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Research Article

Antioxidant, Anti-inflammatory, and Anticancer Potentials of Ethyl acetate Extract of *Andira inermis* Stem Bark

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ABSTRACT

Inflammation is a prominent factor implicated in many diseases. Cancer being one of such disease is the second leading cause of death globally. In this study, the antioxidant activity of different extracts of *Andira inermis* stem bark was investigated. Further, the ethyl acetate extract was studied for anti-inflammatory and anticancer activities. Successive extraction of *A. inermis* stem bark with different solvents of increasing polarity gave significantly ($p < 0.05$) different extracts yields between 3.94 g (1.31%) to 21.66 g (7.22%). Ethyl acetate had a significant amount of total phenolics (133.28 ± 3.20 mg GAE/g) and flavonoids (117 ± 2.96 mg QE/g) when compared to other extracts. The ethyl acetate extract showed significant antioxidant activity using DPPH and ABTS assays with IC_{50} of 56.11 µg/mL and 35.13 µg/mL respectively. Carrageenan administration resulted in a significant increase in the paw thickness of rats compared to control. Ethyl acetate extract treatment gave a significant reduction in paw size from 1st to 5th hour compared to the negative control group. Treatment of HepG-2 and MCF-7 cell lines with different concentrations of ethyl acetate extract produced IC_{50} values of 247.26 µg/mL and 365 µg/mL respectively. The antioxidant, anti-inflammatory and anticancer activities of ethyl acetate extract were evidently due to the high total phenolics and flavonoids. The ethyl acetate extract of *A. inermis* could be used as a potential source of antioxidant, anti-inflammatory, and anticancer agents.

Keywords: *Andira inermis, antioxidant, anti-inflammatory, anticancer*

INTRODUCTION

Medicinal plants have been used by man since time immemorial to solve common health challenges. This is because plant-based traditional medicines are readily accessible and cheaper when compared to orthodox medicines. Despite the scientific and technological advancements in medicine, many people in the world still rely on traditional medicine for their health needs (Kale *et al.*, 2019; Ugwah-Oguejiofor *et al.*, 2019). Plant materials are also utilized in large quantities in cosmetics, as food preservatives, as nutritional supplements, and as natural raw materials in many pharmaceutical industries (Shadid *et al.*, 2021). The phytochemical analysis of medicinal plants gives an idea of the chemical nature of compounds with bioactive principles responsible for their diverse pharmacological activities (Afzal *et al.*, 2022). These bioactive *compounds* like alkaloids, flavonoids, tannins, phenolics and a wide diversity of others are potent phytochemicals that directly affect human metabolism (Sher *et al.*, 2014; Junior *et al.*, 2019). The extraction and purification of phytochemicals from plants with antioxidant activity are generally affected by factors such as solvent concentration and solvent polarity (Nawaz *et al.*, 2020).

Antioxidant, anti-inflammatory, and anticancer activities are notable pharmacological actions of many medicinal plants. Antioxidants are known to scavenge free radicals and form non-toxic ions or molecules (Ding *et al.*, 2021). Recently, much attention has been generated among scientists by studying antioxidants and their effects on harmful free radicals that cause oxidative stress (Shadid *et al.*, 2021).

Inflammation is the body's response against foreign substances such as pathogens, allergens, and chemical irritants as well as an injury that involves infiltration of leukocytes and generation of pro-inflammatory factors to the injured site. Inflammation helps in mitigating the effects of harmful irritation and allows the injured tissue to recover to normal condition (Li *et al*., 2020). Uncontrolled chronic inflammation is responsible for several disorders such as atherosclerosis, rheumatoid arthritis, ischemic heart disease, and many others (Wahid *et al*., 2021). Commonly used drugs for the treatment of inflammation are non-steroidal anti-inflammatory drugs (NSAID) and corticosteroids. Continuous use of these drugs in the management of inflammation is associated with toxicity and side effects. There is a need to explore medicinal plants as potential new sources of compounds for the development of an alternative to the earlier mentioned drugs in the management of inflammation.

