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Research Article

ANTI-INFLAMMATORY, ANALGESIC AND ANTI - PYRETIC ACTIVITY OF CASSAVA LEAVES EXTRACT

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ABSTRACT

Objective: The objective of this study was to evaluate the *in-vivo* anti-inflammatory, anti - pyretic, analgesic activities and phytochemical analysis of ethanol cassava leaf extract (ECLE).

Material and method: The leaf extract was prepared with ethanol filter and rotary evaporated and stored in desiccator. Preliminary phytochemical screening terpernoids, tannins, flavonoids, anthraquinones, alkaloids, and cardiac glycosides and carotenoids were carried out. A different concentration (100,250,500mg/kg) of the leaf extract was evaluated for their anti-inflammary, analgesic and anti-pyretic effect using carrageenan and histamine induced oedema, acetic acid induced writhing and yeast induced pyrexia in rats respectively.

Result and Conclusion: The extract at concentration100, 250,500mg/kg was able to inhibit carrageenan and histamine induced oedema, acetic acid induced writhing and yeast induced pyrexia in rats. Terpernoids, tannins, flavonoids, carotenoids were found present in the ECLE.

Keywords: cassava leaves, anti-inflammatory, anti pyretic, analgesic, phytochemicals

INTRODUCTION

Cassava also known as Manihot esculentaCrantzis categorized under the family of phorbiaceae; this plant is a heterozygous, vegetative propagated root crop with a wide variety of uses. Cassava was introduced in Africa and Asia, as a consequence of the Portuguese trading activities in the southern hemisphere. Today, Africa produces more cassava than the rest of the world combined. Cassava is to African peasant farmers as rice is to Asian farmers, or wheat and potatoes are to European farmers [1,2,3].Cassava is a staple food for at least 500 million people and animal feed in tropical and subtropical Africa, Asia, and Latin America, with an estimated total cultivated area greater than 13 million hectares, of which more than 70% is in Africa and Asia. Cassava is the third most important food source after rice and maize [4].It is one of the plants that is believed to protect itself; it does so by producing poisonous latex which is mainly found in its leaves. The main reason this plant is grown is because of its roots which are highly reach in starch. Cassava may be a useful source of starch for people who are suffering from celiac disease (gluten intolerance) as it does not contain any gluten[4].The leaves are very rich source of protein (up to 30%, compared to only 1-3% in the roots), but must be cooked thoroughly in order to render the prussic acid harmless. The young leaves are rich in vitamin B, C, Carotene, Calcium and Iron.

METHODS

The leaves of cassava were gotten from the local market in Kuala Lumpur. They were identified by a botanist.

ETHANOL EXTRACTION

Grinded powder leaves of 895.97g were weighed accurately using an analytical balance (EL-2000S, Setra, USA) and poured into a large conical flask. The sample was extracted by adding ethanol to the flask. The sample was left for three days for maximal extraction after which the solution was evaporated to dryness under reduced pressure and controlled temperature of 40ºC water bath by using Buchi rotary evaporator model R-200. The extraction process was repeated until the extraction medium turned pale green. The extract was stored in a refrigerator at 4 °C in a flask covered with aluminum foil, for further use.

PHYTOCHEMICALS SCREENING

Alkaloids

Alkaloids were measured by accurately weighing 0.5g of the (ECLE).. The extract was mixed with 5ml of 1% aqueous hydrochloric acid on a steam bath and was properly stirred. The solution was filtered and 1ml of the filtrate was treated with a few drops of the Mayer's reagent. Formation of cream or precipitate colour indicated the presence of alkaloids [5].

Tannins

A dried extract of 5g was stirred with 100ml of distilled water. The solution was filtered. Ferric chloride was added to the filtrate. The presence of blue black precipitate was taken as the presence of tannins [5].

Saponins

A dried extract of 0.1g was boiled with 5ml of distilled water for five minutes. The mixture was filtered while still hot and the filtrate was used for the following tests. To 1ml of filtrates, two drops of olive oil was added, the mixture was shaken and carefully observed for the formation of emulsion.1ml of filtrate was diluted with 4ml of distilled water. The mixture was vigorously shaken and then observed on a stand for formation of stable froth [5].

Anthraquinones

The ECLE of 0.5g was boiled with 1ml of 10% sulphuric acid and filtered.2.5ml of benzene was added to the filtrate and shaken; few drops of 10% ammonia solution were added. The presence of pink or red violet color in the lower ammonia phase was taken as the presence of anthraquinones [5].

Cardiac glycosides

A dried ECLE of 0.5g was dissolved in 2ml of glacial acetic acid containing one drop of 1% ferric chloride solution. This was then underlayed with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of cardenolides [5].

