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Phytochemical and anti-snake venom characteristics of the leaves extract of *Hibiscus radiatus*

Isaac Enemali Shaibu and Simon Koma Okwute

Abstract
The leaves *of Hibiscus radiatus* are traditionally used as a remedy for the treatment of snake bike, inflammation and related disorders in the form of decoction by the Auta tribal community in Wamba, Nassarawa state, Nigeria. This study was designed to investigate the anti-snake potency against *Naja naja* snake species, anti-bacterial activity and anti-inflammatory potential of the crude methanol leaf extract. The crude methanol extract was subjected to phytochemical screening which revealed the strong presence of phenols, tannins, gums and mucillages, and alkaloids, but moderate presence of sterols, terpenoids, saponins, amino acids, flavonoids and carbohydrates. Fixed oils and fats were however not observed. The crude was screened for anti-snake venom activity by oral administration of *Hibiscus radiatus* (10 mg/kg). It exhibited anti- snake venom potential without preventing total death in the experimental animals after 4 days count by its anti-microbial and anti-inflammatory properties. The crude extract showed a significant ($p<0.05\%$) antibacterial properties against selected clinical isolates, *Staphylococcus aureus, Bacillus subtilis, and Escherichia coli,* but the activity of the extract against the fungus, *Candidas albicans* was not significant ($p>0.05$). The anti-inflammatory property of the leaves extract was evaluated by carrageenan-induced paw edema among other models in six groups of albino rats for 6 hours. The crude extract reduced paw edema (87.79%) within the dose range of 150-300 mg/kg) comparable with the standard drug, diclofenac sodium (10 mg/kg, 93.89%). The results obtained therefore suggest that the methanolic extract of *Hibiscus radiatus* leaves can be considered to be a valuable source of remedy for the treatment of snake bites, bacterial infections associated with wounds at the site of snake bites and inflammations associated with the poisons. The bioactivities of the leaves extract may be attributable to the presence of the phytochemicals and thus confirms the claimed traditional application of the leaves as an anti-snake venom agent.

Keywords: *Hibiscus radiatus*, leaves, anti-snake venom, antibacterial, anti-inflammatory and analgesia

Introduction
Snake bites causes injury and death worldwide and therefore pose a very serious yet neglected threat to public health with a significant burden in sub-Saharan Africa [1]. A common symptom of a bite from a venomous snake is the presence of two puncture wounds from the animal's fangs [2]. Sometimes, venom injection from the bite may occur [1] and this may result in redness, swelling, and severe pain at the area, which may take up to an hour to appear [3] Vomiting, poor vision, tingling of the limbs and sweating may result [1]. Most bites are on the hands or arms [4]. The fear following a bite is common with symptoms of a racing heart and feeling of fainting [1]. The venom may cause bleeding, kidney failure, a severe allergic reaction, tissue death around the bite or breathing problems [5]. Bites may also result in the loss of a limb or other chronic problems [6]. The outcome depends on the type of snake, the area of the body bitten, the amount of venom injected and the health conditions of the victim [7].
Despite the availability of anti-snake serums, they are insufficient and hardly reach the deserving population. The anti-snake venoms available are often specific to particular species of snakes. Various drugs are therefore required to manage the symptoms of snake bites. Key among the drugs used are the synthetic analgesics and anti-inflammatory agents. The side effects of analgesic and anti-inflammatory agents, which include gastrointestinal upset, gastric ulcer, bleeding and liver damage, are a major concern in clinical setting. Thus, the search for safe and effective newer agents is growing. As one of research areas, screening medicinal plants with claimed anti-snake, analgesic and anti-inflammatory activities may create the opportunity of discovering new compounds with greater safety and efficacy [8]. The traditional healers use different practices, including herbs, to manage snake bites and associated pain and inflammation in many countries of the world. Herbal remedies are widely used in developing countries to manage snake bites, pain, inflammation and other associated ailments because of their accessibility, affordable costs and their nature friendly advantages [9].

