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RESEARCH ARTICLE

Antioxidant and Gastro-protective Activity of Ethanolic Rind Extract of *Manihot Esculenta Crantz*

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ABSTRACT

Manihot Esculenta Crantz or better known as cassava has been widely used to cure headache, hypertension, conjunctivis and cancer. In this study, we investigated the antioxidant and gastro-protective effect of ethanol rind extract of Manihotesculenta Crantz (Cassava) on ethanol and stress induced ulcer. The rind was dried in an oven and pure enthanol was used to extract the crude and simple biochemical and colorimetric assays were used to study the its antioxidant activity. Gastric ulcer were induced in the rat using absolute ethanol and stress. Oral administration of 100, 250 and 500mg/kg of the extract showed gatro-protective of 36.3%, 54.6% and 63.7% respectively for stress induced ulcer and 42.2%, 49.1% and 70.2% respectively for ethanol induced ucler. The pH of the stomach in both ulcer models were increased from 2.84-7.13 after oral administration of the extracts. It showed total Antioxidant Capacity (TAC) of 36.13 ± 5.76 µg AAE/mg DW, total Proanthocyanidin (TPR) 25.9±1.41 µg CATE/mg DW and Total Phenolic Content (TPC) 28.9µg GAE/mg DW. The concentration needed for 50% scavenging activity of DPPH, ABTS, SO, and NO was found to be 32.22, 0.161, 107.3 and 0.372 mg/ml, respectively and reducing power of 1.126. Cassava rind extract showed gastroprotective effect against ethanol and stress induced ulcer and this effect maybe because of its antioxidant property.

KEYWORDS

Cassava, Peels, Antioxidant activity, Total phenolic content, Gastro-protective

INTRODUCTION

Cassava (*Manihotesculenta*), also called manioc, tapioca or yuca, is one of the most

important food crops in the humid tropics, being particularly suited to conditions of low nutrient availability and able to survive drought[1]. It is a perennial shrub that is cultivated mainly for its starchy roots. Not only is it used for human consumption but also for biofuel[2]. The largest producer of cassava is Nigeria, followed by Brazil, Thailand, Zaire and Indonesia.

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It is the staple food for over 500 million people in western and Central Africa with an average consumption of approximately 500cal/day[2].

The Cassava rind (a thin brown outer covering and thick leathery parenchymatousinner covering which constitutes about 20-35% of the weight of the tuber is usually regarded are waste. Cassava rind is a lignocellulolytic material and has been used for ethanol production. Many human diseases are known to be as a result of an imbalance between the formation and neutralization of pro-oxidants[3]. Antioxidant compounds like polyphenols, flavonoids and phenolic acids scavenge free radicals such as superoxide, hydroperoxide and

thus inhibit oxidative mechanisms that may lead to degenerative diseases. To the best of our knowledge cassava rind have not been used before for it folklore uses or tested for gastro-protective and antioxidant properties. Therefore, in this study we explored the antioxidatant and gastro-protective effect ethanol rind extract on stress and ethanol induced ulcer animal model.

MATERIALS AND METHOD

Preparation of Crude Rind Extract

Cassava rinds were purchased from Chow Kit, Kuala Lumpur, Malaysia. The cassava rinds were washed, weighed and dried using an oven (Memmert, Germany) set at 40ºC for four days. The dried rinds were ground into powder and dried further with oven set at 75º C to deactivate linamarase. 124 grams of the dried and deactivated linamarase was soaked in ethanol (sigma USA) for 2 days. The mixture was filter using whatman filter paper and the filtrate evaporated using rotary evaporator (BUCHI, Switzerland) set at 40ºC to obtain the ethanol rind extract with is in paste-like state. The paste-like were freeze dried and stored in a desiccator for future use.

Total Antioxidant Capacity

The total antioxidant capacity of the ethanol cassava rind extract were determined according to xxx using the phosphomolybdenum assay proposed by[4]. The absorbance was read at 695 nm against the control. Ascorbic acid was used as standard.

