Phytochemical identification and analgesic potential of the seed extract of *Irvingia gabonensis*

Abstract. *Irvingia gabonensis* is used ethno-medicinally in some West Africa culture in the treatment of pain. This analgesic potential of the stem bark has been reported in the literature. In order to evaluate the analgesic potential of the seed extract and the phytochemicals that may be responsible for this effect. This study was aimed at investigating the phytochemical content and evaluation of the analgesic effect of *Irvingia gabonensis* seed. The seeds of *Irvingia gabonensis* were screened for phytochemicals using standard procedures and GC-MS analysis. It central and peripheral analgesic potentials were evaluated at doses of 50 mg/kg, 100 mg/kg and 200 mg/kg using acetic acid induced abdominal writhing and hot plate methods in Swiss albino mice. Acetylsalicylic acid (100 mg/kg), morphine hydrochloride (2 mg/kg) and pentazocine (15 mg/kg) were used as the standard drugs. ANOVA was used to analyze data from the results and level of significance was set at $P \leq 0.05$. Phytochemical screening showed the presence of alkaloid, flavonoid, cardiac glycoside, triterpenoid and saponin. GC-MS analysis revealed thirty compounds mostly fatty acids. At 200 mg/kg of the methanolic extract, there was a dose dependent decrease in writhing response which also compared well with acetylsalicylic acid. From the results, *Irvingia gabonensis* seed contains compounds that could be responsible for the precieved analgesic activity.

Keywords: Writhing test; hot plate; *Irvingia gabonensis*; analgesic; GC-MS analysis

1. Introduction

Irvingia gabonensis belong to Irvingiaceae family [1], also known as African or Bush mango and it is a tree of height 28 ± 10 m. It is one of the six species found in tropical Africa [2,3] and grows wild in the forest especially in the humid lowland area and widely cultivated in Nigeria, Cameroun and Cote d'Ivoire [4]. It has a yellow spherical smooth fruit with fibrous mesocarp when ripe (sweet pulp) and hard endocarp [5]. The seed is use as food in preparation of soup use in eating starchy pastes in many households in Africa [6] and also in medicine due to the presence of phytochemicals and pharmaceutical excipients [7].

Different parts of this plant is use in the treatment and management of ailments. The seed can be powdered and made into an astringent cream that is applied on burns and minor abrasion to soothe and reduce bleeding from the skin [8], and it also used in the treatment of wounds [9]. The powdered stem bark of *Irvingia gabonensis* is use to relieve pain, extracts from both water and ethanol of the seeds are used to improve kidney and liver functions [9,10], management of type II diabetics and reducing incidence of obesity [11]. Consumption of *Irvingia gabonensis* seeds can cause slow absorption of dietary sugar as a result of delayed stomach emptying, with consequential reduction in elevated blood sugar levels, normally seen after a meal [12].

The chemical composition of *Irvingia gabonensis* ethanol seeds extract was reported by Olorundare and coworkers in 2020 [13] and it was revealed that 4,6-di-O-methyl-alpha-d-galactose, Neophytadiene, 4-Methyl-2,5-dimethoxybenzaldehyde, Cycloheptasiloxane, tetradecamethyl-, Pyrrolidine, 1-(1-cyclohexen-1-yl)-, Naphthalen-4a,8a-imine, octahydro-, Cyclotetrasiloxane, octamethyl-, Z,Z-7,11-Hexadecadien-1-ol, Cyclotetrasiloxane, octamethyl-,1,2-Cyclopentanedione, Ethanol, 2-(ethylamino)- and Oxime-, methoxy-phenyl- were the common compounds. While quercetin, kaempferol, ellagic acid, mono-, di-, and tri-O-methyl-

ellagic acids, and their glycosides were reported earlier [6,14]. Also the seed extract is a good source of macro nutrients (fatty acids, glycerides and proteins) and micro nutrients (vitamin and minerals) [15].