Globally cancer is a leading cause of mortality accounting for nearly 10 million deaths in 2020 (Ferlay, *et al*., 2020). Liver and breast cancers are common among the leading cancers in men and women respectively. Cancer exerts tremendous physical, emotional, and financial burdens on families, and these burdens continue to increase (Charles-Okhe *et al*., 2022). The use of synthetic drugs in the management of cancer is not only expensive but accompanied by serious side effects.

Andira inermis (wright) D.C belongs to the family *Fabaceae* and is commonly called cabbage tree (Ken, 2014). It is an evergreen tree used for different purposes in traditional medicine among the people of North-East Nigeria. The fruits of the plant are eaten, the stem bark is used as a vermifuge, narcotic and purgative, while the inner stem bark is used to treat snake bites (Ken, 2014). Some traditional medicine practitioners in North-Eastern Nigeria use the stem bark in the management of cancer. Recent reported pharmacological activities of the plant include hypoglycaemic and antioxidant activity (Egua *et al*., 2020) and antibacterial activity (Adebisi *et al*., 2021). A detailed search in the literature revealed that no previous studies were carried out to investigate the anti-inflammatory and anticancer activities of *Andira inermis* stem bark extract. The objectives of the current study were to determine the phytochemical constituents, antioxidant activity, total phenols, and flavonoids of different extracts of *Andira inermis* stem bark. The ethyl acetate extract with the highest antioxidant activity was used to determine the anti-inflammatory and anticancer activities to provide a scientific basis for the complementary or alternative use of *Andira inermis* as anti-inflammatory and anticancer agents.

MATERIALS AND METHODS

Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), gallic acid, Folin-Ciocalteu reagent, trichloroacetic acid (TCA), dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyl-2H-tetrazolium bromide (MTT), HepG-2 and MCF-7 cancer cell lines were purchased from Sigma-Aldrich (Germany). All other chemicals and reagents used were of analytical grade.

Collection of plant material

The stem bark of *Andira inermis* was collected in March, 2020 in Talasse, Balanga Local Government Area, Gombe State, Nigeria. The plant material was identified and authenticated by a Botanist in the department of Plant Science, Modibbo Adama University of Technology, Yola. The Plant material was assigned a voucher number PL0722DD/2020.

Preparation of extracts

The stem bark of *Andira inermis* was dried under a shed at room temperature (30 ± 3 ^{0}C) for 14 days. The dried stem bark was powdered using a pestle and mortar then an electric grinder and made to pass through a 0.5 mm mesh sieve. The extraction was carried out as described by Nawaz *et al*. (2020) with slight modification. The dried powder material (300 g) was macerated in 2 L hexane in a 4 L container and properly covered with intermittent shaking for 48 hours at room temperature. Thereafter, the mixture was filtered through Whatman No. 1 filter paper and concentrated. The residue obtained after extraction in hexane was further extracted for 48 hours consecutively with 2 L each of dichloromethane, chloroform, ethyl acetate, methanol, and water. The various solvents filtrate were all concentrated under reduced pressure using a rotary evaporator at 50 ^{0}C. Water filtrate was freeze-dried to produce the water extract. Each solvent extract was weighed and the percentage of total extractable components (TEC) was calculated using the formula:

$$\text{TEC (\%)} = \frac{Weight\ of\ extract}{Weight\ of\ sample}\ x\ 100$$

Phytochemical analysis

In vitro phytochemical screening

The qualitative phytochemical screening was carried out on the extracts using standard methods. Presence or absence of alkaloids, flavonoids, phenols, saponins, tannins and terpenoids were tested following the procedure by Sofowora, (1993). Anthraquinones and anthocyanin were tested as described by Trease and Evans, (2002). While cardiac glycosides and phlobatannins were tested based on the procedures described by Ajayi *et al*. (2011).