In Nigeria, a large number of plants species are traditionally used to treat snake bites and the associated symptoms such as headache, bleeding, bloody cough, fear, shivering, and wounds [10]. Very few of these plants have been scientifically evaluated while most of them remain unexploited [11]. The plant *Hibiscus radiatus* (*H. radiatus*)) belongs to the Malvaceae family. It is an annual or perennial herb or woody-based subshrub growing to 2–2.5 m (7–8 ft) tall. The leaves are deeply three- to five-lobed, 8–15 cm (3–6 in) long, arranged alternately on the stems. The flowers are 8–10 cm (3–4 in) in diameter, white to pale yellow with a dark red spot at the base of each petal, and have a stout fleshy calyx at the base, 1–2 cm (0.39–0.79 in) wide, enlarging to 3–3.5 cm (1.2–1.4 in), fleshy and bright red as the fruit matures. *Hibiscus radiatus* is an allotetraploid of *Hibiscus cannabinus* and perhaps *Hibiscus surattensis* [12]. Two species of *Hibiscus* were distinguished [13] with the following features: *Hibiscus cannabinus* leaves and calyx lobes are glandular and has epicalyx (a series of bracts subtending and resembling a calyx) segments attached to the calyx which are not characteristics of *H. radiatus*. *H. cannabinus* has an elongate nectary gland at the base of the lower leaf midrib and on the midvein of each calyx lobe as well as a whitish tomentellum on the calyx which *H. radiatus* does not have. *H. radiatus* has a tooth-like appendage below the apex on the inner surface of the epicalyx bractlets which is not present on *H. cannabinus*. Ethnobotanical information on *Hibiscus radiatus* and the results from general phytochemical screening of *Hibiscus radiatus* are currently scanty. Nevertheless, the *Hibiscus* species are reputed for their use as abortifacient and to stimulate expulsion of placenta after childbirth [14]. The various parts of the plant including flowers, fruits, leaves and roots are known to possess various bioactivities in traditional medical practices [15, 11, 14].

However, there has been no investigation on the anti-snake venom activity of *Hibiscus radiatus* to confirm the claimed use by traditional healers of northern Nigeria. This paper reports on the results of phytochemical screening and the anti-snake venom characteristics of the leaves extract of *Hibiscus radiatum* using various models with albino mice.

Materials and Methods

Materials

The leaves of *Hibiscus radiatus* (Figure 1) were collected from Wamba, Northern Nigeria, at the homes of traditional healers *(Autas)* in November, 2017, at the village backyard dumpsite. The seeds were also collected from the healers for propagation.

Fig 1: Habitat of *Hibiscus radiatus* Lin.(Malvaceae)

The fresh plant was presented for identification at the herbarium of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria. After authentication, the voucher specimen was deposited in the herbarium with the identification number (NIPRD/6887). Instructions by the traditional healers for packaging the seeds and the leaves were strictly followed. The leaf samples were cleaned by rinsing with copious amounts of water, shade-dried and powdered with Electrical Napress 80 Shredder to 60 mesh size (Cal. USA).

All solvents used in the work were of standard grade and were also redistilled before use.

The experimental animals were Swiss albino female mice (20-25 g) for acute toxicity test and Sprague dawley female rats (150-200g) for the *in vivo* anti-inflammatory activity. The animals were purchased from animal breeding houses in Vom, plateau state and were acclimatized under standard environmental conditions: temperature 25^0C; humidity range of 30-50%; 12 h dark/8h light cycles; and air ventilation. They were fed with standard pellet diet (Unilever Ltd., India) and fresh water. The animals were housed for 7 days prior to experimental use in polyacrylic cages (38×23×10 cm) with not more than four per cage. The experiments conducted followed an approved guideline of international ethical committee (NNREC, 2018).

Snake venom from Tukur & Tukur Snakes Farm Nassarawa, Nigeria was collected by the zoo technologist in line with good collection guideline [16]. The collected snake venom was kept in the refrigerator at a temperature of −20 °C (−4 °F). The Anti-snake venom (Bharat Vaccines limited) standard was purchased from Nigerian Pharmaceutical Sector.

Five clinical isolates including two Gram positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*, two Gram negative bacteria, *Escherichia coli and Enterobacter aerogenes* and a fungus, *Candida albicans* were obtained from National Hospital Microbiology unit, Abuja. All the test organisms were preserved in a refrigerator at 4^0C in nutrient agar slant until required.

Methods

Extraction of plant material

The air-dried powdered leaf sample (100g) was successively subjected to Soxhlet extraction with n-hexane, chloroform, ethyl acetate and methanol to give the various fractions which were evaporated to dryness *in vacuo*. Another portion of leaf sample (100g) was also extracted with methanol to give crude methanol extract to give the dry residue on evaporation *in vacuo.* The crude methanol extract was liberally treated with petroleum to remove wax and a dark brown residue on drying.