Acetylsalicylic acid is an anti-inflammatory drug, grouped under non-steroidal class and prescribed for pain and inflammation. It acts via the peripheral routes by irreversibly disabling the COX enzyme in the inflammatory cascade. However it is limited by major gastrointestinal complications such as gastritis, hemorrhage, peptic ulcer, kidney problem and stomach upset [16]. These side effects can be overcome by administering morphine and pentazocine which are centrally acting pain killers, though they can lead to dependency, addiction, sedation and respiratory depression on prolong use. Due to significant side effects associated with NSAIDs and narcotic medications, there is renew interest in dietary supplements and herbal remedies which long been used in reducing pain and inflammation. Many of which have shown similar mechanism of action but with little or no side effects associated with them [17]. Thus the purpose of this research was to determine the phytochemical content of the seed of *Irvingia gabonensis*, partially characterize the crude extract using GC-MS and evaluate analgesic property of the crude extract of the seed of *Irvingia gabonensis*.

2. Experimental

2.1 Reagents and Materials

The test drugs were purchased from reputable manufacturer and they are acetylsalicylic acid (Alpine Pharmacy, Abuja, Nigeria), morphine hydrochloride (Sigma Pharmaceutical Misouri, USA) and pentazocine (Alpine Pharmacy, Abuja, Nigeria). The solvent used were of analytical grade; methanol was purchased from Sigma Germany. In-house purification was used to obtain

the distilled water used in this experiment and appropriate volume was utilized to produce the various doses of *Irvingia gabonensis* seed extract administered (50 mg/kg, 100 mg/kg and 200 mg/kg).

2.2 **Collection and identification of sample**

Irvingia gabonensis seeds were purchased from Uselu market, Egor Local Government Area, Edo state. Identification and authenticated was carried out by Dr H.A. Akinnibosu in Plant Biology and Biotechnology Department, University of Benin and herbarium number UBHI-153 was issued. The seeds were air dried for four weeks, pulverized using Victoria milling machine and 2.5 L of methanol (Sigma) was used to macerate 1 kg of the powdered seeds for 3 days. The supernatant solvent was decanted after repeated stirring and then passed through Whatman grade 1 (11 µm) filter paper and the filtrate concentrated at a temperature of 50 ^{o}C in-vacuum using rotary evaporator. The crude extract was then stored at a temperature of 4 ^{o}C until used.

2.3 Evaluation of phytochemicals

Seed powder of *Irvingia gabonensis* was screened for the various phytochemicals by standard method [18,19]. Phytochemicals screened for include alkaloid, saponin, tannin, flavonoid, steroids, cardiac glycoside and triterpenoid.

2.4 GC-MS analysis and condition

The identity of the compounds were determined by GC-MS model QP-2010 (Shimadza), equipped with Restek column measuring 30 m length, 0.25 mm internal diameter and 0.25 µm thicknesses. Helium gas was used as the carrier gas as the GC was operated in the splitless mode and flow rate was set at 1 ml/min. Injection temperature was maintained at 250 °C, while sample injected volume was 8 µl. The programmed column temperatures were: 60 ^{o}C (1.50 min), to 260

at 14 ^{o}C min^{-1} (1.50 min) to 300 ^{o}C at 14 ^{o}C min^{-1} held for 3.3 min. Samples were injected automatically into the MS at an interface temperature of 250 ^{o}C with ion source temperature at 230 ^{o}C. Election impact ionization (EI) at of 70 eV was carried out. Retention time and abundance of the confirmation ions relative to that of quantification ion were used as identification criteria [20]. The fragmentation pattern was compared with the data from National Institute of Standard Technology (NIST).

2.5 Experimental Animals

The experiment was done using Swiss albino mice weighing 25-35 g of both sexes. They were purchased from the Department of Pharmacology and Toxicology, Animal House and kept in an environmental friendly conditioned polypropylene cages with unrestricted access to food (regular pellets) *ad libitum* and clean drinking water. Temperatures and humidity were maintained at 24 ± 1 ^{o}C and (50 ± 5 %) on a 12 hrs light-dark cycle. The animals were kept together for 14 day before the experiment was conducted to allow for acclimatization. Experimental protocol of the international accepted principle on laboratory animal use, care and handling was adhered to completely. Ethical approval was applied for and approval was given by the Faculty of Pharmacy, Ethical Committee, University of Benin.

