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Chemical Composition and Analgesic Effects by Acetic Acid Induced Writhing and Hot plate method of Combined Hydro-ethanol Leave Extract of Kigelia africana and Guiera Senegalesis In Mice

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ABSTRACT
Some traditional plants are usually used in some part of developing countries for the management of pain. An ache is common experience observed by the patients, and patient anxiety is a form of warning signal. This work aimed at finding the chemical compositions, antibacterial and analgesic properties of combined hydro-ethanol leave extract (CHELE) of K. africana (KA) and G. Senegalesis (GS) in mice. Antimicrobial screening of combined hydro-ethanol extracts of KA and GS screened against the Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Salmonella spp and Ciprofloxacin used as a standard control. The mice were grouped into 4 and administered with acetic acid intraperitoneal and kept in separate transparent cages for observation of abdominal writhing compared to control group (G). G1 Normal control, G2 treated with standard drug (piroxicam 20mg/kg), G3 received extracts at dose of 250 mg/kg bw and G4 received extract at dose of 500mg/kg bw. Plant extract possess significant analgesic activity by decreasing number of writhing made compared to control group. Percentage of inhibition in G3 treated with 250mg/kg significantly increases (31.48%) compared to G2 with inhibition percentage of 1.85%, so also does G4 extract administered at 500mg/kg, which inhibits writhing percentage by 48.14%. in hot plate method, the administration of piroxicam, combined extracts (250mg/kg and 500mg/kg bw) at 120 and 180 minutes after first (30 minutes) and second jumping (60 minutes) decreased the time in seconds. The mice withstand the heat when compared with the control group. CHELE treated at provided doses showed significant decrease in number of writhes compared to the control group. At higher dose (500mg/kg) the combine extract has the highest effect. Preliminary phytochemical analysis of CHELE record the presence of alkaloids, saponins, glycocides, balsams, tannins, volatile oil among others. The CHELE resulted in notable activity for the used bacterial strains as compared to ciprofloxacin. S. aureus, pseudomonas and Salmonella spp shown highly sensitive strains while E. coli show none. Gas chromatography–mass spectrometry (GCMS) displayed twenty nine (29) compounds in the combine leave extracts of KA and GS. The antibacterial and analgesic activity might be due to the chemical compositions of the combine extracts of KA and GS, and play a role in pain relief.

KEYWORDS: *Chemical Composition, Analgesic Effects, Combined Hydro-ethanol*

INTRODUCTION

Certain disorders that occur commonly in patients that experience pain like hyperalgesia, allodynia and hyperesthesia (Mamun-or-Rashid *et al.*, 2017). A number of compounds that possessed analgesic properties so far were isolated from different plant origin, that led scientists to uncover this therapeutic side with better pharmacokinetic and Pharmacodynamics profile with newer molecule (Mamun-or-Rashid *et al.*, 2017).

The plant *Kigelia africana* is a family member of Bignoniaceae popularly known as the sausage tree. The plant has huge fruits, which hangs from long fibrous stalks (Cragg and Newmann, 2001). The root and stem bark of *k. africana* proved to potentially have anti-diabetic, antioxidant and antibacterial properties (Abdu *et al.*, 2020; Said *et al.*, 2019).

The plant remedies are also used to treat some ailment like, hemorrhoids (Oliver-Bever, 2004). *Guiera senegalensis* is a Family member of Combretaceae popularly known as *Sabara* in Hausa. G. senegalensis is a shrub of the savannah region of west and central Africa (Umma *et al.,* 2023). GS grow with lower rainfall and very lightly dry soils, predominantly found in Western Africa, indigenous to Nigeria, Cameroun and widespread to East Africa in Egypt and Sudan (Umma *et al.,* 2023). GS is used widely in traditional medicine for the cure of many diseases. In Nigeria, the leaves and root extract of GS were scientifically proved to manage or cure dysentery, diarrhea, gastrointestinal pain, rheumatism, and fever among others. Some phytochemical compounds found in GS, might be attributed to the important biochemical properties in the plant.