Carotenoids

A solution of 100ml of the ECLE was made in chloroform, and concentrated sulphuric acid was added. A deep blue color indicates the presence of carotenoids [6].

Preparation of animals

The animals were purchased at the international medical institute in Kuala Lumpur. The rats were kept in groups of five in a standard condition in animal holding units, UCSI University, Kuala Lumpur Malaysia. The animals were fed with standard pellet diet and water ad libitium and left for 2 weeks before starting the experiments. The Project has ethical committee approval code no. ETUCSI 10012

Carrageenan induced Paw Oedema

The test was carried out as described [6], Saline (1ml/kg) was used as negative control ECLE. (100, 250, 500ml/kg) as a test compound while indomethacin (10mg/kg), was used as a positive control. Saline 1ml, test compound and drug were orally administered1 hour before injection into the sub plantar side of right hind paw of the rats to induce paw oedema. The paw diameter of swelling (mm) was measured after carrageenan injection at 1hour interval for 5 hours. The percentage inhibition for each group was calculated according to the formula:

Equation 1

% inhibition=100- [oedema volume in the treated⁄oedema volume in the control]×100

Histamine induced Paw Oedem

Adopting the method described [7]; the paw oedema was produced by sub-plantar administration of 0.1% freshly prepared solution of histamine into the right hind paw of the rats. The paw volume was recorded before the histamine injection. Rats five per group were orally administered with 0.5 ml of the ECLE at 100, 250 and 500 mg/kg body weight, indomethacin 10mg/kg (positive control) and saline (negative control) and histamine was administered 1 hour after the administration of the extract, saline and indomethacin. The right hind paw volume was measured at 1, 2, 3, 4 and 5 h. The anti-inflammatory activity was calculated as described earlier for carrageenan induced oedema.

Acetic acid induced writhing

This study was performed according to the standard protocol [6]. Each rat was intraperitoneally injected with 0.6% aqueous solution of acetic acid (10ml/kg body weight);1 hour after receiving oral administration of the saline 1ml as a negative control ECLE at a concentration 100,250, 500ml/kg and indomethacin (10mg/kg) as a positive control. Immediately after the acetic acid injection, each animal was placed in a transparent observation cage and the number of writhes per rat was counted for 30 minutes. The writhing activity comprised of constriction of the abdominal muscles together with a stretching of the hind limbs .The percentage inhibition was calculated using the following formula:

Equation 2

[(control mean-treated mean)/control mean]×100

Induction of Pyrexia

Yeast induced pyrexia was used to evaluate the antipyretic activity of the ECLE. Before conducting the experiment, the weight of the female rats were measured in order to determine the volume of drugs, extracts and yeast suspension to be given to each rat. The rats were divided into 5 groups of five and the basal rectal (anal) temperature of each rat was recorded by insertion of thermometer probe 3-4cm deep into the rectum. The animals were given a subcutaneous injection of 4ml/kg of 15% w/v Brewer's yeast suspension to induce fever and the animals were returned to their cages. Twenty four hours after yeast injection, the rat's rectal temperature was measured, only rats with elevated rectal temperature of at least 0.5˚C were selected for the study [8]

Equation 3

Percentage of reduction= YIP-PTT⁄ YIP

(YIP=yeast induced pyrexia, PIT=post induced temperature)

STATISTICAL ANALYSIS

The data was expressed as mean± standard deviation. The statistically significant differences between groups were measured using one way ANOVA (one way analysis of variance) followed by Dunnett's test. Statistical analysis was performed using Graph pad prism 6.0(Graph Pad software, San Diego, CA, USA).Values of *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 were considered statistically significant.

RESULTS AND DISCUSSION

Table 1: Effects of ethanol extract of cassava leaves and indomethacin on carrageenan-Induced paw oedema in rats

Treatment	Dose mg/kg	0hr	1hr	2hr	3hr	4hr	5hr
-VE control	-	0.77±0.006	0.788±0.004	0.792±0.0098	0.800±0.0063	0.798±0.010	0.810±0.013
indomethacin	10	0.722±0.004	0.730±0.006	0.738±0.004	0.658±0.004	0.658±0.004	0.640±0.006
			7.30%	6.80%	17.75%	17.54%	21%****
Cassava extract	100	0.720±0.000	0.710±0.000	0.738±0.004	0.658±0.004	0.607±0.008	0.598±0.004
			9.90%	6.80%	17.75%	23.90%	26.2%****
	250	0.780±0.006	0.778±0.010	0.788±0.004	0.688±0.004	0.688±0.004	0.672±0.004
			1.30%	0.50%	14%	3.90%	17%****
	500	0.788±0.004	0.778±0.004	0.778±0.00	0.758±0.007	0.740±0.000	0.731±0.004
			1.30%	1.30%	5.30%	7.30%	9.8%****