2.6 Assessment of writhing response in mice by acetic acid

Analgesic activity of the extract was evaluated by method described earlier by Koster *et al*., 1959 [21] and modified by Igbe *et al*., 2013 [22]. The mice were divided into five groups, with five mice per group. Appropriate doses used for each group was determined from the range finding study. Group I, II, III, IV and V received distilled water (10 ml/kg), 50 mg/kg, 100 mg/kg, 200 mg/kg of extract and 100 mg/kg acetylsalicylic acid respectively. These were given orally, 1 hr

before intraperitoneal administration of 10 ml/kg of 0.6 % acetic acid. Writhing response was observed at intervals of 5 min. for 30 min.

2.7 Hot plate test

Eddy and Leibach method of 1953 [23] was used to determine the analgesic property of *Irvingia gabonensis* by hot plate after slightly modification. The mice were divided into six groups of five mice each. Group I, II and III received 50 mg/kg, 100 mg/kg and 200 mg/kg of the extract orally, while group IV and V received the standard drugs like morphine hydrochloride (2 mg/kg body weight subcutaneously) and pentazocine (15 mg/kg intraperitoneally). The last group (VI) received distilled water. The extract, standard drug and distilled water were administered 1 hr before placing them on the hot plate (Ugo Basile hot plate, model 7280, Germany) with temperature of 55.1 ± 0.1. Pre-drug latency was determined for all the animals and the time, in seconds, for each mouse to respond, by shaking or licking of the paw or jumping was recorded as a response to pain. After the administration of drug, the latency time for responses was measured at 30, 60, 90 and 120 min, also the cut-off time for the hot plate latencies was set at 30 s [24].

2.8 Presentation of results and statistical analysis

Results were presented in mean ± standard deviation (SD) and statistical analysis was carried out by GraphPad InStat 3.06 version using one way analysis of variance (ANOVA post test). Significance level was set at $P \leq 0.05$.

3. Results and discussion

Phytochemical screening of the powdered seed of *Irvingia gabonensis* showed the presence of alkaloid, flavonoid, cardiac glycoside, phenol, triterpenoid and saponin, while tannins and steroids are absent (Table 1). This result is in agreement with study conducted by Mgbemena and

coworker in 2019[25]. Phytochemicals are known to have wide range of biological activities which include immunomodulatory, antioxidative, anti-HIV, hypolipidemic, anti-mutagenic, antitumor, antidysrhythmic and hepatoprotective [26], but extract from the leaf and stem bark of *Irvingia gabonensis* have been reported to show anti-diabetic and analgesic effects that compared well with narcotic analgesic due to the presence of these phytochemicals [21,27].

Table 1. Phytochemical screening of the seed extract of *Irvingia gabonensis*

S/N	Phytochemical	Inference
1	Alkaloid	+
2	Flavonoid	+
3	Cardiac glycoside	+
4	Triterpenoid	+
5	Saponin	+
6	Tannin	-
7	Steroids	-

Key: + = present, - = negative

Chromatogram of the seed extract of *Irvingia gabonensis* revealed the presence of Glycerin, 2-Undecanone, 4-methyl-2-Hexanol, Decanoic acid, methyl ester, n-Decanoic, Caprylic acid, 2-Tetradecanone, Dodecanoic acid, methyl ester, Dodecanoic acid, Tridecanoic acid, 12-methyl-, methyl ester, Tetradecanoic acid, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, 6-Octadecenoic acid, methyl ester, (Z)-, Methyl stearate, Methyl palmitate, Octadecanoic acid, Tetradecanoic acid, 2,3-dihydroxypropyl ester, (Z)-9-Octadecenamide, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, Oleoyl chloride, Ergost-25-ene-3,6-dione, 5,12-dihydroxy-, (5.alpha.,12.beta.)-, 1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenye-

10,14-dimethylene-pentadec-4-enyl)cyclohexane, 17-Octadecynoic acid, 9,12,15-Octadecatrienoic acid, Squalene, Decanoic acid, 2-hydroxy-1-(hydroxymethyl), 4,5-Diethyl-7,9(1,8-naphtho)-2,2,3-trimethyl-, β and γ-tocopherol (figure 1). The identities of these compounds were obtained from the value of the retention time and information from NIST. The GC analysis provided the retention time and percentage area of the analyzed compounds while the MS confirmed the identity of these compounds from the fragmentation pattern with the base peaks and molecular weights.