METHODOLOGY

Collection and Extraction of Plant Leave: The *G. senegalensis* and *K. africana* samples were collected from Safana Local Government Area, Katsina State, Nigeria. Four hundred grams (400g) of the combined sample was extracted with 50% hydro-ethanol (50/50 v/v) by cold maceration for 1 week in order to ascertain maximum extraction with constant stirring. In order to remove the mac, the extract was filtered, then evaporated to dryness at 50˚C, over a water bath. The extract would be stored and freshly prepared when required for an experiment.

Animals: The experimental animals used were forty (40) adult wister mice weighing between 20-30g of either sex, 5-6 weeks both sexes used during the study. The mice were purchased from animal houses of ABU, Zaria, Nigeria. The mice were kept and monitor at the separate cages at Federal University Dutsin-Ma, animal house. The mice were kept at normal conditions with standard feed and acclimatized for 1 week before the experiments.

Part 1 group (writhing method) consist of 20 mice which was grouped into 4 with 5 mice each while part 2 group (Hot plate method) were also grouped into 4 with 5 mice in each category.

Chemicals/Drugs: The Chemicals supplied in the experiment were of an analytical grade. This include Distilled water, Normal saline, Ethanol, Piroxicam (Hovid), and Acetic acid.

Preliminary Phytochemical Analysis: The screenings for phytochemical components were carried out as reported in the work of Sisidharan *et al.,* (2011). Standard methods were used to know the nature of phytochemicals present in the hydro-ethanol extract of *K. africana* and *G. senegalensis.* Phytochemical analysis carried out according to standard procedures detects the presence of secondary metabolites like alkaloids, saponins, tannins, glycosides, Balsams, volatile oil, among others.

ANALGESIC STUDIES

Acetic Acid (AA) Solution: For the preparation of AA solution, about 0.7 ml AA was diluted with 100 ml distilled water.

Standard Sample: Preparation of piroxicam at the dose of 20-mg/kg-body weight, 10 mg of piroxicam capsule was taken and a suspension of 10 ml was made.

Acetic-Acid Induced Abdominal Writhing in Mice: The writhing method adopted in the study was described by Gaertner *et al.,* (1999). Twenty (20) wister mice were grouped into 4 with 5 mice in each cage. Group 1 was the normal control treated with 1ml/kg normal saline, Group 2 another control administered piroxicam (10mg/kg i.p), group 3 orally administered CHELE 250mg/kg and group 4 orally administered 500mg/kg respectively. Administration of 1% AA solution (0.1ml i.p) was done after 30 minutes to the four groups. Five minutes after the administration of AA, the number of writhes was counted for 10 minutes.

Percentage inhibition of writhing inhibition was calculated in relation to the control as follows:

$$\%\ \text{inhibition} = \frac{\text{inhibition (control)} - \text{inhibition (extract)}}{\text{inhibition (extract)}}\ x100$$

Test in Mice (Hot plate method): The method employed and adopted by Wilson *et al.,* (2003), with some modifications. The pain threshold was used to select the mice, and all the mice would be placed at temperature of 45 ± 1ºC singly on the hot plate. The mice responded within 2 sec were selected. Group 1 administered normal saline 30 minutes prior to the pretreatment. Piroxicam for group two, test sample extract for group three and four. The animals were placed individually on hotplate and time taken to react to the heat by the mouse either by licking paws or jumping away from the hotplate was noted. The experiment was repeated after 60, 120 and 180 minutes respectively and the reaction time was noted.

The percentage protection against thermal pain:

$$\%\ \text{protection against thermal pain} = \frac{(\text{test mean} - \text{control mean})}{\text{control mean}}\ x100$$

Statistical Analysis: The data expressed as Mean ± Standard Error of Mean. The results were analyzed by Analysis of Variance (ANOVA). The significance level at $P< 0.05$.

RESULTS

Qualitative Phytochemicals of *Kigelia africana* and *Guiera senegalensis* extracts.