Total Proanthocyanidins

Total proanthocyanidins was determined based on the procedure of[3]. The absorbance of resulting mixture was measured at 500 nm after 15 min at room temperature. Total proanthocyanidin content was expressed as catechin equivalents (mg/g)

Total Phenolic Content

The total phenolic content was determined with FolinCiocalteau reagent using the modified method of [5]. The absorbance of the samples was measured at 765 nm. Total content of phenolic compounds in sample was expressed in gallic acid equivalent [GAE].

DPPH Radical Scavenging Activity

DPPH was determined according to the method described by of [3]. The absorbance of the mixture was measured using spectrophotometer at 517 nm.

ABTS Radical Scavenging Activity

The ABTS scavenging activity of the cassava rind extracts was determine according to the method of[3]. The percentage inhibition of ABTS radical by the extract was calculated using the following equation:

ABTS scavenging activity = {(Abs control – Abs sample)/ (Abs control)} × 100

Where; Abs control: absorbance of ABTS + methanol; Abs sample: absorbance of ABTS + extract or standard.

Scavenging Activity of Nitric Oxide

The method of[3] was used to determine the nitric oxide radical scavenging. The amount of nitric oxide radical inhibited by the extract was calculated using the following equation:

NO radical scavenging activity = {(Abs control – Abs sample) / (Abs control)} × 100

Where; Abs control is the absorbance of NO radical + methanol; Ab sample is the absorbance of NO radical + extract or standard.

Superoxide Radical Scavenging Activity

To measure the superoxide radical scavenging activity we used the riboflavin-light-NBT systemdescribed by[5,6].

Absorbance was measured immediately at 590nm. Ascorbic acid was used as standard.

Determination of Reducing Power

The reducing power radical scavenging effect was assayed according to the method of[7] with slight modifications.

Increased absorbance indicated an increase in reducing power. Ascorbic acid was used as reference drug.

Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide scavenging activity was determined using method of[8]. The reaction mixture without test sample was used as control. Percentage hydrogen peroxide scavenging activity was calculated using the formula below;

% Inhibition = $(V_0-V_1)/V_0$ x 100; Where V_0 = volume of NaS_2O_3 used to titrate the control, V_1= volume of NaS_2O_3 solution used in titrate the test mixture

Animal Preparation

Health Sprague Dawley rats of either sex, 6-8 weeks old weighing 200-250g were purchased from institute of medical research (IMR), Kuala Lumpur. The animals were divided into five groups each group had five rats (N=5) and were left for a week to acclimatized. They were fed with standard pellet and had free access to water. The animal handling was done according the UCSI University animal ethical committee guide line.

Ethanol Induced Ulcer

Overnight fasted rats were randomly divided group: 1 (negative control) treated with saline (1mL), group: 2 (positive control) treated with cimetidine (100mg/kg) group 3- 5 (test groups) was treated with cassava rind extract at concentration of 100, 250 and 500mg/kg respectively. After one hour, gastric lesions were induced in animals by oral administration of 1ml of absolute ethanol in all groups[9,10]. The gastro-protective effect was measured as mean ulcer score which was expressed as ulcer index (UI), which was calculated using equation below;

Ulcer index (UI) = Total lesion length / Number of ulcerated animals

% inhibition = [UI control – UI treated / UI control] x 100

Stress Induced Ulcer

Similarly as in ethanol induced ulcer, the rats were divided into five groups (n=5) and they were treated according to group: 1 (negative control) treated with saline (1mL), group: 2 (positive control) treated with cimetidine (100mg/kg) group 3-5 (test groups) were treated with cassava rind extract at concentration of 100, 250 and 500mg/kg respectively after overnight fasting. Ulcers were induced by subjecting the rats to water restraint stress. Stress induced ulcer was done by immobilizing the rats in a cylindrical cage, supported by a retort stand and immersed in water maintained at 28°C in the presence of intense light for 3hrs[12]. The rats were killed with overdose of diethyl ether, after 3hrs and the stomach of the rats were opened along the greater curvature to measure the ulcer. The gastric injury was scored on a scale of 0-4 based on the severity of formation of haemorrhages, according to the method used by[13] Table 2.