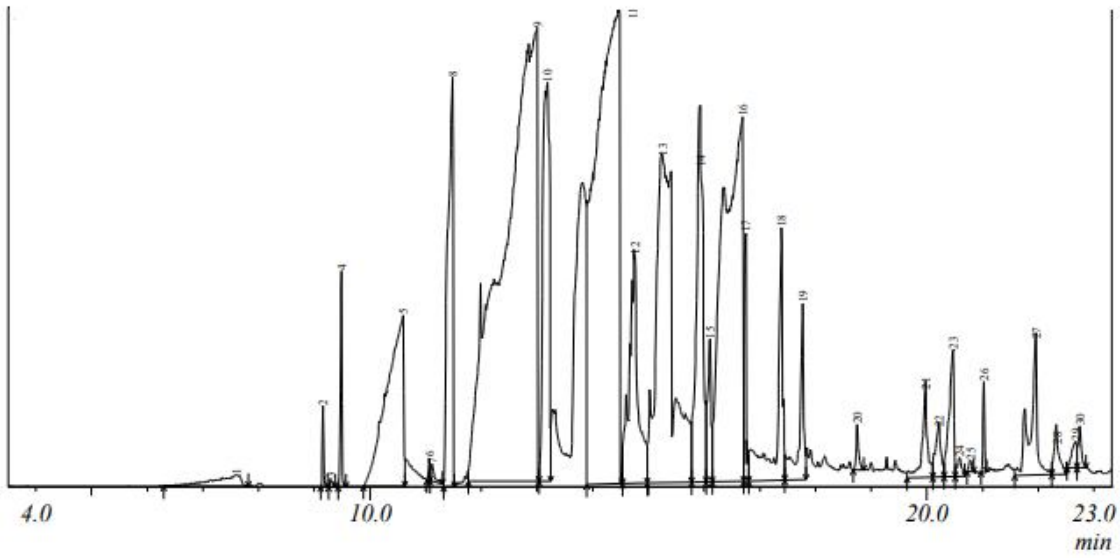

Figure 1. Chromatogram of the methanolic extract of *Irvingia gabonensis*

From table 2, the compounds can be grouped base on their functional group into acids, alcohol, ester, amide, alkyl halide and amine. The acids include saturated and unsaturated fatty acids and this can be seen from the molecular formula of most of the compounds containing two oxygen atoms which is part of the carboxylic acid functional group. Perusing through the result revealed

fatty acid with base peak ion at 73 (m/z) and esters with base peak ion of 55 and 74 (m/z). Fatty acids have been implicated in pain reduction associated with rheumatoid arthritis, dysmenorrhea and neuropathy [28]. Vitamin E was also identified and it has the potential of preventing or been used in the treatment of osteoarthritis due to its antioxidant and anti-inflammatory effects [29]. Apart from the medicinal properties of these compounds identified stearic and oleic acid also have protective effect on the plant [22]. Also fatty acids from the seed of *Irvingia gabonensis* are used as excipient in tablet formulation as binding, emulsifying and suspending agents [7].

Table 2. GC-MS analysis of crude extract from *Irvingia gabonensis* seed

S/N	Compounds	RT	% A	MF	BP ion	MW
1	Glycerin	7.60	0.54	$C_3H_8O_3$	61	92
2	2-Undecanone	9.16	0.17	$C_{11}H_{22}O$	58	170
3	4-methyl-2-Hexanol	9.29	0.03	$C_7H_{16}O$	45	116
4	Decanoic acid, methyl ester	9.49	0.57	$C_{11}C_{22}O_2$	74	186
5	n-Decanoic	10.60	4.93	$C_{10}H_{20}O_2$	73	172
6	Caprylic acid	11.07	0.05	$C_8H_{16}O_2$	73	144
7	2-Tetradecanone	11.11	0.11	$C_{14}H_{28}O$	58	212
8	Dodecanoic acid, methyl ester	11.48	3.53	$C_{13}H_{26}O_2$	74	214
9	Dodecanoic acid	13.00	25.96	$C_{12}H_{24}O_2$	73	200
10	Tridecanoic acid, 12-methyl-, methyl ester	13.19	4.60	$C_{15}H_{30}O_2$	74	242
11	Tetradecanoic acid	14.46	19.18	$C_{14}H_{28}O_2$	73	228
12	Hexadecanoic acid, methyl ester	14.76	3.43	$C_{17}H_{34}O_2$	74	270
13	n-Hexadecanoic acid	15.26	9.98	$C_{16}H_{32}O_2$	73	256