Quantitative phytochemical screening

Determination of total phenolics

The Folin-Ciocalteueu method reported previously by Badrulhinormal *et al*. (2020) with little modification was adopted. Briefly, 0.5 ml of the extract in DMSO (mg/mL) was mixed with 2.5 mL of 10% aqueous solution of Folin-Ciocalteu reagent. The mixture was left to stand for 5 min and 2.0 mL of 5% aqueous solution of Na_2CO_3 was added. The mixture was stirred and incubated for 1 hour in the dark at room temperature. Thereafter, the absorbance was measured at 765 nm using a UV-Vis spectrophotometer. Gallic acid was used as standard (0.1-5 mg/mL) and methanol as blank. Results were expressed as mg gallic acid equivalent per gram (mg GAE/g).

Determination of flavonoids

The aluminium chloride method described earlier (Subhasree *et al*., 2009) with slight modification was used to determine the flavonoid contents of the extracts. Each extract of 0.1 g was dissolved in 20 mL methanol and then filtered using Whatman filter paper No. 1. An aliquot of the filtrate 0.5 mL was mixed in a test tube with 3 mL of distilled water and 0.3 mL of 0.5% sodium nitrate. The mixture was allowed to incubate at room temperature for 5 min. After incubation, 0.6 mL of 10% aluminium chloride was added and allowed to stand for 6 min followed by the addition of 2 mL of 1 M sodium hydroxide. The volume of the entire mixture was made up to 10 mL with distilled water. Absorbance was read at 510 nm using a UV-Vis spectrophotometer. Flavonoid contents of the extracts were calculated using the quercetin standard calibration curve and expressed as mg quercetin equivalent per gram (mg QE/g).

Determination of DPPH radical scavenging activity

The free radical scavenging activity of the extracts was determined using the 2, 2-diphenyl-1-picrylhydrazy (DPPH) method described by (Dudonne *et al*., 2009). DPPH in oxidized form gives deep violet colour in methanol which becomes yellow when reduced with an antioxidant. A 3.0 mL of freshly prepared 0.002% solution of DPPH was added to 1 mL of different concentrations of extracts (20-100 µg/mL in appropriate solvent) in a test tube and mixed. The mixture was allowed to incubate at room temperature in the dark for 20 min after which the absorbance was measured at a wavelength of 517. The absorbance of the DPPH solution (without extract) was taken as a control. Measurements were carried out in triplicate and gallic acid as standard. The percentage inhibition of DPPH by extracts was calculated using the formula below. The extract concentration that inhibited 50 percent (IC_{50}) of the radical was calculated from a linear regression plot.

$$\% \text{ inhibition} = \frac{Abs.\ of\ blank - abs.\ of\ sample}{Abs.\ of\ blank}\ x\ 100$$

Determination of ABTS radical scavenging assay

ABTS radical scavenging assay was conducted as described (Arteeaga-Crespo *et al*., 2020) with slight modification. ATBS solution was prepared by reacting 5.0 mL of 7 mM ATBS and 88 mL of potassium persulfate in deionized water. The mixture was allowed to stand in the dark for 14 hours at room temperature to give a blue solution. Thereafter, 250 mL of 99.5% ethanol was added to the mixture to produce the $ABTS^+$ solution which gave an absorbance of 0.7 at 734 nm. To determine the ABTS radical scavenging activity, 3.0 mL of ABTS solution was mixed with 100 µL of different concentrations of extracts (20-100 µg/mL) and incubated at 37 ^{0}C for 10 min in the dark. The absorbance was measured at a wavelength of 734 nm using a UV-Vis spectrophotometer. Trolox was used as a standard and measurements were carried out in triplicates. The percentage inhibition of ABTS radicals was calculated using the formula:

$$\% \text{ inhibition } = \frac{Abs.\ of\ blank - abs.\ of\ sample}{Abs.\ of\ blank}\ x\ 100$$