Table 1: Stress induced ulcer scores

Score	Explanation
0	Almost normal (no spots)
1	Mild (1-5spots)
2	Moderate (6-10 spots)
3	Severe (more than 10 spots)
4	Very severe (spots all over the mucosa)

Phytochemical Analysis

The phytochemical test was performed with a simple biochemical and colorimetric tests identify the possible bioactive component present in the cassava rind extract. The phytochemical tested are; flavonoids, alkaloids, tannins, terpenoids, saponins, steroids and reducing sugars.

Statistical Analysis

All values were reported as mean ± S.D. The statistical significance of differences between sample and control, between groups was assessed according to Graph Pad Prism 6.0. A value of $p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ were considered significant.

RESULTS AND DISCUSSION

Phytochemical Analysis

Preliminary phytochemical screening of cassava rind extract of indicated the presence of flavonoids, alkaloids, tannins, terpenoids, steroids, saponins and reducing sugars which contributed to the antioxidant and gastro-protective activity.

Total Proanthocyanidins and Antioxidant Capacity

The total proanthocyanidins, antioxidant capacity and phenolic content of cassava rind extract are shown in Figure 1. The plant extract possessed high phenol contents (28.9µg Gallic acid equivalent/mg) followed by proanthocyanidins (15.3µg Catechin equivalent/mg). The total Antioxidant capacity was (22.93 µg Ascorbic Acid equivalent/mg). Many authors have reported that phenolic compounds, proanthocyanidins possess antioxidant activity, anti-cancer, anti-ulcer, anti-inflammatory, anti-diabetic, anti-aging and prevention of cardiovascular diseases[16,17,18,19]. The antioxidant mechanism of these polyphenols are believed to be mainly due to their redox properties allowing them to act as reducing agents, hydrogen donors, free radical quenchers, metal chelators and decomposers of peroxides[19].

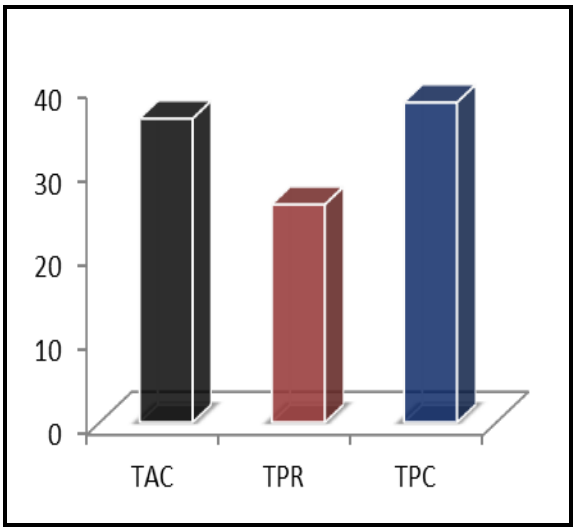

Figure 1: Total antioxidant capacity, phenolic and Proanthocyanidins content ofethanolic crude rind extract of *ManihotesculentaCrantz.*

In Vitro Antioxidant Activity

DPPH Radical Scavenging Activity

DPPH free radical scavenging assay has been one of the most commonly used assays to determine the antioxidant activity properties of plant samples. 1, 1-Diphenyl-2-picrylhydrazyl is a stable free radical. It accepts a hydrogen atom from the donor resulting in the loss of its deep purple color[20]. The DPPH activity was recorded in terms of EC_{50} (concentration of test compound decreasing the absorbance of a DPPH solution by 50%) as shown in Table 2. According to[21] EC_{50} between 50 and 100 µg/ml possess significant antioxidant activity and those above 200µg/ml have little activity. The Cassava peel extract showed H-donor activity and significant free radical scavenging activity with an EC_{50} value of 95.12µg/ml compared to Rutin 36.99 µg/ml.

ABTS Radical Scavenging Activity

The peroxidase substrate 2, 2'-azino-bis(3-ethylben- zthiazoline-6-sulphonic acid (ABTS) which forms a relatively stable radical upon one-electron oxidation is another popular antioxidant testing method. This radical has characteristic absorbance maxima at 734 nm which decreases with the scavenging of the proton radicals. In this experiment, the cassava rind extract was effective in scavenging the ABTS radical as shown in table 2. The IC_{50} values of the extract compared to ascorbic acid were 0.174 and 0.158 respectively. The extracts ability to scavenge the ABTS radical indicates that the mechanism of antioxidant action of this test sample was as a hydrogen donor[19].