14	6-Octadecenoic acid, methyl ester, (Z)-	15.94	4.24	$C_{19}H_{36}O_2$	55	296
15	Methyl stearate	16.11	0.85	$C_{19}H_{38}O_2$	74	298
16	Methyl palmitate	16.69	11.87	$C_{17}H_{34}O_2$	55	264
17	Octadecanoic acid	16.75	0.82	$C_{18}H_{34}O_2$	55	284
18	Tetradecanoic acid, 2,3-dihydroxypropyl ester	17.39	2.41	$C_{17}H_{34}O_4$	98	302
19	(Z)-9-Octadecenamide	17.77	1.27	$C_{18}H_{35}NO$	59	281
20	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	18.75	0.18	$C_{19}H_{38}O_4$	43	330
21	Oleoyl chloride	19.98	0.81	$C_{18}H_{33}ClO$	55	300
22	Ergost-25-ene-3,6-dione, 5,12-dihydroxy-, (5.alpha.,12.beta.)-	20.22	0.50	$C_{28}H_{44}O$	55	444
23	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenye-10,14-dimethylene-pentadec-4-enyl)cyclohexane	20.47	1.06	$C_{33}H_{56}$	55	452
24	17-Octadecynoic acid	20.59	0.15	$C_{18}H_{32}O_2$	55	280
25	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	20.78	0.06	$C_{11}H_{16}O_4$	73	322
26	Squalene	21.02	0.24	$C_{30}H_{50}$	69	410
27	Decanoic acid, 2-hydroxy-1-(hydroxymethyl)	21.97	1.62	$C_{13}H_{26}O_4$	155	246
28	2,6-Di[p-cyanophenyl]-4-picoline	22.34	0.40	$C_{20}H_{13}N_3$	295	295
29	γ-Tocopherol	22.67	0.23	$C_{28}H_{48}O_2$	151	416
30	β-Tocopherol	22.75	0.21	$C_{28}H_{48}O_2$	151	416

Key: BP ion = Base peak ion, MF = Molecular formula, MW = Molecular weight, % A = Percentage area, RT = Retention time

Figure 2 showed the acetic acid induced mouse writhing for 50 mg/kg, 100 mg/kg and 200 mg/kg for *Irvingia gabonensis* seed extract and controls which include acetylsalicylic acid (aspirin) used as positive control and distilled water used as negative control. The extract revealed significant reduction ($P < 0.05$) in the writhing reflexes as the dose of the methanolic extract increases from 50 to 200 mg/kg when compared to the placebo (distilled water). The decrease responses observed with 200 mg/kg crude extract compared well with acetylsalicylic acid of 1.5 ± 2.38. At time 10 min., 100 mg/kg of the extract reduces reflexes more than acetylsalicylic acid and the extent of reduced reflexes was also significant ($P \leq 0.05$) when compared to the placebo. Decrease refluxes was also seen at 15 min. but at this time the decrease reflexes was inversely proportional to the dose of the extract administered. At 25 min., the extract reduces reflexes better than aspirin but uniformly at 50 mg/kg, 100 mg/kg and 200 mg/kg, while at 30 min. 100 mg/kg and 200 mg/kg reduces reflexes more than aspirin but not significant, it is expected that at this time the effect of extract is wearing out.