Determination of anti-inflammatory activity

The anti-inflammatory effect of ethyl acetate extract of *Andira inermis* stem bark was evaluated as described (Omowunmi *et al*., 2017) with slight modification. Briefly, 30 male rats were divided into 6 groups of 5 rats each. Group I and II received 1 mL physiological saline each as normal and negative controls respectively, Group III received 100 mg/kg bw aspirin by gavage as standard drug control. Groups IV, V and VI were treated with 50, 100 and 200 mg/kg bw of ethyl acetate extract of *Andira inermis* respectively. Inflammation was induced in Groups II, III, IV, V and VI, 1 hour after the treatments by injecting into the sub plantar tissue of the right hind paw, 1% carrageenan suspension in 0.9% NaCl (0.1 mL). Thereafter, the diameter of the hind paw was measured at intervals of 1, 2, 3, 4 and 5 hours and compared to the diameter of the paw of normal control. The percentage inhibition of inflammation was measured by the change in paw size as calculated using the formula:

$$\% \text{ inhibition of oedema} = \frac{T - T_o}{T} \text{ x } 100$$

Where T is the thickness of the paw in the negative group and T_o is the thickness of the paw in the treated and negative groups at a particular time after induction of inflammation.

Determination of anticancer activity

The method previously published by Paudel *et al.* (2019) was used with slight modification for the anticancer activity of the ethyl acetate extract against human hepatocellular

carcinoma (HepG2) and human breast carcinoma (MCF-7). The cells were sub-cultured in an RPI-1640 medium containing 10% foetal bovine serum (FBS), 2 mM L-glutamine, penicillin (100 U/L), and streptomycin (100 µL) and incubated at 37 ^{0}C, 5% CO_2 in a humidity incubator for 48 h in the dark. The cell suspension, 5 x 10^3 cells (100 µL) were seeded into a 96-well cell culture plate and added different concentrations (25, 50, 100, 200, and 400 µg/mL) of the extract in triplicate then re-incubated for 24 h. The extract was removed followed by the addition of 100 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) solution (0.5 mg/mL in PBS) and re-incubated for 4 h. MTT was then discarded and 100 µL dimethyl sulfoxide (DMSO), was added to each well and placed on an orbital shaker for 30 min to dissolve formazan crystals. The absorbance of the dissolved formazan crystal was read at 595 nm using a microplate reader. Cisplatin was used as a standard drug control. Percentage cell viability was calculated using the formula (a) while inhibition of cell growth was calculated using (b):

Cell viability (%) = $\frac{Absorbance\ of\ treated}{Absorbance\ of\ control}\ x\ 100$-------- (a)

% inhibitory activity = ($[\frac{Absorbance\ of\ control}{Absorbance\ of\ treated} - 1]\ x\ 100$) ------- (b)

RESULTS

Extraction of plant material

Successive extraction of the stem bark of *Andira inermis* with hexane, dichloromethane, chloroform, ethyl acetate, methanol, and water yielded different amounts of extracts ranging between 3.94 g (1.31%) to 21.66 g (7.22%) as shown in Table 1. Methanol gave the highest yield followed by ethyl acetate while hexane had the lowest yield. The different yields of the extracts were significantly different ($p < 0.05$) when compared to each other except between dichloromethane and chloroform extracts.

Phytochemical content

Phytochemical constituents of the different solvent extracts are given in Table 2. The phytochemicals present include alkaloids, cardiac glycosides, flavonoids, phenols, Phlobatannins, saponins, tannins, terpenoids, and anthraquinones, while anthocyanins was absent in all the extracts. Alkaloids was absent in hexane, dichloromethane, and chloroform extracts while cardiac glycosides was absent in hexane and dichloromethane extracts. Saponins were found to be present in methanol and water extracts only.

Total phenolics and flavonoid contents

Quantitative determination of total phenols and flavonoids of the extracts gave different results, which were significantly different when compared with each other (Table 3). Ethyl acetate extract produced significant amounts of both total phenols (133.28 ± 3.20 mg GAE/g) and flavonoids (117 ± 2.96 mg QE/g) when compared to other extracts.