Preliminary phytochemical screening of *Hibiscus radiates*

The crude methanol extract was subjected to photochemical screening using the method of [17] for the presence of secondary metabolites, which include alkaloids, steroids, phenols, tannins, saponins, flavonoids, cardiac glycosides and anthraquinones.

Acute toxicity screening

Acute toxicity screening was performed using the method described by [18]. The experimental animals were divided into seven groups of 4 animals each (n=4) each. The first group served as control and was treated with normal distilled water. Groups 2-4 were orally treated with the crude methanolic leaf extract at doses of 50, 150, 300mg/kg, respectively, while groups 5-7 received oral doses of 5, 20, and 100mg/kg, respectively. Observations were made for mortality and reaction signs at hourly and daily counts for 7 days.

Anti-snake venom activity screening

The anti-snake venom activity screening was based on the snake venom model for death records [19, 20]. The extract and standard anti snake venom (ASV) were administered orally using gastric canula and the treatment continued for seven consecutive days while the ASV was given only on the first day.

Antimicrobial activity screening

The bacterial strains were selected on the basis of the diseases against which the *Hibiscus radiatus* is locally used for. Testing of the plant extracts for antibacterial activity was done by the agar-disc diffusion method [21].

Anti-inflammatory Test

Carrageenan-induced hind paw edema method

Carrageenan-induced hind paw edema model was used for evaluating the anti-inflammatory activity [8]. The animals were fasted overnight prior to the experiment and were divided into six groups of four animals each: as detailed below: Group I: normal control rats treated with distilled water; Group II: carrageenan (1% w/v in 0.9% normal saline) induced animals as negative control; Group III: carrageenan-induced animals pretreated with methanol leaf extract of *Hibiscus radiatus* (50 mg/ kg); Group IV: carrageenan-induced animals pretreated with methanol leaf extract of *Hibiscus radiatus* (150mg/k); Group V: carrageenan-induced animals pretreated with methanol leaf extract of *Hibiscus radiatus* (300 mg/kg); Group VI: carrageenan-induced animals pretreated with the standard drug, indomethacin (50 mg/kg, diclofenac sodium). Acute inflammation was induced after an hour in groups II to VI on the left hind paw by sub plantar injection of 0.1 ml carrageenan (1% w/v) in 0.9% saline. After the administration of carrageenan, paw volume was measured using digital Plethysmometer (Ugo Basile, Italy). The readings were recorded for a total period of 4 hours and the percentage of paw edema inhibition was determined.

Acetic Acid Induced Writhing Method

The test models of [23] for nociception evaluation was employed in this method. Mice of either sex were divided into six groups with each consisting of four animals. Four groups were given different doses (50-500mg/kg) of the plant extract, while the control group was given a vehicle and the reference group was given 50 mg/kg of diclofenac sodium just one hour before 0.6% acetic acid (10 ml/kg, i.p) administration. Five minutes after acetic acid intraperitoneal (i.p.) administration, the number of writhes was counted to determine analgesic activity of the extract. The animals were individually placed in a glass jar and the contractions of abdominal muscles together with stretching of the hind limbs were cumulatively counted over a period of 30 minutes. The percentage protection against writhing was taken as an index of analgesia.

Hot Plate Method

In this test, mice of either sex were divided into six groups, each consisting of four animals. All animals were fasted overnight. Four groups were given different doses (50-500mg/kg) of extract of *Hibiscus radiatus* while one group was given a vehicle 0.9% normal saline as control per oral (p.o) and the other group was given standard drug diclofenac sodium as reference group (50 mg/kg, p.o). The animals were placed on a hot plate maintained at a temperature of 40°C. Before the treatment, the reaction time of each animal was recorded. The latency to lick the paw or jump from the hot plate was noted as the reaction time. The reaction times were noted at 30, 60, 90, and 120 min.

Tail immersion test

The procedure is based on the observation that morphine-like drugs selectively prolong the reaction time of the typical tail withdrawal reflex in mice. In this study, we used tramadol (50mg/Kg) as the reference drug. The test animals were treated with *Hibiscus radiatus* leaves extract at 250 mg/kg and 500mg/kg and the control group was treated with solvent. The tail of mice (1 to 2 cm) was immersed in warm water kept constant at 55°C. The reaction time was the time taken by the mice to deflect their tails. A latency period of 20 seconds was defined as complete analgesia and the measurement was then stopped to avoid injury to mice. The latent period of the tail-flick response was determined before and 0, 30, 60 and 90 minutes after the administration of drugs.