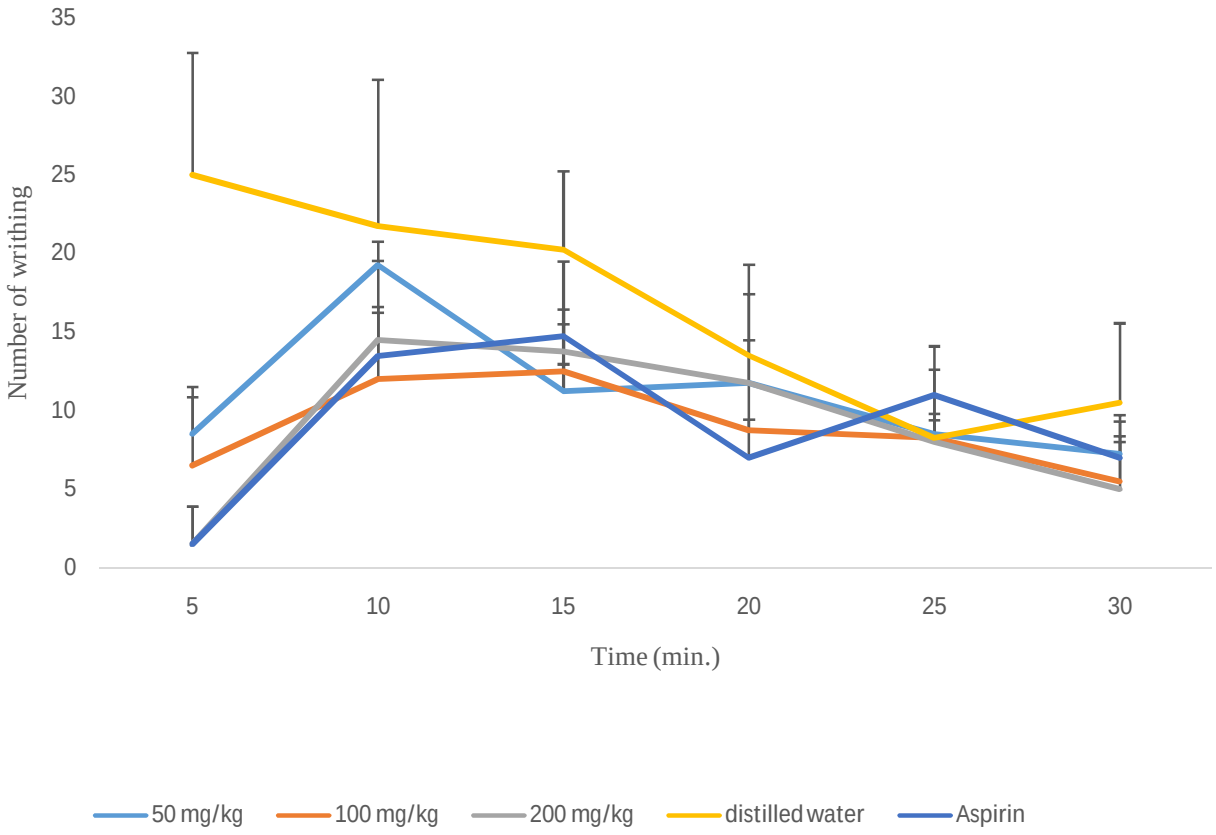

Figure 2: Acetic acid induce writhing responses for different doses of *Irvingia gabonensis* and acetylsalicylic acid (aspirin).

Acetic acid writhing test is a chemical nociceptive method used to evaluate the potential of a test drug to alter pain peripherally. This was achieved by injecting a pretreated mouse intraperitoneally with acetic acid, this induced pain which results in stretching and extension of the legs which term as writhing. This process is an indirect nonselective release of endogenous mediators which stimulate the nociceptive neurons.

Acetylsalicylic acid (NSAIDs) acting via inhibition of cyclo-oxygenase enzyme (COXs), making them the most widely used drugs for the treatment of pain and inflammation. COXs catalyse the conversion of arachidonic acid into prostaglandins, which is implicated in homeostatic regulation such as inflammatory response [30].

Figure 3 showed how administering different doses of *Irvingia gabonensis* seed extract altered the responses time of the Swiss mouse to temperature in a hot plate experiment. Perusing through the result revealed increase reaction time (at 120 min.) for the seed extract of *Irvingia gabonensis* at 100 mg/kg and 200 mg/kg. However, when compared with morphine it was not statistically significant. At this same time, the extract revealed better reaction time at 50 mg/kg, 100 mg/kg and 200 mg/kg, which were also not significant when compared with pentazocine.

