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Phytochemical Screening of Hemp and its Anti-Inflammatory Activity, Antibacterial Activity and Cytotoxic Activity

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ABSTRACT

Background and Objective: Hemp is one of the most popular plants commonly used by people since time immemorial due to its wide applications. It is a species of *Apocynum cannabinum*, of the family Apocynaceae reported to be effective in the treatment of nausea and vomiting and also strongly linked with cancer chemotherapy. The aim of this study was to investigate the phytochemical constituents of the methanolic crude extract of hemp and its anti-inflammatory, antibacterial and cytotoxicity activity from its polar and non-polar solvent extracts. **Materials and Methods:** Phytochemical screening of Hemp was carried out using GC-MS. Evaporation was done with a retovap. Antibacterial cultures were inoculated with Mueller-Hinton agar (MHA) using the disc diffusion method, lethality test was then carried out using Brine Shrimp (Artemia salina). After which carrageenan pedal inflammation was induced in rats. **Results:** Phytochemical screening of methanol crude extract of Hemp dogbane leaves revealed a total number of 100 chemical constituents among which are (E)-beta-famesene, caryophyllene, eucalyptol, caryophyllene oxide and tetrahydrocannabinol. The anti-inflammatory property of the crude extract of hexane, dichloromethane, chloroform and methanol extracts on carrageenan-induced paw reduced the incidence of paw oedema by 77.57, 69.66, 76.12 and 81.72%, respectively. The anti-bacterial screening showed a significant inhibition value, with higher inhibition obtained with methanol crude extract of 27.31±0.14 μg mL^{-1} on *Escherichia coli* at 500 μg mL^{-1} and the lower inhibition was observed at 50 μg mL^{-1} on *Salmonella typhi* 8.70±0.00 μg mL^{-1}, while the value of the mortality rate of larvae was 77.57, 69.66, 76.12 and 81.72%, respectively for the solvent crude extract. **Conclusion:** Hemp leaf extract showed high antibacterial, anti-inflammation and cytotoxic activities and thus added scientific information on the medicinal use of hemp.

KEYWORDS

Hemp, *Apocynum cannabinum*, phytochemicals, antibacterial, cytotoxicity, anti-inflammatory

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INTRODUCTION

Hemp dogbane is a species of *Apocynum cannabinum*, local to North America of the dogbane own circle of relatives Apocynaceae. It is a branched perennial plant that grows as much as 1.5 m tall with easy contralateral leaves and small greenish-white flowers[1]. The fruit is a pair of long, slender pods. The nodding bell-shaped, drooping fragrant flowers grow in terminal cymes and are pink outside, pink and white striped inside. Flowering occurs from late June through August[2]. Each flower produces two, brown, slender, sickle-shaped pods which may be 2 1/2 to 4 inches in length. The pods produce about 200 small, spike-shaped, reddish-brown seeds which have a tuft of soft, silky hairs at one end. The fibers from the stem bark are typically utilized by Indians for making bags, mats and nets[3]. The plant produces milky juice and latex rubber. All parts of the plant contain a milky juice. These components, the dried roots and associated components of the plant are utilized in making pills that act as coronary heart stimulant[4]. This plant dogbane differs from its close relative Indian Hemp (*A. cannabinum*) in that its leaves are mostly stalkless and the flowers are both in leaf axils and terminal.

Dogbane has been used to relieve dyspepsia, constipation, fever, gallstones and dropsy. It is also used in the treatment of liver disorders. Given in large doses, it is cathartic and emetic and may cause other symptoms of poisoning[4]. Dogbane is so named, they say because it is said to be poisonous to dogs[5]. When used, it is generally combined with less harsh medications suitable for the intended purpose. Hemp dogbane is used as medicine in the treatment of heart failure but even in small doses, it is dangerous[6]. Many researchers have reported that the chemical components of Hemp dogbane are medicinal, serving as a remedy for nausea and vomiting and additionally related to most cancers chemotherapy, anorexia and cachexia. The HIV/AIDS sufferers in addition to sufferers affected by neuropathic pains and spasticity were advised to apply these compounds[7-9].