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Table 1. Qualitative analysis of phytochemical parameters of combined hydro-ethanol extracts of *K. africana* and *G. senegalensis.*

Parameters	*Kigelia africana* + *Guiera senegalensis*
Alkaloid	+++
Flavanoids	+
Tannins	+++
Anthraquinone	+
Saponins	+++
Steroids	+
Glycosides	++
Balsams	++
Volatile oil	++

KEY: +++ Highly Present; ++ Moderate; + partial present

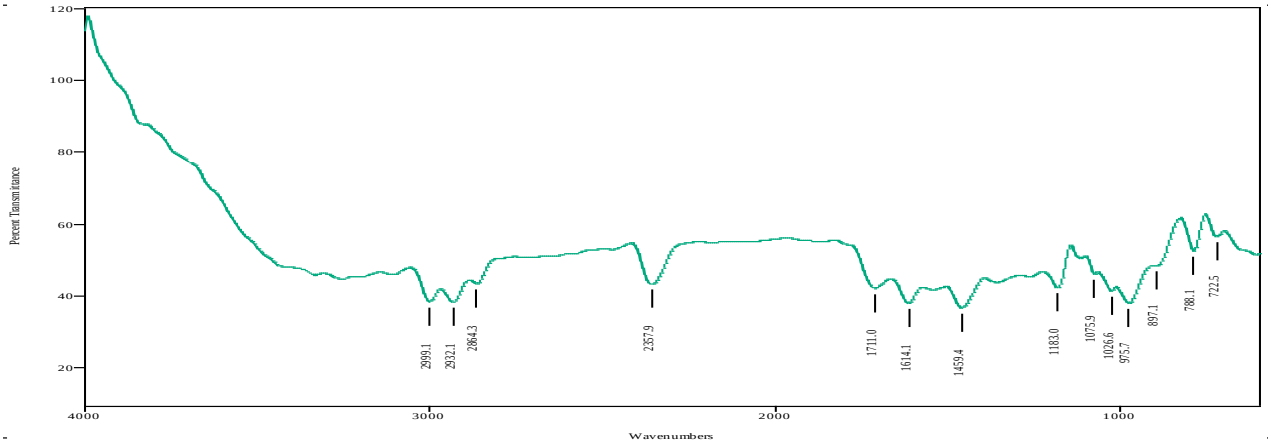

Figure 1: Fourier Transform infrared spectroscopy (FTIR) of the combined hydro-ethanol extracts

Table 2: GS-MS analysis of the combined extracts of *K. africana* and *G. senegalensis*

NO.	RT	NAME OF COMPOUND	MF	AREA	QUALITY
1	5.567	Furan-2-carbohydrazide, (1-methylhexylideno)	$C_{12}H_{16}N_2O_2$	4.16	38
2	6.34	5-Deoxypyridoxal	$C_8H_9NO_2$	0.23	87
3	6.558	1,3-Butadiene-1-carboxylic acid	$C_5H_6O_2$	0.19	43
4	6.843	4-Phenylsemicarbazide	$C_7H_9N_3O$	0.48	44
5	7.019	4,6-Dimethyl1,3-nitro-2 (1H)-pyridin one	$C_7H_8N_2O_3$	0.21	43
6	7.522	Hydroquinone	$C_6H_6O_2$	0.50	46
7	7.65	Butanedinitrile	C_4H_7N	0.89	46
8	8.017	Methyl 4-pentynoate	$C_6H_{10}O_2$	2.03	47