Results and Discussion

Results

Phytochemical screening of the crude methanol extract of the leaves of *Hibiscus radiatus* showed the presence of saponins, alkaloids, flavonoids, tannins, terpenoids, and phenols, mucillages and gums, and phytosterols (Table 1).

Table 1: Preliminary phytochemical screening of leaf of *Hibiscus radiates*

Phytochemicals	Remarks
Phytosterols	+
Terpenoids	+
Fixed oils and fats	
Saponins	+
Phenolic compounds	++
Tannins	++
Amino acids	+
Flavonoids	+
Gums and mucillages	++
Alkaloids	++
Carbohydrates	+

++ = Strong presence
+ = Likely presence
-- = weak or absent

The crude extract was administered to the animals up to a dose of 250mg/kg and their behavioral responses observed for 7 days for toxicity. The results are recorded in Figures 1 and 2.

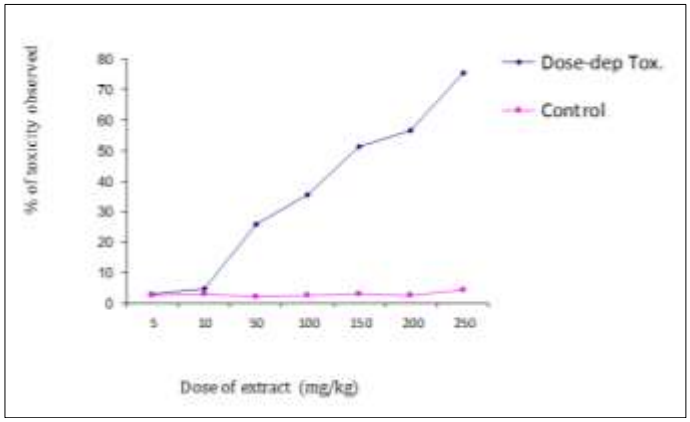

Fig 1: Dose dependent toxicity of *Hibiscus radiatus* leaf extract

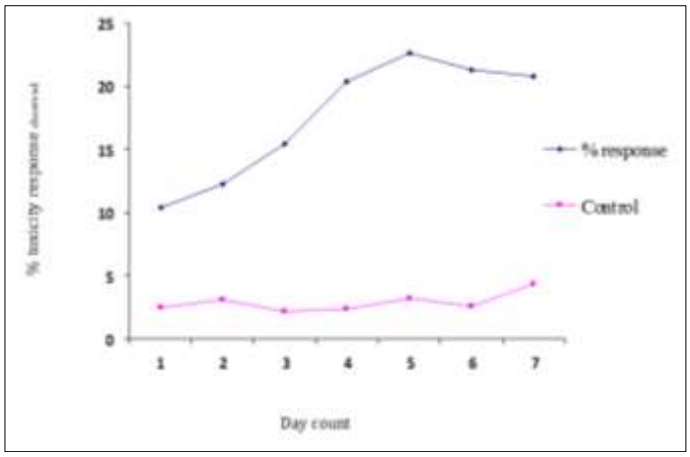

Fig 2: Toxicity pattern of *Hibiscus radiatus* leaf extract on day count basis

The results of the administration of various doses of *Hibiscus radiatus* (*H. radiatus*) leaf extract at doses 25mg/kg to 250mg/kg to albino mice injected with snake venom (SV) are shown in Table 2 and Figure 3.

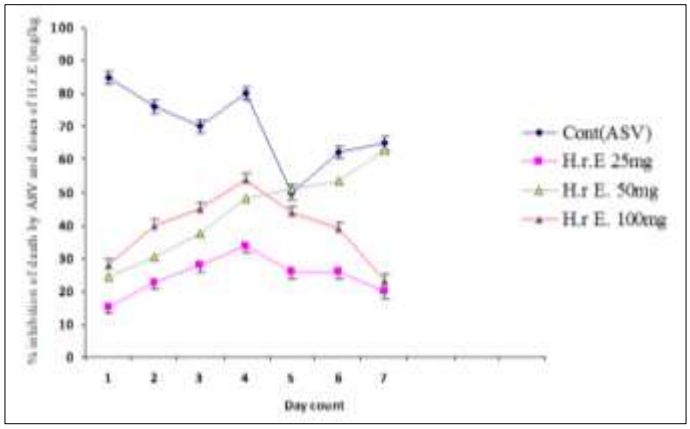

Fig 3: Inhibition of death by *Hibiscus radiatus* extract (H.r.E) of albino mice injected with snake venom (SV) with anti-snake venom (ASV) as control