In the past, Hemp dogbane was controlled by successive tillage operations, but with the advent of pre-emergence herbicides, mechanical methods of weed control are used less frequently[8]. Established Hemp dogbane is not susceptible to the common pre-emergence herbicides though seedlings may be controlled. As farmers increase their use of herbicides, they are decreasing the use of cultivation for weed control. Planting crops in narrow rows prevents a farmer from using mechanical weed control. This revolution in weed control practices is allowing Hemp dogbane to become a troublesome weed in many areas[9].

This study was aimed at ascertaining the phytochemicals present in Hemp dogbane leaves and their anti-inflammatory, antibacterial and cytotoxicity activity from polar and non-polar solvent extracts.

MATERIALS AND METHODS

Study area: The study was carried out in the Central Research Laboratory located at Federal University Wukari, Taraba State, Nigeria, between November and March, 2021.

Plant collection: The leaves of Hemp dogbane were purchased from the National Drug Law Enforcement Agency (NDLEA) in Nigeria. The plant samples (140 Hemp dogbane leaves) were shade dried for 10 days. The dried samples were ground to powder with the aid of mortar and pestle and taken to the laboratory for analysis.

Preparation of Hemp dogbane leaves extracts: The method of Audu *et al*.[10] was used in the preparation of methanolic extract of Hemp dogbane leaves. As 500 g of the leaves were soaked in 95% of four solvents of polar and nonpolar grade: Hexane, dichloromethane, chloroform and methanol for 72 hrs. The extracts were obtained by subjecting the mixtures to rotary evaporation to eliminate the solvent. The extracts were preserved in the fridge and later used for phytochemical screening, anti-microbial, anti-inflammatory and cytotoxic assays.

Phytochemical screening: Gas Chromatography-Mass Spectrometry (GC-MS) (version Clarus 680) was used to attain diverse chemical elements primarily based on the mass/charge (m/z) ratio of the chemical substances as described by researchers[11,12].

Antibacterial assay: The pathogens used were received from an expert medical institution Yola, Adamawa State and diagnosed in the Natural Product Research Laboratory Federal Housing Estate Bajabure, No. 10 Sanitation Road, Gerie, Adamawa State as *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia*. These bacterial cultures were inoculated on the floor of Mueller-Hinton agar (MHA) plates. Subsequently, on the surface of each inoculation plate, filter paper discs (6 mm diameter) saturated with extracts (25 µL) were inserted. The positive and negative controls used were chloramphenicol and 95% ethanol of the plant extract respectively. The checks had been executed in triplicates. At 37°C for 24 hrs, the plates were incubated. At the end of incubation, a transparent ruler was used to measure zones of inhibition. Zones susceptible to the extracts were zones of clearing greater than 6 mm.

Brine shrimp lethality assay

Preparation of brine shrimp: Artificial seawater was prepared by dissolving 40 g of sodium chloride (AR) introduced into one liter of distilled water.

Hatching the shrimp egg (*Artemia salina*): The synthetic seawater prepared earlier was used to fill a shallow oval-shape plastic container (35×15×10 cm). Small several shrimp eggs (*Artemia salina*) were scattered into the container, which was then covered with plastic cellophane and perforated with many holes and kept lighted by a fluorescent lamp for 48 hrs. After 48 hrs, hatched brownish orange nauplii larvae from the illuminated container were pipette out and transferred using a micropipette to a Petri dish with shallow saline water for later administration of the treatments.