Table 1. Different Solvent Extraction Yields of *Andira inermis* Stem Bark

Solvent	Yield (%)
Hexane	3.94 ± 0.12^e (1.31)
Dichloromethane	9.33 ± 0.21^d (3.11)
Chloroform	10.39 ± 0.04^d (3.46)
Ethyl acetate	17.74 ± 0.19^b (5.91)
Methanol	21.66 ± 0.30^a (7.22)
Water	12.81 ± 0.21^c (4.27)

Values are mean ± SEM. Different superscripts indicate significance ($p < 0.05$) when means of solvents yields are compared.

Table 2. Phytochemical Constituents of Different Extracts of *Andira inermis* Stem Bark

Constituents	Extracts					
	HE	DCM	CLM	EA	MTH	WR
Alkaloids	–	–	–	+	+	+
Anthraquinones	–	–	–	–	–	–
Anthocyanin	–	–	–	–	–	–
Cardiac glycosides	–	–	+	+	+	+
Flavonoids	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Phlobatannins	+	+	+	+	+	+
Saponins	–	–	–	–	+	+
Tannins	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+

(+) indicate presence and negative (–) absence. He: Hexane, DCM: Dichloromethane, CLM: Chloroform, EA: Ethyl acetate, MTH: Methanol, WR: Water.

Table 3. Total phenols and Flavonoids Contents of *Andira inermis* Stem Bark Extracts

Solvent	Phenolics (Mg GAE/g)	Flavonoids (mg QE/g)
Hexane	61.72 ± 2.05[d]	37.75 ± 1.55[d]
Dichloromethane	111.98 ± 4.41[b]	68.13 ± 1.25[c]
Chloroform	80.67 ± 3.83[c]	62.23 ± 2.28[c]
Ethyl acetate	133.28 ± 3.20[a]	117.85 ± 2.96[a]
Methanol	119.77 ± 2.64[b]	88..10 ± 1.46[b]
Water	32.20 ± 1.82[e]	24.35 ± 0.56[e]

Values are mean ± SEM for triplicate determinations. Different columns superscripts letters indicate significant difference in means when compared at $p < 0.05$.

Antioxidant activity

The DPPH radical scavenging activity of the extracts (Fig. 1) produced significant ($p < 0.05$) difference when ethyl acetate extract and gallic acid were compared with other extracts. However, when ethyl acetate and methanol extracts were compared at the highest concentration used, they were found to be statistically similar. The IC_{50} value of ethyl acetate extract and gallic acid against DPPH radical were 56.11 µg/mL and 15.98 µg/mL respectively and both values were significant when compared to IC_{50} values of other extracts. Result for radical scavenging activity of the extracts against $ABTS^{\pm}$ radical is presented in Fig. 2, with ethyl acetate extract and trolox values being significantly different ($p < 0.05$) when compared to other extracts at 60 – 100 µg/mL. Ethyl acetate and trolox gave IC_{50} values of 35.13 µg/ mL and 25.89 µg/mL respectively and were also significant when compared to other extracts. The significant antioxidant activity of the ethyl acetate extract made it a choice for subsequent studies. The potential anti-inflammatory, and anticancer activities of the ethyl acetate extract were further determined.

Anti-inflammatory activity

The anti-inflammatory activity of different concentrations (50 – 200 mg/kg bw) of ethyl acetate extract of *Andira inermis* was determined with values indicated in Table 4. Carrageenan administration in the paw of rats resulted in significant ($p < 0.05$) increase in the paw thickness of both treated and non-treated rats compared to normal control (group not administered carrageenan). Rats treated with different concentrations of extract or standard drug (aspirin) showed significant ($p > 0.05$) reduction in paw size from the 1^{st} – 5^{th} hour with percentage reduction ranging from 4.79 – 41.58% when compared to rats in negative control group. The standard drug was found to show significant reduction in paw thickness at 1^{st}, 2^{nd}, and 3^{rd} hours compared to extract treated groups. However, no significant reduction ($p > 0.05$) was observed at 4^{th} and 5^{th} hours between standard drug and 200 mg/kg bw of ethyl acetate extract.