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9	8.778	Bicyclo[3.1.1]heptane,2,6,6-trimethyl-[1R(1.alpha.,2.beta.,5.alpha.)]	$C_{10}H_{18}$	6.20	48
10	9.009	1,6-Heptadiene, 2,5,5-trimethyl	$C_{10}H_{18}$	3.10	52
11	9.300	Dodecanoic acid, 10-methyl-,methyl 1 ester	$C_{14}H_{28}O_2$	8.58	74
12	9.626	Butane, 1-(ethenylthio)	$C_6H_{12}S$	5.97	50
13	10.46	Heptadicanoic acid, 16-methyl-,methyl ester	$C_{19}H_{38}O_2$	10.46	58
14	11.188	Tert-hexadecanethiol	$C_{48}H_{99}AuS_3$	17.69	50
15	11.914	Eicosane	$C_{20}H_{42}$	2.27	74
16	12.145	Tetradecanal	$C_{14}H_{28}O$	3.24	51
17	12.457	Methoxyacetic acid,tetradecyl ester	$C_{17}H_{34}O_3$	1.59	56
18	12.898	2-amino-4-azido-5-[3,4,5-trimethoxybenzyl] pyrimidine	$C_{14}H_{19}N_{403+}$	17.59	91
19	15.994	oleic acid	$C_{18}H_{34}O_2$	0.89	84
20	16.795	oleic acid	$C_{18}H_{34}O_2$	0.40	95
21	16.999	2-methyl-z-z-3, 13-octadecadieol	$C_{19}H_{36}O$	0.10	94
22	17.148	oleic acid	$C_{18}H_{34}O_2$	0.05	64
23	17.501	1-octadecanesulphonylchloride	$C_{18}H_{37}CIO_2S$	2.98	87
24	13.842	1-chloroeicosane	$C_{20}H_{41}CL$	0.87	96
25	13.985	2,6,10-dodecatrien-1-ol, 3,7,11-trimethy	$C_{15}H_{26}O$	2.56	55
26	17.177	(Z)-9,17-octadecadienal	$C_{18}H_{32}O$	0.029	94
27	27.1343	Stannane,tetraethyl-	$C_{18}H_{20}Sn$	0.131	91
28	28.1515	3-(3,4-dimethoxyphenyl)propylamine,PFP	$C_{11}H_{17}NO_{20}$	17.167	41
29	28.7466	1H,3H-furo[3,4-c]furan,1,4-bis (3,4dimethoxyphenyl)	$C_{22}H_{26}O_7$	13.053	93

KEY: = RT (Retention Time), MF (Molecular Formula).

Table 3: Antibacterial assay of combined leave extract of *K. africana* and *G. senegalensis* and their zone of inhibition.

Bacteria	Hydro-ethanol combined extract	Standard drug (ciprofloxacin)
Escherichia coli	-	11.2mm
Staphylococcus aureus	12mm	10mm
Salmonela spp	7mm	9mm
Pseudomona aeruginosa	9mm	9mm

KEY: = (-) No Inhibition

Table 4: Results showing Number of Writhing and percentage inhibition of *K. africana* and *G. Senegalensis* extracts at different doses against standard drug

Group	Dose	Writhing	% Inhibition
NC	0.1ml	13.50 ± 0.87^a	-
Piroxicam	20mg/kg	13.25 ± 1.25^a	1.85%
CHELE (250mg/kg)	250mg/kg	17.75 ± 1.89^b	31.48%
CHELE (500mg/kg)	500mg/kg	20.00 ± 1.08^b	48.14%

Keys: NC = Normal control. CHELE= Combined hydro-ethanol extract of leave extract. Values are mean ± SEM (n = 5). Values with different superscripts within a row differ significantly from each other ($P < 0.05$).

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Table 5. The effect of combine hydro-ethanol extract of KA and GS on hot plate reaction time test in mice

Treatment kg/mg	First jump (30 min)	Reaction Time Second jump (60 min)	Sec Third jump (120 min)	Fourth jump (180 min)
Control	0.1633 ± 0.22[a]	0.9133 ± 0.35[a]	0.5100 ± 0.72[a]	0.6533±0.44[a]
Piroxicam 20mg/kg	0.2433 ± 0.15[a]	1.0667 ± 0.67[b]	0.8033±0.10[a]	0.7733±0.14[a]
CHELE (250mg/kg)	0.1633 ± 0.88[a]	1.8600 ± 0.14[b]	1.0433±0.21[b]	0.9833±0.18[a]
CHELE (500mg/kg)	0.2867 ± 0.27[a]	1.3667 ± 0.27[b]	1.1433±0.23[b]	0.8300±0.20[a]

Values are ± SEM (n=5). Values with different superscript in the same column represent significance different ($p<0.05$).

DISCUSSION:

The preliminary phytochemical screening of combined hydro-ethanol leave extracts (CHELE) of *K. africana* (KA) and *G. Senegalensis* (GS) detected the presence of some secondary metabolites that are highly present (alkaloids, tannins and saponins), moderately present (glycosides, balsams and volatile oil) and partially present (flavanoids, anthraquinones and steroids). Some of These phytochemical constituents possess different pharmacological properties like saponins, glycosides, alkaloids among others were found to have anti-inflammatory, anti-allergic effects, antimicrobial, anti-nociceptive, antioxidants, anticancer, antidepresant, antidiarrheal and hepatoprotective effects (Soetan *et al.,* 2006; Akkol *et al.,* 2007; Singh *et al.,* 2010 and Yassin *et al.,* 2013).