Treatment of brine shrimp (*Artemia salina*): The experimental setup included six treatments, including a negative control of fake seawater (T1) and five dosages of powdered samples: 1 ppm (T2), 10 ppm (T3), 100 ppm (T4), 1000 ppm (T5) and 10000 ppm (T6). Each treatment was done in three replicates and the treatment duration lasted for 24 hrs in which 0.1 g of a powdered sample of Hemp leaves methanol extract was added to the first well and shaken by inverting the test tube. As 1 mL of the mixture was taken to be added to the succeeding test tubes in a tenfold dilution process. The pipette was used to transfer fifteen brine shrimp nauplii into each vial. During the treatment time, fluorescent light was used to illuminate the vials. Using a magnifying glass, the treated were counted macroscopically in the stem of the pipette against a bright backdrop to determine the number of dead and alive nauplii larvae.

Statistical analysis: Toxicity was determined by using a 3X magnifying lens to count the dead and alive nauplii larvae and computing the average % death of nauplii larvae for each treatment using the formula described by Lee *et al.*[13] and the computation of the lethal concentration (LC_{50}) was by using Probit Statistical Analysis by linear regression. The statistical difference was considered significant at $p<0.05$:

$$\text{Death (\%)} = \frac{\text{Death in treated tube or control tube}}{\text{Number of treated nauplii}} \times 100$$

Abbot's formula given below was used to correct the data gathered in cases where control deaths occurred:

$$\text{Death (\%)} = \frac{\text{Death in treated tube} - \text{Death in control tube}}{\text{Total death}} \times 100$$

Anti-inflammatory activity of carrageenan-induced rat paw oedema: The method described by Stark *et al*.[14] was used to induce carrageenan pedal inflammation in rats. Five groups of rats (n = 5) were formed. The animals in the test group were treated orally, 1 hr before carrageenan injection with 150 mg kg^{-1} of plant extracts. At the same time, the control group received 0.9% saline and the reference group received 150 mg kg^{-1} aspirin. An injection of 0.1 mL of 1% carrageenan was given into the right hind foot of each rat under the sub-plantar aponeurosis. The measurement of the increase in paw size was done immediately before and after 3 hrs following carrageenan injections. The inhibitory activity after 3 hrs was taken as a measure of paw oedema.

Ethical consideration: All ethical matters as concerned animal handling were observed following the animal ethical policies of the Department of Biochemistry, Federal University Wukari, Taraba State, Nigeria.

Statement of informed consent: Participants of this study provided their written informed consent to participate in this study.

RESULTS

The results of the phytochemical constituents of methanol crude extract of Hemp dogbane leaves revealed a total number of 100 chemical constituents among which are (E)-beta-famesene, caryophyllene, eucalyptol, caryophyllene oxide, tetrahydrocannabinol, (-)-globulol, tumerone, 3-ethyl-3-methyl heptane, cholest-22-ene-21-ol, 3,5-dehydro-6-methox, 3-tetradec-n-5-yne, (E)- and (6R,7R)-bisabolone. The n-nonadecanol-1 had the highest retention time (36.215) while Hydroperoxide,1-ethylbutyl had the lowest retention time (7.953) as shown in Table 1.

The LC_{50} of hexane, dichloromethane, chloroform and methanol crude extract of Hemp dogbane Leaves. Dichloromethane produced the highest LC_{50} rate (204.36) while hexane produced the lowest LC_{50} rate (48.95) shown in Table 2.

The effect of Hemp dogbane leaves crude extract of Aspirin, Hexane, Dichloromethane, Chloroform and Methanol on carrageenan-induced paw oedema rats. 150 mL kg^{-1} methanol produced the highest percentage inhibition rate (81.72%) while 10 mL kg^{-1} normal saline showed the lowest percentage inhibition rate (0. 005%). as shown in Table 3.

The effect of Hemp dogbane leaves extract (µg mL^{-1}) on Gram-positive and Gram-negative bacteria. It was observed that all the solvent extracts showed a significant inhibition value, with the highest inhibition (27.31±0.14) obtained with methanol crude extract of 500 µg mL^{-1} on *Escherichia coli* and the lowest inhibition (8.70±0.00)observed at 50 µg mL^{-1} hexane on *Salmonella typhi* as shown in Table 4.