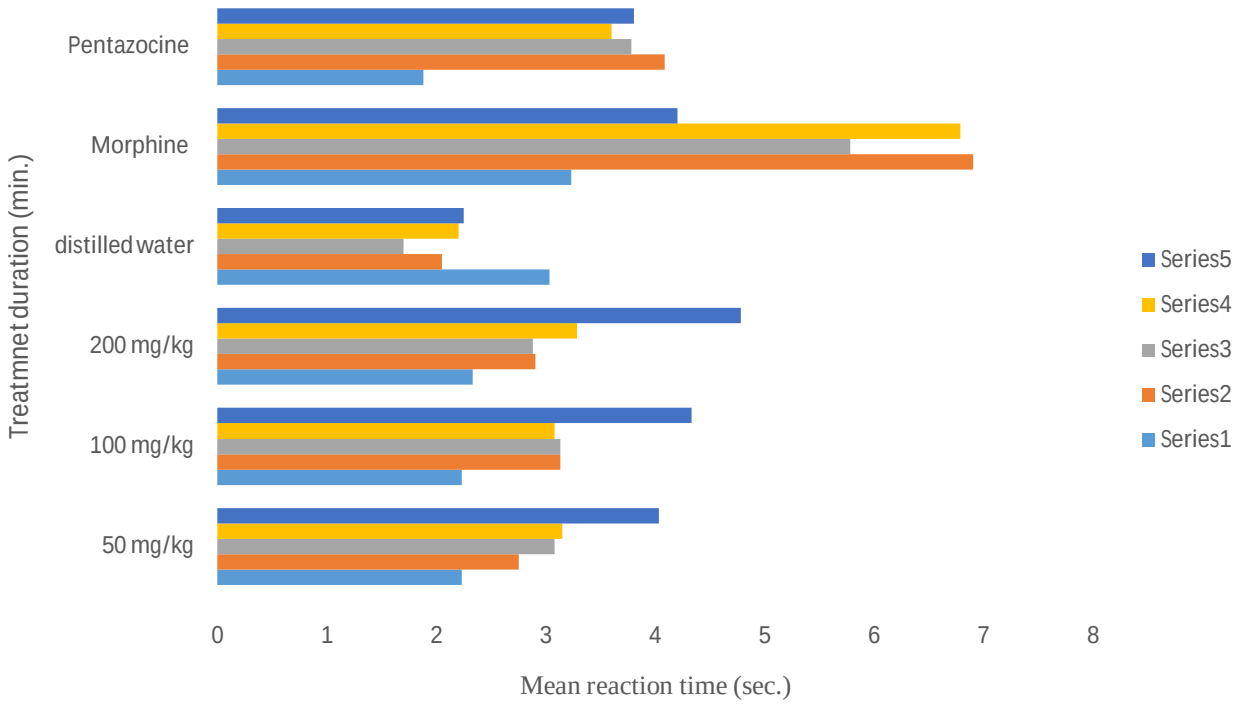

Figure 3. Hot plate analgesic effect of different doses of *Irvingia gabonensis* seed, morphine and pentazocine

Thermal method is an extensively used test [31] where mice are placed on a heated surface and their responses are recorded [32]. This method is used in determining the site of action of centrally acting analgesic such as morphine and pentazocine, which are highly potent and clinically relevant analgesics. Morphine is an agonists that are limited to clinical application and

produce varying degrees of central nervous system disturbance which manifest primarily as respiratory depression and sedation, tolerance and dependence could also develop. Morphine is the naturally occurring opiate analgesic from which other agents have been synthesized. It was used as a standard to evaluate the potency of the different doses of seed extract. It was observed that the extract possess activity that were not significant.

4. Conclusion and recommendation

Apart from the fact that *Irvingia gabonensis* seeds is used in the preparation of slimy soup that can be used to consume starchy meal in some Africa home, this study have shown that it possess different classes of phytochemicals, which were shown to be majorly fatty acids and peripheral analgesic potential at 200 mg/kg. It is possible that the analgesic property may be due to the presence of these fatty acid. There is need to be isolate the particular compounds eliciting this activity.

Conflict of interest: There was none.

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