Nitric Oxide Scavenging Activity

According to[22] Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity also in inflammatory processes such as carcinomas, juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis[3]. In this experiment nitric oxide a reactive free radical generated from sodium nitroprusside in aqueous solution at

physiological pH and reacts with oxygen to form nitrite. The cassava peels had IC_{50} of 0.372 µg/ml compared to that of ascorbic acid 0.0467 µg/ml. The extract has shown moderate activity in scavenging nitric oxide activity.

Superoxide Oxide Scavenging

Superoxide has been reported to be very harmful to cellular and acts as a precursor of reactive species[5]. It has also been known to initiate Lipid peroxidation[23]. The superoxide free radical scavenging activity assay is based on the ability of the test extract to inhibit the formazan formation by scavenging the superoxide radical generated in riboflavin-light-NBT system[5]. The illumination of light on riboflavin in the presence of EDTA generates superoxide radicals with nitro blue tetrazolium to form a blue colored complex. The cassava peel extract showed a dose dependent free radical scavenging activity with (IC_{50} 107.3µg/ml) was lower than that of standard Ascorbic acid (103.8µg/ml).

Reducing Power Activity

In this assay the presence of antioxidants in the sample would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. The antioxidant compound forms a blue colored complex with potassium ferricynanide, trichloroacetic acid and ferric chloride[24]. And an increasing absorbance indicates the increase of reducing ability[24]. The absorbance reading at 700nm of ascorbic acid showed an increase in a dose dependent manner from 0.248 at 10ppm to 0.979 at 50ppm, while, the cassava peels increased from 0.084 at 10ppm to 0.315 at 50ppm as shown in table 2.

The reducing properties of the plant extracts are generally associated with the presence of reductones, which have been shown to exert antioxidant action by donating a hydrogen atom by breaking the free radical chain[24]. Cassava has been reported to contain ten antioxidant compounds: coniferaldehyde, isovanillin, 6-deoxyjacareubin, scopoletin, syringaldehyde, pinoresinol, *p*-coumaric acid, ficusol, balanophonin and ethamivan and were previously found to have DPPH and ABTS free radical scavenging activity in the stems of the cassava plant[25]. These antioxidant compounds are currently being researched for their role in the prevention of many free radical damage caused diseases like cancer, immune system decline, heart disease and many others[25].

Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually of essential thiol groups. Hydrogen peroxide can cross cell membranes rapidly, once inside a cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects [8]. The cassava rind extract was able to inhibit hydrogen peroxide in a dose dependent manner with IC_{50} 1.126 µg/mL compared to ascorbic acid IC_{50}0.634µg/mL as shown in Table 3. Hydrogen peroxide is not very reactive but it sometimes becomes toxic because it can give rise to hydroxyl radicals in the cells[26]. Therefore, it is important to remove hydrogen peroxide for the antioxidant defense in cell or food systems.

Ethanol Induced Ulcer

Ethanol and stress induced ulcer model was used to evaluate the gastro-protective abilityof cassava rind extract. Oxygen-derived radicals and agents with antioxidant properties have been implicated in the pathogenesis of ethanol-induced gastric ulcer. The exposure of ethanol to the gastric mucosa has been shown to affect cellular integrity which is associated with oxidative stress and mitochondria damage. The result of cassava rind extracts (100, 250, and 500mg/kg) on gastric ulcer induced by absolute ethanol results are shown in Table 5 and Figure 2. As reported by other authors, oral administration of cassava rind extract at 100, 250, 500mg/kg and cimetidine (100mg/kg) was able to significantly increase the pH of stomach to 5.77 ± 0.40, 5.19 ± 0.41, 5.84 ± 0.32 and 7.1 ± 0.08 respectively compared to negative control 2.84 ± 0.12 Table 5. The extracts were able to significantly protect the mucosa of the rat from ulcer formation.