Table 1: GC-MS phytochemical profile of methanol Hemp dogbane crude

Peak#	R.Time	Area	Height	Name
Peak Report TIC				
1	7.953	222500	64674	Hydroperoxide,1-ethylbutyl
2	8.219	156756	48186	Hydroperoxide,1-methylhexyl
3	8.964	108173	30712	beta.-Pinene
4	9.176	257191	64159	beta.-Myrcene
5	9.826	308222	67864	3-Carene
6	10.362	308528	81490	Benzene,1-methyl-3-(1-methylethyl)-
7	10.452	213994	56830	Cyclobutane,1,2-bis(1-methylethenyl)-,tran
8	10.580	1086380	239492	Eucalyptol
9	11.823	177990	39712	2-Furancarboxylicacid,tetrahydro-3-methyl-
10	12.144	87623	27032	Cyclopenta[c]pyran-1,3-dione,4,4a,5,6-tetra
11	12.572	616673	157330	Linalool

Table 1: Continue

Peak#	R.Time	Area	Height	Name
Peak Report TIC				
12	12.725	105343	27898	1,8-Octanediol
13	13.862	394139	97663	4-Ethyl-4-methyl-1-hexene
14	14.003	82806	23714	Bicyclo[3.1.1]heptan-3-ol,6,6-dimethyl-2-m
15	14.981	24292993	6118787	Bicyclo[2.2.1]heptan-2-ol,1,7,7-trimethyl-,(
16	15.142	5710206	1811162	3-Cyclohexen-1-ol,4-methyl-1-(1-methyleth
17	15.396	459367	151345	Benzamide,4-methyl-
18	15.517	443592	158565	Cycloundecanone
19	15.593	1543660	450854	.alpha.-Terpineol
20	16.246	122710	37205	1,6-Octadien-3-ol,3,7-dimethyl-,formate
21	17.122	113849	46543	4,7,7-Trimethylbicyclo[4.1.0]hept-3-en-2-on
22	17.318	640828	190555	Oxirane,decyl-
23	17.505	154014	49201	Benzaldehyde,4-methoxy-
24	17.570	55708	25409	2,3,6-Trimethylhept-3-en-1-ol
25	17.986	1910462	470346	trans-Ascaridolglycol
26	18.135	70334	16305	Pentanoicacid,5-hydroxy-,p-t-butylphenyl
27	18.378	485575	124519	1,2-15,16-Diepoxyhexadecane
28	18.529	795113	217316	trans-Ascaridolglycol
29	18.585	689642	194906	2-Cyclopenten-1-one,3,4-dimethyl-
30	18.656	808996	167366	5H-1-Pyrindine
31	19.226	245561	83589	1,3,6-Heptatriene,2,5,6-trimethyl-
32	19.294	298411	110742	Cyclohexene,4-ethenyl-4-methyl-3-(1-methy
33	19.806	517171	193954	Benzene propanoic acid, ethylester
34	20.176	195810	45581	2-Octenal,(E)-
35	20.302	266049	57149	p-Mentha-1,5-dien-8-ol
36	20.465	1447131	385036	Phenol,2-methoxy-4-(2-propenyl)-,acetate
37	20.605	169396	49955	1,3-Bis(cinnamoyloxymethyl)adamantine
38	20.685	108968	28433	Benzoic acid,3-methoxy-,methyl ester
39	20.809	1381791	436145	Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-
40	20.964	5959392	1851458	2-Propenoicacid,3-phenyl-,methyl ester
41	21.305	382049	131392	1H-Cycloprop[e]azulene,1a,2,3,4,4a,5,6,7b-
42	21.503	945008	177608	1,6,6-Trimethyl-8-oxabicyclo[3.2.1]octan-2-
43	21.634	282242	102595	(-)-Aristolene
44	21.710	955711	349913	Caryophyllene
45	21.846	507069	188408	.gamma.-Elemene
46	21.957	114997	22358	GermacreneD
47	22.200	407071	156619	Aromandendrene
48	22.295	94630	29742	(E)-.beta.-Famesene
49	22.352	75566	32895	Isoledene
50	22.432	204796	58029	S-(+)-5-(1-Hydroxy-1-methylethyl)-2-methyl
51	22.659	525248	191233	Humulene
52	22.772	403126	88328	Aromandendrene
53	22.945	222277	58142	Cyclooctane, methyl-
54	23.271	145151603	10340067	2-Propenoicacid,3-phenyl-,ethylester
55	23.364	42113022	10650405	2-Propenoic acid,3-phenyl-,ethylester,(E)-
56	23.444	13502769	5478567	Heptadecane
57	23.567	845195	203782	Bicyclo[3.1.1]hept-2-ene,2,6-dimethyl-6-(4-
58	23.672	733383	209969	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexah
59	23.746	534210	154402	.alpha.-Guaiene
60	23.873	2866308	684911	(1S,2S,4S)-Trihydroxy-p-menthane
61	24.126	1627766	591693	.beta.-copaene
62	24.181	2992297	710944	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexah
63	24.337	358455	135693	Cycloisolongifolene,8,9-dehydro-
64	24.403	387319	144804	Cycloheptane,4-methylene-1-methyl-2-(2-m
65	24.558	2119362	704372	(3S,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-di
66	24.980	3492154	1286044	Cyclohexanemethanol,4-ethenyl-.alpha.,.alp