Table 2: Inhibition of death in albino mice groups by day count using various concentrations of extract and SV compared with ASV control group

				Days			
Group	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Control ASV [2%v/v = 0.2mL]	4.90 ± 0.22	3.90 ± 022	3.16 ± 0.33	2.62 ± 012	2.00 ± 0.30	1.59 ± 0.23	1.44 ± 0.06
SV (2%v/v)+Extract(25mg/kg)	4.16 ± 023	3.05 ± 0.19	2.0 ± 0.22	1.62 ± 0.25	1.35 ± 0.22	1.09 ± 0.27	1.11 ± 0.21
% inhibition	15.25	22.82	27.91	33.87	25.93	25.89	20.11
Extract (50mg/kg)	1.89 ± 022	3.63 ± 0.18	2.99 ± 0.08	1.92 ± 0.10	1.77 ± 0.27	1.89 ± 0.22	1.17 ± 0.32
% inhibition	72.17	30.22	37.35	48.03	51.13	24.26	24.26
Extract (100mg/kg)	5.13 ± 0.07	3.96 ± 0.15	3.45 ± 0.14	2.27 ± 0.13	1.96 ± 0.09	2.97 ± 0.11	2.93 ± 015
% inhibition	28.03	39.76	45.03	53.61	43.91	38.91	20.55

Key: SV = Snake venom; **ASV** = Anti snake Venom (polyvalent)

The crude methanol extract and the isolate were subjected to antimicrobial screening. The results using Agar diffusion and Disc diffusion methods are shown in Table **3** and Figure **4**.

Table 3: Anti-bacterial activity of crude methanolic extract and the isolate (zone of inhibition in mm)

Test organisms	Agar diffusion		Disc diffusion	
	Extract	Isolate	Extract	Isolate
Escherichia coli	10.7±0.87	9.00±2.94	11.30±2.86	2.40±1.74
Enterobacter aerogenes	7.21±2.28	1.76±0.88	6.55±1.16	1.80±0.57
Enterobacter aerogenes	5.50±2.28	1.00±1.41	4.01±1.63	2.16±0.60
Staphylococcus aureus	2.04±0.86	1.82±0.65	1.00±0.81	1.26±0.73
Candida albicans	0.00±00	1.86±0.65	1.06±0.89	0.44±0.02

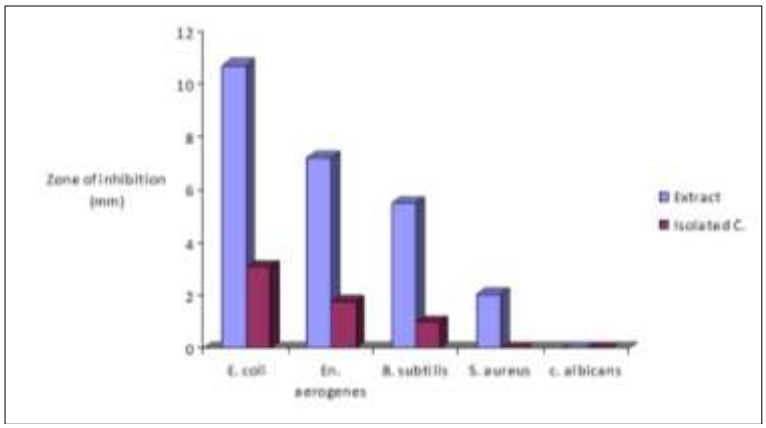

Fig 4: Antibacterial activity of crude methanolic extract and the isolate

The crude extract was investigated for analgesic and anti-inflammatory activities. The results are presented in Tables 4-6 and Figures 5-8.