Table 2: Reduced power activity of the ethanolic Cassava peel extract

Concentration (ppm)	Ascorbic Acid	Peels
10	0.248 ± 0.009	0.084± 0.0006
20	0.380 ± 0.028	0.097± 0.0006
30	0.574 ± 0.029	0.101± 0.0021
40	0.856 ± 0.059	0.207± 0.0012
50	0.979 ± 0.094	0.315± 0.0017

Reducing power activity of ethanolic extracted cassava leaves and peels and ascorbic acid values expressed as mean ± standard deviation (n=3)

Table 3: Hydrogen peroxide reducing activity of ethanolic Cassava Peel extract

Sample Concentration	Ascorbic Acid (% Inhibition)	Peels (% Inhibition)
10	1.692	19.90
50	6.55	21.04
100	12.12	22.30
150	13.98	23.38
200	16.17	25.22

Table 4*: In Vitro* antioxidant assay results

Group	Cassava Peels	Rutin	Ascorbic Acid
DPPH (EC_{50} mg/mL)	32.22	0.028	-
SO(IC_{50} µ/mL)	107.3	-	103.8
NO(IC_{50} µg/mL)	0.372	-	0.046
ABTS(IC_{50} µg/mL)	0.161	-	0.159

Table 5: Gastroprotective effect and gastric pH of crude rind extract on ethanol-induced ulcer, ulcer index and inhibition percentage in rat model

Treatment	Dose	Ulcer index in mm (Mean ± S.D)	% Inhibition	pH
Negative Control	5ml/kg	34.6mm ± 5.03mm	-	2.84 ± 0.12
Positive Control	100mg/kg	22.0mm ± 2.82mm **	36.4	7.13 ± 0.08
Rind extract	100mg/kg	20.0mm ± 1.41mm***	42.2	5.77 ± 0.40
Rind extract	250mg/kg	17.6mm ± 2.33mm***	49.1	5.19 ± 0.41
Rind extract	500mg/kg	10.3mm ± 3.21mm****	70.2	5.84 ± 0.32

All value are represented as mean ± S.D (N=5). Statistical significance with One Way ANOVA followed by Dunnett's test (**p<0.05, ***p<0.001) in comparison to negative control group.

Percentage inhibition of 42.2, 49.1 and 70.2% were shown by oral administration of 100, 250 and 500mg/kg. The result shows that cassava rind extract may have protected the mucosa by increasing the mucus production which helps to protection of surface epithelial cells and there by inhibiting ulcer formation[28]. Ethanol induces ulcer by generation of oxygen derived free radicals such as superoxide anions, lipid peroxides, and hydroxyl radicals. Gastro-protective effect of cassava rind extract may have been due to its antioxidant property and phytochemical present which have been reported previously to have similar effect.

Stress Induced Ulcer

Water immersion stress is one of the best models of stress in rats to induce ulcer. This provides both emotional and physiological stress to the animal[29].

Stress induced gastric ulcers have been used for evaluation of anti-ulcer activity in animals mediated by histamine release thereby increasing gastric acid secretion and reduction in mucous production, it also causes disturbances in the gastric mucosal microcirculation, motility and mucus production[30,31].

Stress is a major ulcerogenic factor and stress ulceration arises from diffuse lesions of the mucosal layer of the stomach occurring due to stressful events, such as severe trauma, extensive burns, shock, anxiety, and depression. Clinical fact has been proven that stress-induced gastric mucosal damage is mediated through reactive oxygen species (ROS) such as hydroxyl radicals (·OH) which is a toxic ROS, as it only needs a millisecond to cause DNA damage[32].