Table 1: Continue

Peak#	R.Time	Area	Height	Name
Peak Report TIC				
67	25.091	379745	93587	Tetrahydrocannabinol
68	25.250	429633	140089	Ledol
69	25.404	168628	68277	1H-Cycloprop[e]azulen-4-ol,decahydro-1,1,
70	25.611	728673	162957	cis-Thujopsene
71	25.720	107654	43276	(-)-.beta.-Bourbonene
72	25.788	369088	133740	1H-Cycloprop[e]azulen-7-ol,decahydro-1,1,
73	25.912	1546337	481650	Caryophylleneoxide
74	25.998	1210748	334816	(-)-Globulol
75	26.220	9537793	2884756	1H-Cycloprop[e]azulen-4-ol,decahydro-1,1,
76	26.453	1353297	437396	Ledol
77	26.692	6379553	1634139	Apiol
78	26.793	1816811	606628	(2E,4S,7E)-4-Isopropyl-1,7-dimethylcyclode
79	26.890	3204078	431669	Selin-6-en-4.alpha.-ol
80	27.267	1449200	336012	.tau.-Cadinol
81	27.459	1078318	227391	cis-7-Dodecen-1-ylacetate
82	27.675	7373907	1055562	aR-Turmerone
83	27.781	9732533	2530917	Tumerone
84	27.921	982628	283247	Neointermedeol
85	28.095	5219535	945405	Methylp-methoxycinnamate,cis
86	28.554	3559740	1255685	Curlone
87	28.644	383657	117218	1-Naphthalenol,decahydro-1,4a-dimethyl-7-
88	28.800	169360	24598	Tumerone
89	28.921	189552	57096	3-Ethyl-3-methyl heptane
90	29.010	76219	20541	Cholest-22-ene-21-ol,3,5-dehydro-6-methox
91	29.350	80047	14015	3-Tetradecen-5-yne,(E)-
92	29.468	116515	43744	(6R,7R)-Bisabolone
93	29.570	77487	18388	Isoamyl cinnamate
94	30.454	321804006	10887380	Ethylp-methoxycinnamate
95	31.915	258352	36900	9-Hexadecyn-1-ol
96	32.139	428362	104396	3-Methoxycinnamicacid
97	33.792	213963	44026	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione
98	34.241	470411	29885	(E)-3-Methyl-5-((1R,4aR,8aR)-5,5,8a-trimet
99	35.615	274346	64135	2-Propenoic acid,3-(4-methoxyphenyl)-,2-e
100	36.215	391704	62766	n-Nonadecanol-1
		656418560	72994384	