Value are express as mean ± SD for N=5

Anti-inflammatory activity of ECLE was evaluated using two very popular assay; carrageenan and histamine induced paw oedema. Carrageenan-induced rat paw oedema is used widely as a working model of inflammation in the search for new anti-inflammatory drug [9]. Oedema induced by carrageenan is believed to be biphasic: the first phase (1 hour after the induction) involves the release of serotonin and histamine and the second phase which is determined (after the first 1 hour) is believed to be mediated by prostaglandins cyclooxygenase products leukotriene and kinin. ECLE was able to show a significantly (P<0.0001) decrease paw oedema induced by carrageenan by at the fifty hour compared to negative control saline. 100mg/kg exhibited a highest percentage inhibition 26.2%, which similar to indomethacin (21%), while 500mg/kg showed the lowest inhibition at the fifth hour (table1). The effects shown by ECLE maybe through the inhibition 5-lipoxygenase and/or cyclooxygenase [10].It may also be through the inhibition of the release or synthesis of histamine and serotonin.[9]

Table 2: Effects of ethanolic extract of cassava leaves and indomethacin on Histamine-induced paw oedema in rats

Treatment	Dose	0hr	1hr	2hr	3hr	4hr	5hr
-ve control	-	0.713±0.010	0.608±0.004	0.620±0.010	0.652±0.004	0.628±0.004	0.640±0.006
indomethacin	10	0.620±0.006	0.552±0.004	0.542±0.004	0.550±0.006	0.558±0.004	0.558±0.004****
			9.20%	12.60%	15.60%	11.10%	12.90%

Cassava extract	100	0.690±0.006	0.580±0.006 4.60%	0.568±0.004 8.40%	0.560±0.006 14.10%	0.540±0.006 14%	0.538±0.004**** 15.90%
	250	0.658±0.004	0.658±0.007	0.620±0.006	0.612±0.007 6.10%	0.610±0.000 2.90%	0.598±0.004**** 6.60%
	500	0.670±0.006	0.658±0.004	0.642±0.004	0.618±0.004 5.20%	0.608±0.004 3.20%	0.598±0.004**** 6.60%

Value are express as mean ± SD for N=5,

Histamine is an important inflammatory mediator as well as a potent vasodilator which increases vascular permeability [11]. ECLE was able to significantly (P<0.001) decrease paw oedema induced by histamine at the fifty hour compared to negative control saline. 100mg/kg showed the highest percentage 15.9%, which was higher than indomethacin (12.9%), while the lowest effect was showed by 250 and 500mg/kg (table 2). The effects shown by ECLE maybe through the inhibition of the release or synthesis of histamine. Although ECLE at 100mg/kg showed a stronger inhibition of carrageenan induced paw oedema compared to histamine induced paw oedema but the result correlate with the earlier assumption that ECLE inhibition the phase one by inhibiting histamine release or synthesis [9]

Table 3: Effects of ethanol cassava extract leaves and indomethacin on Acetic acid-induced writhing in rats

Treatment	Drug(mg/kg)	Within 30 minutes	% inhibition
-ve control	-	134± 1.00	-
Indomethacin	10	46.667±4.163****	65.2%
Cassava extract	100	33.00±2.0****	75.4%
	250	46.667±2.082****	65.2%
	500	41.333±1.528****	69.2%

Value are express as mean ± SD for N=5,

A non-specific but widely used method for analgesic test is the acetic acid induced writhing test[12].Acetic acid has been found to cause an increase in peritoneal fluid levels of prostaglandins (PGE2 and PGF2), hence causing inflammatory pain by inducing capillary permeability[13].Writhing consists of a constriction of the abdominal muscle together with stretching of the hind limbs due to tissue damage and sensitization of the nociceptors nerves by inflammatory mediators [14, 15, 16]. ECLE was able to show a significantly (P<0.001) decrease in acetic acid induced writhing compared to negative control saline. 100mg/kg showed the highest percentage inhibition 75%, which is higher than indomethacin (65.2%), while the lowest effect was showed by 250 mg/kg (table 3). The inhibition of acetic acid induced writhing in the rat by ECLE may therefore be due to inhibition of the activity of the COX and decrease in the synthesis of inflammatory mediators; prostaglandins and thromboxane's from arachidonic acid [17]. It may also be as a result of blockage of calcium influx and or intracellular calcium dependent mechanisms [18].