Table 4: Anti-inflammatory activities of various extracts by carragennan paw edema method

Group	Paw volume(Mean ±S.E)	Change in Paw volume (mL)		
		Time (hrs).		
		1	2	3
Blank (0.9% D/S)	1.21±0.15	1.25±0.11	2.27±0.14	2.29±0.17
Dicl. Na+ (50mg/kg)	1.12±0.19	1.11±0.09	1.32±0.13	1.37±0.51*
Hr.M(250mg/kg)	1.31±0.17	1.21±0.15	1.43±0.12	1.55±0.19**
Hr.M (500mg/kg)	1.41±0.01	1.19±0.16	1.27±0.11	1.45±0.13**
Hr.PE (250mg/kg)	1.17±0.51	1.65±0.94*	1.75±0.16	1.95±0.06
Hr.PE (500mg/kg)	1.13±0.08	1.52±0.02	1.28±0.09	1.43±0.31*
Hr.C (250mg/kg)	1.66±0.02	1.92±0.01	1.62±0.17	1.79±0.45
Hr.C (500mg/kg)	1.53±0.37	1.68±0.12	1.74±0.31	1.88±0.16

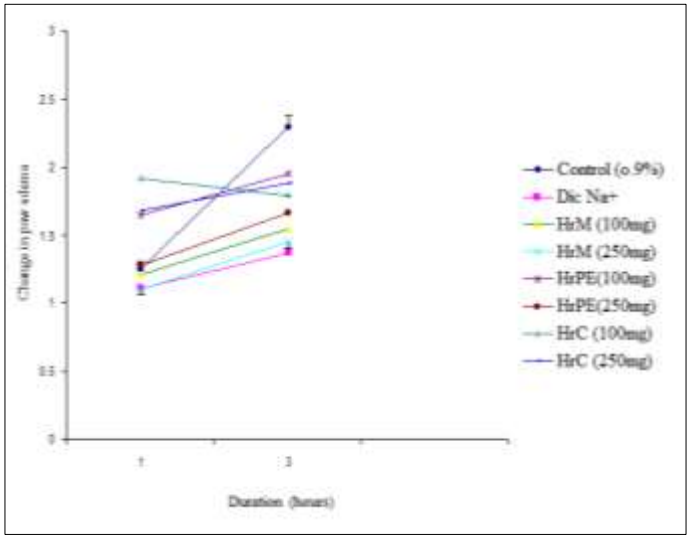

Fig 5: Inflammatory activities of various extracts using carragenan paw edema method

Table 5: Effect of various extracts of *Hibiscus radiatus* on acetic acid induced writhing albino mice

Group	Number of writhing (Mean ± S.E)	% Inhibition
Blank (0.9% D/S)	52.31 ± 0.16	0
Dicl. Na^+ (50mg/kg)	48.12 ± 0.53*	65.14
Hr.M (250mg/kg)	45.82 ± 0.17**	54.17
Hr.M (500mg/kg)	50.13 ± 1.12**	60.85
Hr.PE (250mg/kg)	12.55 ± 1.19	18.35
Hr.PE (500mg/kg)	18.41 ± 1.21	23.37
Hr.C (250mg/kg)	7.89 ± 1.05	9.17
Hr.C (500mg/kg)	14.54 ± 0.88	18.26

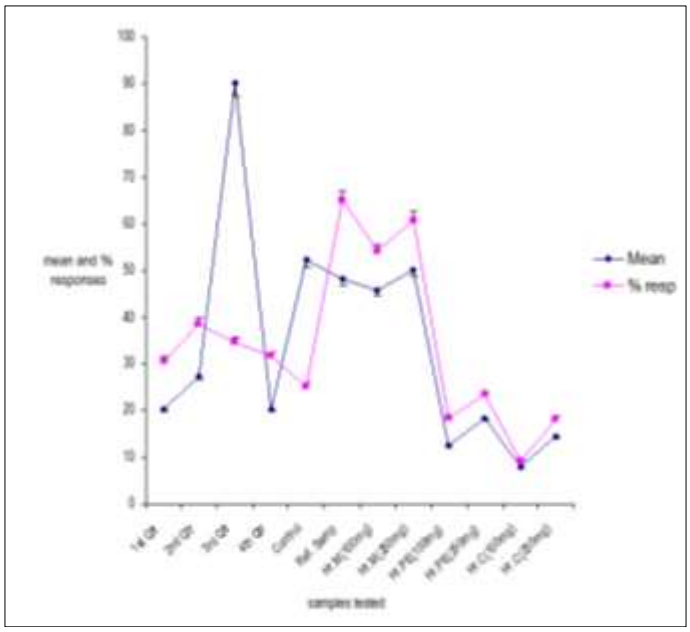

Fig 6: Analgesic and antinociceptive potency test of various extract using Hot plate method

Table 6: Analgesic and antinociceptive potency test of extract using hot plate method