Anticancer potential

The anticancer activity of different concentrations of ethyl acetate extract on 2 cancer cell lines, HepG-2 and MCF-7 are shown in Tables 5a and 5b respectively. Treatment of HepG-2 cells with the extract produced highest percentage cell viability (98.80%) at the 25 µg/mL and highest inhibition of cell growth (72.31%) at the 400 µg/mL. The concentration of the extract that inhibited 50% of HepG-2 cell growth (IC_{50}) was found to be 247.26 µg/mL compared to the standard drug (cisplatin) 23.44 µg/mL. The percentage inhibition of MCF-7 cells by different concentration of extract ranged from 1.62 – 52.12% and standard drug was 46.78 – 100%. The IC_{50} of the extract against MCF-7 was 365 µg/mL compared to the standard drug 24.74 µg/mL. The anticancer activity of the ethyl acetate extract was found to be dose dependent.

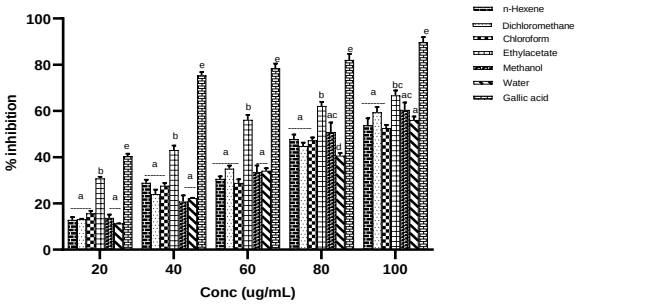

Fig. 1: DPPH radical scavenging activity of different extracts of Andira inermis stem bark. Values are mean ± SEM for triplicate determinations. Two way ANOVA and Turkey multiple comparison test were used. Bars having different letters are significantly different ($p < 0.05$) at the same concentration.

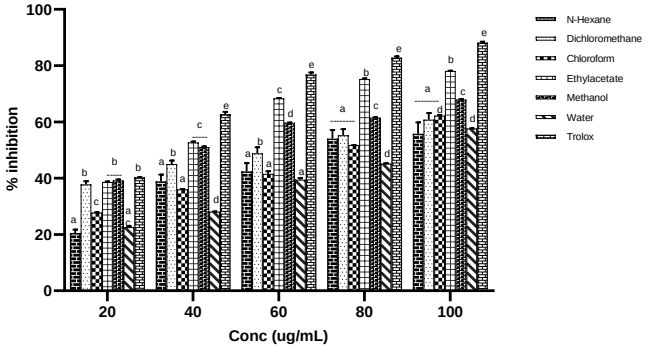

Fig. 2: ABTS radical scavenging activity of different extracts of *Andira inermis*. Values are mean ± SEM for triplicate determinations. Data was analyzed using two-way ANOVA followed by Turkey multiple comparison test with significance at $p < 0.05$. Bars having different letters are significantly different ($p < 0.05$) at the same concentration.

