of *Hannoa klaineana* Pierre & Engl. for the relief of pain and its associated diseases. Further studies should be done to isolate and characterize active compounds responsible for the analgesic activity and to elucidate their mechanisms of action.

Conflict of interest

The authors declared no conflict of interest.

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