Group	Basal reaction time (minutes) (Mean ± S.E)	Reaction time after drug admin. (Standard and Extract)			
		Time (Mean ± S.E)			
		15	30	60	120
Blank (0.9% D/S)	3.78 ± 0.14	3.82 ± 0.11	4.94 ± 0.13	5.05 ± 0.25	4.75 ± 0.15
Dicl. Na+ (50mg/kg)	3.81 ± 0.32	5.34 ± 0.23	6.47 ± 0.32	7.01 ± 0.36*	8.29 ± 0.13
Hr.M(250mg/kg)	3.26 ± 0.32	3.29 ± 0.40	3.36 ± 0.17	3.90 ± 0.27**	4.53 ± 0.21
Hr.M (500mg/kg)	3.32 ± 0.36	3.39 ± 0.24	4.47 ± 0.19	6.62 ± 0.17*	4.76 ± 0.17
Hr.PE (250mg/kg)	3.68 ± 0.25	3.55 ± 0.22	3.73 ± 0.09	6.02 ± 0.28	4.83 ± 0.31
Hr.PE (500mg/kg)	3.49 ± 0.32	4.02 ± 0.11	4.44 ± 0.18	7.39 ± 0.33*	4.67 ± 0.18
Hr.C (250mg/kg)	3.50 ± 0.41	3.63 ± 0.20	3.73 ± 0.16	5.53 ± 0.26	3.41 ± 0.27
Hr.C (500mg/kg)	3.33 ± 0.33	3.47 ± 0.12	2.57 ± 0.12	3.88 ± 0.12*	3.29 ± 0.19

Key: n = 6; ***p*<0.05 (significant); Hr = *Hibiscus radiatus*; Hr.M = treatment with methanol; Hr.PE = treatment with pet. Ether; Hr.C = treatment with chloroform

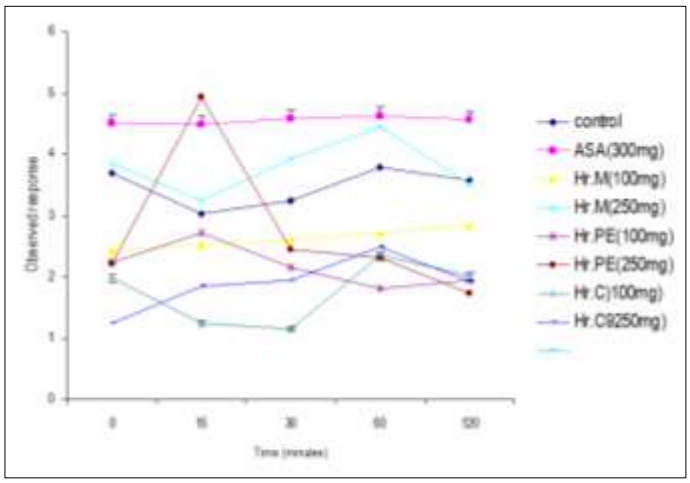

Fig 8: Antinociceptive activities of various extracts by tail immersion test

Discussion

Extraction of the leaves of *Hibiscus radiatus* with methanol gave the corresponding crude extract which was subjected to preliminary phytochemical screening. The results (Table 1) showed the presence of the following classes of phytochemicals: sterols, phenolics, saponins, tannins, flavonoids, alkaloids and carbohydrates. Some of these phytochemicals are known to exhibit anti- oxidant, anti-inflammatory and anti-tumor properties [24]. The results are in agreement with those previously reported for other species of *Hibiscus* with the predominant phytochemicals being alkaloids, phenolics and glucopyranosides [25, 26, 27]. Though, the mortality is low at lower doses, behavioral changes shown by the experimental animals indicated toxicity pattern of *Hibiscus radiatus* as illustrated in Figures 1 and 2. Normally, substances with LD_{50} range between 5,000 and 15,000 mg/kg are considered to be non-toxic [18]. Therefore, *Hibiscus radiatus* is considered to be toxic at the doses (<250 – 1500mg/kg) at which toxic reactions were observed.