Table 4. Anti-inflammatory Activity of Ethyl Acetate Extract of *Andira inermis*

Group	Paw edema thickness (mm) and percentage inhibition of paw thickness at hourly time interval				
	1st hour	*2nd hour*	*3rd hour*	*4th hour*	*5th hour*
Normal control	1.332 ± 0.015	1.338 ± 0.020	1.332 ± 0.017	1.332 ± 0.017	1.326 ± 0.016
Negative control	2.631 ± 0.021[a]	2.658 ± 0.027[a]	2.672 ± 0.028[a]	2.746 ± 0.018[a]	2.762 ± 0.019[a]
Standard drug	1.842 ± 0.019[b] (30.65)	1.794 ± 0.009[b] (32.51)	1.624 ± 0.009[b] (39.58)	1.600 ± 0.005[b] (41.73)	1.564 ± 0.014[b] (43.74)
50 mg/kg bw extract	2.504 ± 0.014 (4.79%)	2.408 ± 0.010 (9.41%)	2.342 ± 0.009 (12.87%)	2.238 ± 0.017 (18.35%)	2.094 ± 0.018 (24.68%)
100 mg/kg bw extract	2.242 ± 0.012[c] (14.75%)	2.194 ± 0.038[c] (17.46%)	2.024 ± 0.014[c] (24.70%)	1.946 ± 0.016[c] (29.13%)	1.828 ± 0.004[c] (34.24%)
200 mg/kg bw extract	2.032 ± 0.007[d] (22.74%)	1.948 ± 0.020[d] (26.71%)	1.844 ± 0.015[d] (31.40%)	1.674 ± 0.020[d] (39.04%)	1.623 ± 0.026[d] (41.58%)

Values are mean ± SEM (n = 5). Two-way ANOVA was used and Tukey's multiple comparison post hoc test for significance between group means. [a] is significantly higher compared to normal control, [b] significantly lower compared to the negative control and treated groups group, [c] significantly lower compared to 50 mg/kg bw group, [d] significantly lower compared to 100 mg/kg bw group.

Table 5a. *In vitro* Anticancer Activity of Ethyl Acetate Extract of *Andira inermis* Stem Bark against HEGP-2 Cancer Cell Line

Conc. (µg/mL)	HepG-2		Cisplatin	
	% cell viability	% inhibition	% cell viability	% inhibition
25	98.80	1.21	65.74	52.12
50	97.61	2.45	53.78	85.93
100	82.34	21.45	45.82	> 100
200	68.79	45.37	41.04	> 100
400	58.03	72.31	36.25	> 100
R^2		0.965		0.855
Equation		y = 0.195*X – 1.7		y = 1.352 + 18.31
IC_{50} (µg/mL)		247.26		23.44

Table 5b. *In vitro* anticancer activity of ethyl acetate extract of *Andira inermis* stem bark against MCF-7cancer cell line

Conc. (µg/mL)	MCF-7		Cisplatin	
	% cell viability	% inhibition	% cell viability	% inhibition
25	98.41	1.62	68.13	46.78
50	93.76	6.66	60.16	66.23
100	86.45	115.67	56.57	76.76
200	74.63	33.99	48.20	> 100
400	65.74	52.12	45.42	> 100
R^2		0.969		0.855
Equation		y = 0.135*X + 1.13		y = 0.384*X + 40.50
IC_{50} (µg/mL)		362.00 ±		24.74 ±

DISCUSSION

Extractions of medicinal plant using different solvents and protocols accounts for variations in data within literature (Johnson *et al*., 2020). The different solvents used in this study gave different yields which were significantly different when compared. The use of different solvents makes it possible to establish the best solvent for the extraction of phytochemicals based on the ability of the solvent to stabilize the chemical structures of the targeted compounds (Monton and Luparasong, 2019). The absence of alkaloids and cardiac glycosides in the non-polar solvents such as hexane and dichloromethane could account for the nature of the phytoconstituents being more soluble in semi polar and polar solvents rather than non-polar solvents. Different polarity solvents used in this study shows that extraction was done based on the solvent or solvents that were able to dissolve different phytochemicals extracted. Extraction of phytoconstituents of *Andira inermis* stem bark was best achieved with semi polar solvents like ethyl acetate and methanol rather than non-polar solvents such as hexane

or completely polar solvent like water. Recent studies have shown that solvent polarity significantly affects the yield and pharmacological activities of the bioactive compounds (Afzal *et al*., 2022; Naqbi *et al*., 2022; Wairata *et al*., 2022). Methanol and ethyl acetate can be recommended for the extraction of bioactive compounds from *Andira inermis* stem bark.