FTIR analysis was detected in the above spectrum: sp2 -hybridized CH bonds (2999.1-2357.9 cm^{-1}), sp3 -hybridized CH bonds (1711.0-1459.4 cm^{-1}). Absorption bands due to the nitro group: 1183.0 and 722.5 cm^{-1}. The nitro group is conjugated with the benzene ring because they were at lower wave numbers than usual. These absorptions usually determine the nature of the functional group present in the compound being considered by the spectrum. Many functional groups require the presence of several characteristic absorptions. Result from the GC–MS analysis showed 29 compounds detected in the CHELE of KA and GS. The compounds are coming from carbohydrate, lipids and nucleic acid. Some of the compounds present are Furan-2-carbohydrazide, (1-methylhexylideno), 5-Deoxypyridoxal, 1,3-Butadiene-1-carboxylic acid, 4-Phenylsemicarbazide, 4,6-Dimethyl1,3-nitro-2 (1H)-pyridin one, Hydroquinone, Butanedinitrile, Methyl 4-pentynoate, Methyl 4-pentynoate, 1,6-Heptadiene, 2,5,5-trimethyl, Heptadicanoic acid, 16-methyl-,methyl ester Eicosane, oleic acid, 2-amino-4-azido-5-[3,4,5-trimethoxybenzyl] pyrimidine among others. The compounds detected were 29 and oleic acid present in 3 different areas of 0.89, 0.40 and 0.05. These compounds might act mutually, trigger to protect the organ damage during oxidative stress, by either inhibiting or scavenging free radicals such as superoxide anion radical (O_2^-), hydroperoxyl radical ($HOO^{\cdot}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$). Compounds like oleic acid, 2-methyl-z-z-3, 1,3-octadecadieol, 1-octadecanesulphonylchloride, 1-chloroeicosane, benzene, carbonic acid, as part of their functional groups and might help in satisfying the free radicals by donating the electron.

The antibacterial properties of CHELE against bacterial strains E. coli, *Salmonella spp*, *P. aeruginosa*, and *S. aureus* found that the CHELE exhibit different remarkable antibacterial properties. CHELE showed no activity on E. coli and observed maximum activity on *S. aureus*, *P. aeruginosa*, and *Salmonella spp.* Standard antibiotic drug (ciprofloxacin) was used to compare these results. The plant stem bark so also the root and fruits extracts of KA using microtiter plate bioassay, possesses good antibacterial properties (Said *et al.,* 2022 and Owolabi *et al.,* 2011).

In the mice writhing assay, the CHELE significantly ($P < 0.05$) decreased the acetic acid induced abdominal writhing in mice. The group that was treated at the highest dose of 500mg/kg bw showed highest percentage inhibition of about 48.14% when significantly compared the inhibition of 1.85 % by Piroxicam (20mg/kg). The visceral pain model is acetic acid induced abdominal writhing model used generally for screening plants and new agents for analgesic activities (Gene *et al.,* 1998; Aliyu and Sama'ila, 2015). The non-specific nociceptive model is also associated with acetic acid test (Bighetti *et al.,* 1999). Pain mediators release like prostaglandin and cytokines occur when administration of intra-abdominal injection of AA, that might be cause for the pain inducement (Ikeda *et al.,* 2001).

In Table 5, the CHELE observed in mice by model of hot plate showed a significantly ($P < 0.05$) increased analgesic activity in all the extract and standard drug treated groups at the second jump (60 minutes) when compared with the normal control (group 1). Then, at third jump (120 minutes) and fourth jump (180 minutes) the effect of CHELE dropped. Standard drug treated group showed significantly ($P < 0.05$) much reduction at 180 minutes. At dose of 250 mg/kg with reaction time of 60 minutes has the highest protection.

CONCLUSION

It can be concluded that, CHELE of KA and GS possess a dose dependent analgesic activity and antimicrobial properties. Because of the chemical compounds detected and bioactive secondary metabolites with significant analgesic and anti-microbial properties can justify its use in pain.

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