Table 2: Brine shrimp lethality assay on a crude extract of hexane, dichloromethane, chloroform and methanol from Hemp dogbane leaves

	Percentage mortality of different concentrations (µg mL^{-1})					
Crude extract	10	100	1000	10,000	100, 000	LC_{50}
Hexane	100	68.44	62.47	54.34	49.37	48.95
Dichloromethane	100	59.67	53.21	48.36	39.46	204.36
Chloroform	100	77.12	69.27	62.34	48.13	58.32
Methanol	100	79.18	68.37	60.11	37.16	53.39

Table 3: Effects of Hemp dogbane leaves crude extract of hexane, dichloromethane, chloroform, methanol and aspirin on carrageenan-induced paw oedema rats

Group	Dose (mL kg^{-1})	Change in paw size (cm)	Inhibition of paw thickening (%)
Normal saline	10 mL kg^{-1}	0.96±0.29	0.005
Aspirin	150 mL kg^{-1}	0.19±0.09	81.45
Hexane	150 mL kg^{-1}	0.28±0.12	77.57
Dichloromethane	150 mL kg^{-1}	0.29±0.11	69.66
Chloroform	150 mL kg^{-1}	0.28±0.07	76.12
Methanol	150 mL kg^{-1}	0.19±0.03	81.72

Data are Means±SD of triplicate determinations, N = 5, values are Mean±SD and $p<0.05$ is considered significant

Table 4: Effect of Hemp dogbane leaves extract on Gram-positive and Gram-negative Bacteria

Conc. (µg mL^{-1})	Organism	Chloramphenicol	Hexane	DCM	Chloroform	Methanol
50	*Salmonella typhi*	20.77±0.03	8.70±0.00	9.63±0.15	8.67±0.06	10.00±0.20
	Escherichia coli	19.79±0.06	14.83±0.06	15.03±0.06	15.07±0.06	18.70±0.00
	Staphylococcus aureus	21.16±0.11	12.93±0.15	13.90±0.10	12.80±0.10	13.60±0.00
	Klebsiella pneumonia	20.76±0.18	10.73±0.06	10.70±0.00	10.70±0.17	12.80±0.10
100	*Salmonella typhi*	20.77±0.03	10.73±0.06	9.67±0.15	10.73±0.21	11.93±0.06
	Escherichia coli	19.79±0.06	17.60±0.00	16.50±0.00	18.73±0.06	20.80±0.10
	Staphylococcus aureus	21.16±0.11	15.97±0.06	14.00±0.10	13.90±0.20	14.83±0.06
	Klebsiella pneumonia	20.76±0.18	11.80± 0.10	11.77±0.06	10.83±0.06	13.77±0.21
250	*Salmonella typhi*	20.77±0.03	13.83±0.12	14.93±0.15	12.77±0.66	14.70±0.20
	Escherichia coli	19.79±0.06	19.73±0.06	20.70±0.10	21.83±0.06	23.03±0.06
	Staphylococcus aureus	21.16±0.11	15.03±0.06	15.10±0.10	15.03±0.12	16.03±0.06
	Klebsiella pneumonia	20.76±0.18	11.87±0.06	10.97±0.06	11.03±0.12	13.97±0.06
500	*Salmonella typhi*	20.77±0.03	13.87±0.23	16.77±0.12	15.87±0.15	17.99±0.06
	Escherichia coli	19.79±0.06	24.73±0.06	24.93±0.06	26.00±0.10	27.31±0.14
	Staphylococcus aureus	21.16±0.11	17.10±0.10	18.23±0.06	15.03±0.06	16.06±0.06
	Klebsiella pneumonia	20.76±0.18	12.97±0.06	13.00±0.10	14.13±0.06	16.07±0.06

*Data are Means±SD of triplicate determinations, values are Mean±SD and $p<0.05$ is considered as significant