The results of anti-snake venom screening are shown in Table **2** and Figure **3**. From the results the extract of *Hibiscus radiatus* produced mortality at doses ranging from 5mg/kg to 250mg/kg, and therefore confirmed the observation by the traditional healers that this species of *Hibiscus* is often avoided by cattle and most herbivores because of its poisoning effects [10]. The direct oral administration of methanolic extract of various concentrations delayed deaths by snake venoms in albino mice as % inhibition of death by *Hibiscus radiatus* leaf extract ranges from 50% to about 70% on day 3 to day 4 in groups of albino mice exposed to snake venom compared to the group exposed to snake venom and concomitant administration of polyvalent antisnake. Crude extract concentration of 2mg/kg exhibited the least inhibition potency against mortality. It should be noted that the venom at the dose 2.25mg/kg (LD_{99}) produces 100% death in the albino mice. The *Hibiscus radiatus* leaves extract increased mean survival time, but could not protect the animals from death when used alone. The plant extract when used alone at the dose of 100 g/kg was found to be more effective against the venom showing mean survival of 4.72±0.29 on day 1 when compared with 1.89±0.22 achieved at the dose of 25 g/kg. Thus, the anti-snake venom activity is concentration-dependent.

The *Hibiscus radiatus* leaf extract displayed potent antibacterial activities against *Escherichia coli* but its activity against other organisms was not significant as shown in Table 3. The results of *in vitro* antimicrobial activity study *of Hibiscus species* by some researchers [28, 29] support the results of the antibacterial studies with *Hibiscus radiatus* against some human pathogens in this work. The rather weak antibacterial potency of the isolated compound against the same clinical isolates suggests that it does not contribute to the antibacterial activity of the crude extract of *Hibiscus radiatus* leaves.

The inhibition of development of paw edema by the crude methanol extract of *Hibiscus radiatus* leaves against Carragennan-induced paw edema is as shown in Table 4 and Figure 5). The treatment with *Hibiscus radiatus* methanolic (Hr.M) extract and diclofenac sodium each significantly (p<0.005) inhibited the carragennan-induced rat paw oedema formation compared to petroleum ether (Hr.PE) and chloroform (Hr.C) leaves extracts at 100 and 250 mg/kg) measured at 1, 2, and 3 hr of experiment. From the results the methanol leaf extract generated a dose-dependent inhibition of edematous volume (25 and 250 mg/kg by 62.43 and 74.31%, respectively), which might perhaps be in part due to the synergetic action of phytochemical constituents present in it (Table 4).

Similarly, oral administration of methanolic leaves extract of *Hibiscus radiatus* at doses 100 and 250 mg/kg inhibited the writhing of albino mice by 54.17 % and 60.85%, respectively, as against petroleum ether extract which showed a low inhibition of 18.35 % and 23.37 % at the same doses. The chloroform extract, however, recorded the lowest and diminished number of writhings of 9.17 % and 18.26 % at the same doses (Table 5 and Figure 6). Animals tested with the leaves extract (100 and 250 mg/kg) of *Hibiscus radiatus* on the hot-plate presented a longer latency time than the control group, with the dose of 500 mg/kg provoking the longest latency (Table 6 and Figure 7. The hot-plate test is commonly used for assays of analgesics and antinociceptives. Tail withdrawal reflex time after administration of methanolic extract was found to be highest among the tested extracts (35.21 % and 69.83 % at doses of 250 mg/kg and 500 mg/kg) compared with the control (52.71% at a dose of 50mg/kg) (Table 8).

The reaction time with petroleum ether extract increased from 33.55 % to 39.61 % at doses of 250 and 500 mg/kg, respectively, while the chloroform extract tail withdrawal reflex time increased at 250 & 500 mg/kg dose with % reflex found to be 25.83 % and 45.45%, respectively. The screening for analgesic and anti-inflammatory revealed that though all extracts caused writhing responses as compared to control at a dose of 500 mg/kg, the significant (p<0.005) result was obtained with the methanolic extract.

Conclusion

This study has reported the presence of important phytochemicals. However, toxicity screening has revealed that the leaves of *Hibiscus* are very toxic, explaining why they are not eaten by cattle and other animals, including man. Also, revealed in this study are the results that showed that the leaves extract possesses significant anti-snake, analgesic, anti-inflammatory and ant-bacterial activities which may be attributable to the presence of some of the phytochemicals. The nature of the isolate from the biologically active methanolic fraction with anti-snake venom activity is still being investigated for structural characterization. Thus, the study for the first time has established that *Hibiscus radiatus* leaves extract significantly increases mean survival of death by snake venom in the groups of animals studied. Though the extract could not totally protect animals from snake venom investigated, the extract has antivenom activity against *Naja naja* snake venom with comparable result with polyvalent antivenom.

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