Phytochemicals present in the extracts of *Andira inermis* such as saponins, terpenes tannins, flavonoids, phenols, and alkaloids have been reported (Egua *et al*., 2020). The medicinal properties of plants used as herbal remedies are due to the presence of these bioactive metabolites and a host of other phytoconstituents (Kpemissi *et al*., 2020). Phytochemicals are produced by plants as defence mechanisms against external factors (Mani *et al*., 2022). The presence of these phytochemicals in *Andira inermis* further explains its possible use as a medicinal plant for the treatment of various diseases.

The DPPH and ABTS radical scavenging assays have been utilized in the evaluation of antioxidant activity of medicinal plants. The ethyl acetate extract demonstrated significant reduction in DPPH and ABTS radicals compared to other extracts possibly due to the high content of total phenols and flavonoids. The role of flavonoids in human nutrition and health could be due to their antioxidant activity (Naqbi *et al*., 2022). Antioxidants inhibit the oxidation of biological molecules to provide a defence against reactive oxygen species (ROS) (Brindza *et al*., 2019).

Administration of carrageenan subplantar injection resulted to oedema formation in the paw of rats. Carrageenan subplantar injection is known to act as a local irritant that increases membrane phospholipase activity thereby triggering the synthesis of several pain and inflammatory cytokines like histamine, 5-hydroxytryptamine, bradykinins prostaglandins, leukotrienes, tumour necrotic factor alpha (TNF-α) which increases the paw size (Wahid *et al*., 2021). The reduction in paw size of rats pre-treated with ethyl acetate extract could be due to the phytochemical compounds present in the ethyl acetate extract. The presence of these phytochemical constituents act through different mechanisms to achieve their antioxidant and anti-inflammatory activities (Baloch *et al*., 2019). Flavonoids and phenols have been suggested to have anti-inflammatory activity by inhibiting prostaglandin synthase, which in turn reduces prostaglandin synthesis and release (Brito *et al*., 2021). The anti-inflammatory property of the ethyl acetate extract of *Andira inermis* could have been achieved through the inhibition of phospholipase activity, prostaglandin synthase and reduction of oxidative stress associated with inflammation by phenolic compounds. The treatment of rats with 400 mg/mL of ethyl acetate extract inhibited inflammation at 4th, and 5th hours in a non-significant manner when compared to the standard drug aspirin. Aspirin is a known NSAID and a cyclooxygenase (COX-1 and 2) inhibitor (Li *et al.*, 2020).

The ethyl acetate extract demonstrated anticancer potential against HepG-2 and MCF-7 cells with IC_{50} of 247.26 µg/mL and 365 µg/mL compared to the standard drug (cisplatin) 23.44 µg/mL and 24.74 µg/mL respectively. The main mechanism of anticancer potential of plant phytoconstituents has been suggested to be mediated by the induction of apoptosis and cell cycle arrest (Gheena and Ezhilarasa, 2019). Polyphenols and flavonoids play a significant role in the medicinal properties of many plants through antioxidant, anti-inflammatory, and anticancer activities (Wang *et al*., 2019).

CONCLUSION

Extraction of *Andira inermis* stem bark using different solvents revealed that methanol and ethyl acetate were the best extracting solvents. The ethyl acetate extract had a significant amount of total phenols and flavonoids and antioxidant activity compared to other extracts. The ethyl acetate extract exhibited anti-inflammatory activity against carrageenan-induced inflammation and anticancer potential against HepG-2 and MCF-7 cell lines. The anti-inflammatory and anticancer activities of the ethyl acetate extract could be due to the presence of phenols and flavonoids that exhibited significant antioxidant activity.

AUTHORS' CONTRIBUTIONS

The study was designed by DD, and supervised the antioxidant and anticancer determination. MSN and HAU supervised the anti-inflammatory determination while MIB handled the extraction and phytochemistry. All authors contributed to the development of the manuscript and approved it.

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CONFLICT OF INTEREST

The authors declare that there is no conflicting interest whatsoever.

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