DISCUSSION

The results of the phytochemical constituents of methanol crude extract of Hemp dogbane leaves revealed a total number of 100 chemical constituents among which were (E)-beta-famesene, caryophyllene, eucalyptol, caryophylleneoxide, tetrahydrocannabinol, (-)-globulol, tumerone, 3-ethyl-3-methylheptane, cholest-22-ene-21-ol, 3,5-dehydro-6-methox, 3-tetradecen-5-yne, (E)- and (6R,7R)-bisabolone (Table 1). Most medicinal plants possess a variety of bioactive chemicals, most of which are flavonoids, alkaloids and phenolics[15]. Flavonoids are made up of natural substances with different phenol groups found mainly in vegetables and some grains, stems and flowers. They are well known for their valuable health benefits, especially for their antioxidant, antimutagenic, anti-inflammatory, anti-cancer and enzyme-regulating properties[16]. The presence of bioactive compounds such as flavonoids, saponins and phenolics in Hemp dogbane leaves may be responsible for the anti-inflammatory effects of plants. This is in tandem with the findings of Ameh *et al.*[17]. Both reported that the flavonoid glycosides showed modulation in calcium transport in isolated inflamed rat liver, thereby showing a reduction in inflammation.

In this study, the result for anti-inflammatory propertiesof Hemp dogbane leaves crude extract of hexane, dichloromethane, chloroform and methanol extracts on carrageenan-induced paw oedema in rats was presented in Table 3. The solvent extracts and aspirin were found to inhibit paw oedema in rats. Aspirin had an inhibition value of 81.4%. The extracts from the polar and non-polar solvents reduced the incidence of paw oedema in rats as follows: Hexane (77.57%), Dichloromethane (69.66%), Chloroform (76.12%) and Methanol (81.72%). A marked anti-inflammatory activity was produced by methanol and all other solvent extracts. They reduced the size of pedal swelling induced by carrageenan in the rats. This was in consonance with the report of Owoyele *et al.*[18]. The implication of this was that Hemp dogbane leaves have significant potential as an agent for malignant cells.

The effect of Hemp dogbane leaves extract (µg mL^{-1}) on certain bacterial species, *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia* for the following solvents: Hexane, dichloromethane, chloroform and methanol was presented in Table 4. From the results, it was observed that all the solvent extracts showed a significant inhibition value, with the highest inhibition obtained with methanol crude extract of 27.31±0.14 on *Escherichia coli* at 500 µg mL^{-1} and the lowest inhibition (8.70±0.00) was observed at 50 µg mL^{-1} on *Salmonella typhi*. This agreed with the report of Umaru*et al.*[11], Okwu and Iroabuchi[19] and Amar[20] who observed particularly that the extract of methanol solvent has higher effects on pathogens at a higher concentration.

This study revealed the anti-inflammatory activity, antibacterial activity and cytotoxic activity of Hemp dogbane crude extracts. The bioactive compounds present in Hemp dogbane crude extracts were also revealed using phytochemical screening. These discoveries have revealed the potential use of Hemp dogbane as traditional medicine.

A limitation of this research work is that possible effects that may result from prolonged use of Hemp dogbane were not looked into. However, researchers interested in this field may explore this gap.

CONCLUSION

The results obtained from this study revealed the chemical constituents of Hemp dogbane crude extracts and their potential as anti-inflammatory and antibacterial substances against four pathogenic bacteria, *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia*. The phytochemical screening of Hemp dogbane methanol crude extract showed a high amount of alkaloids and flavonoids which are most likely responsible for the antibacterial and anti-inflammatory potential of the plant. This proved added scientific information to the use of Hemp dogbane as traditional medicine.

SIGNIFICANCE STATEMENT

This study discovered the active compounds present in Hemp dogbane leaves that can be beneficial in traditional medicine as they possess anti-inflammatory and antibacterial activities against certain bacterial species. This study will help researchers to uncover the critical areas of phytomedicine associated with the Hemp dogbane plant that many researchers were not able to explore. Thus a new theory on drug discovery may be arrived at.

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