Table 4: Antipyretic activity and inhibitory values (100,250,500 mg/kg) cassava ethanol leaves extract, Paracetamol against yeast induced pyrexia (fever)

Treatment	dose	Initial temp	Post yeast temp	1hr	2hr	3hr	4hr
-ve control	-	35.0	36.8±0.9	36±0.00	36± 0.00	36.167±0.41	36.167±0.41
Paracetamol	10	32.2	34.8±0.3	34.167±0.41	34.167±0.41	33.167±0.41	33.167±0.41****
Cassava extract	100	34.1	36± 0.3	35± 0.00	34± 0.00	34.833±0.41	34.833±0.41****
	250	35.3	36.8±0.3	35± 0.00	35± 0.00	34.667±0.52	34.167±0.41****

Value are express as mean ± SD for N=5,

Fever is thought to be produced by several endogenous substances including interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), tumour necrosis factor-α (TNF-α), macrophage protein-1 (MIP-1) and prostaglandins. Brewer's yeast induces both TNF-α and prostaglandin synthesis. Non-steroidal anti-inflammatory drugs (NSAIDs) reduce fever by depressing inflammatory messages at both peripheral sites of tissue inflammation and within central nervous system thermoregulatory sites. These agents suppress peripheral production of pyrogenic cytokines such as TNF-α and interleukin-1β, while lowering the thermoregulatory set point by blocking central cyclooxygenase production of prostaglandin E2 [19]. Antipyretics on the other hand, such as aspirin and paracetamol have been widely used since the late 19th century. It is now clear that most antipyretics work by inhibiting the enzyme cyclooxygenase and reducing the levels of PGE2 within the hypothalamus .Recently, other mechanisms of action for antipyretic drugs have been suggested, including their ability to reduce pro-inflammatory mediators, enhance anti-inflammatory signals at sites of injury, or boost antipyretic messages within the brain. It is a well-established fact that free radicals play an important role in pain antipyretic agents are better understood, the indications for their clinical use are less clear. ECLE was able to show a significant (P<0.0001) reduction in fever induced in rats by subcutaneous injection of 15% suspension of brewers yeast.100 and 200mg/kg showed percentage reduction of 34.83% and 34.66%, which is lower than paracetamol33.16 %(table 4).The reduction of fever induced by brewer's yeast in the rat by ECLE may be due to inhibition of the activity of the enzyme cyclooxygenase and reducing the levels of PGE2 within the hypothalamus or other mechanism.

Table 5: Phytochemicals Present or absent in the cassava leaf extract

TEST	RESULTS
Carotenoids	+
Flavonoids	+
Tannins	+
Terpernoids	+
Anthraquinones	-
alkaloids	-
saponins	-
Cardiac glycosides	-

(+) present, (-) absent

Carotenoids, flavonoids, tannins and terpernoids were present in the ECLE (table 5). Flavonoids have been determined to produce various biological activities like anti-allergic and anti-inflammation effects. They do so by suppressing the cyclooxygenase and lipooxygenase activities, lipid peroxidation, capillary permeability and platelet aggregation. Moreover flavonoids like myricetin and kaempferol are known to be potent xanthine oxidase inhibitors and useful to treat reperfusion injury [20, 21].The above results on anti-inflammatory and analgesic effects of ECLE corresponds to these findings. Studies have shown that tannins are potent xanthine oxidase inhibitors and good remedy as anti-allergic and anti-

inflammatory. Its biological activities are mediated through inhibition of lipid peroxidation and plasma activities. Several studies indicate that tannins are able to induce the analgesic effects and decrease the paw oedema induced by formalin and carrageenan [22, 23, 24] Carotenoid sare more than mere pigments; they play an important role as antioxidants as well. In their capacity as antioxidants, carotenoids protect cells and tissues from harmful radical oxygen species (ROS), acting as scavengers of singlet molecular oxygen, peroxyl radicals. Researchers discovered that α- and β-carotene enhanced the anti-nociceptive activity of morphine [25], Terpenoids significantly inhibit the development of chronic joint swelling. Terpenoids may affect different mechanism relevant to inflammations arising in response to etiological factors [26].The presence of anti-inflammatory effects of the cassava leaves extract may be due to the presence of this terpenoids.

CONCLUSION

The ECLE has shown a significant anti-inflammatory, anti- pyretic and analgesic effects. These effects maybe because of the presence of phytochemicals; flavonoids, tannins, carotenoid and terpenoids present in the ECLE. However purification and mechanism of action of the bioactive component present in ECLE should be elucidated.

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