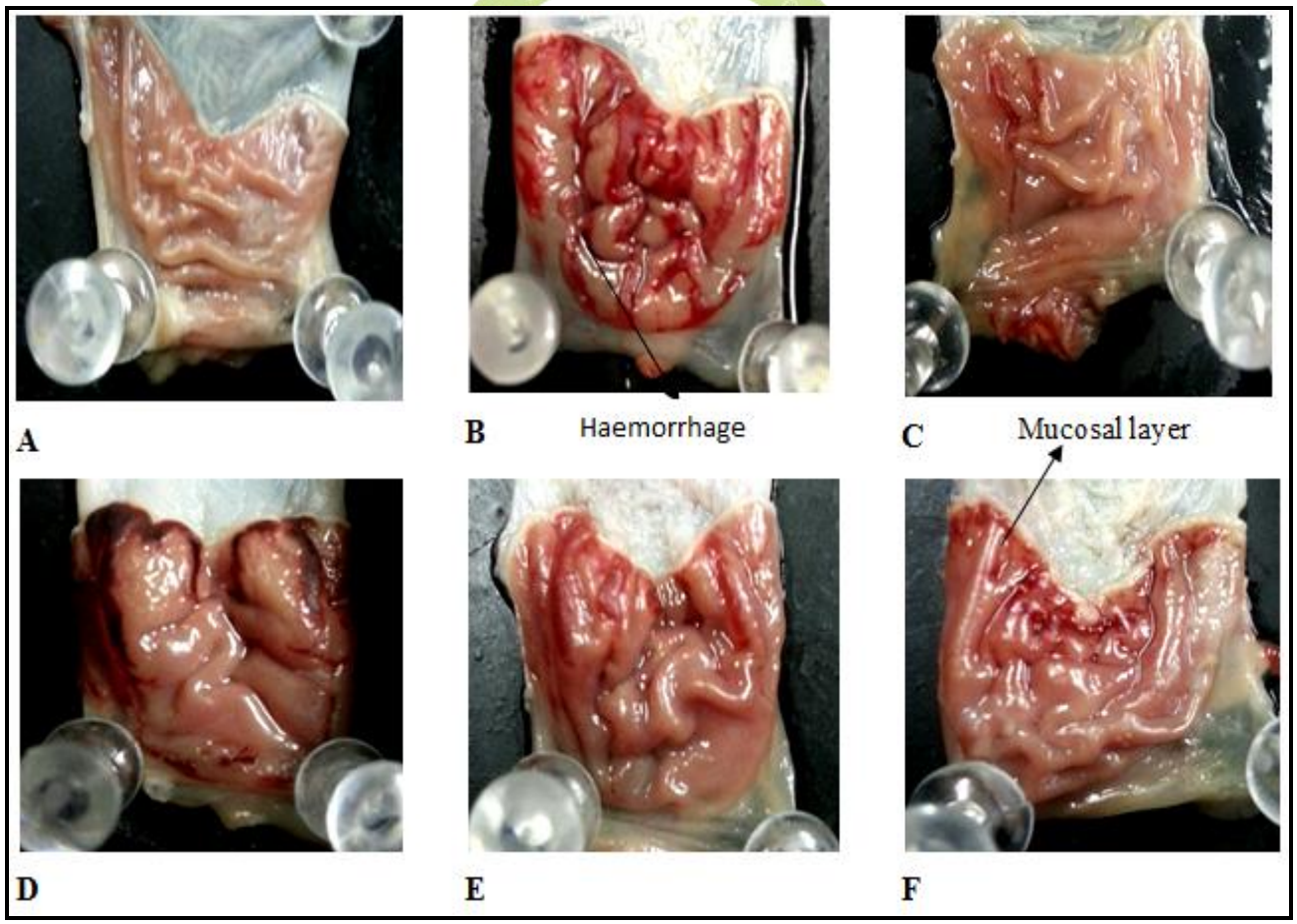

Figure 2: Mucosa lining with no ulcer formation (A). Severe visible hemorrhagic necrosis of gastric mucosa caused by absolute ethanol in ulcer control animals (B). Positive control (C). The ethanolic crude rind extract 100, 250 and 500 mg/kg dose (D-F).

Table 6: Gastro-protective effects of crude rind extract on stress-induced ulcer, ulcer index and inhibition percentage in rat model

Treatment	Dose	Ulcer index in mm (Mean ± S.D)	% Inhibition
Negative Control	5ml/kg	4.00 ± 0.00	-
Positive Control	100mg/kg	0.66 ± 0.57***	82.0
Crude cassava rind extract	100mg/kg	2.33 ± 0.58*	36.3
Crude cassava rind extract	250mg/kg	1.66 ± 0.57**	54.6
Crude cassava rind extract	500mg/kg	1.33 ± 0.57***	63.7

All value are represented as mean ± S.D (n=3). Statistical significance with One Way ANOVA followed by Dunnett's test (**p<0.05, ***p<0.001) in comparison to negative control group.

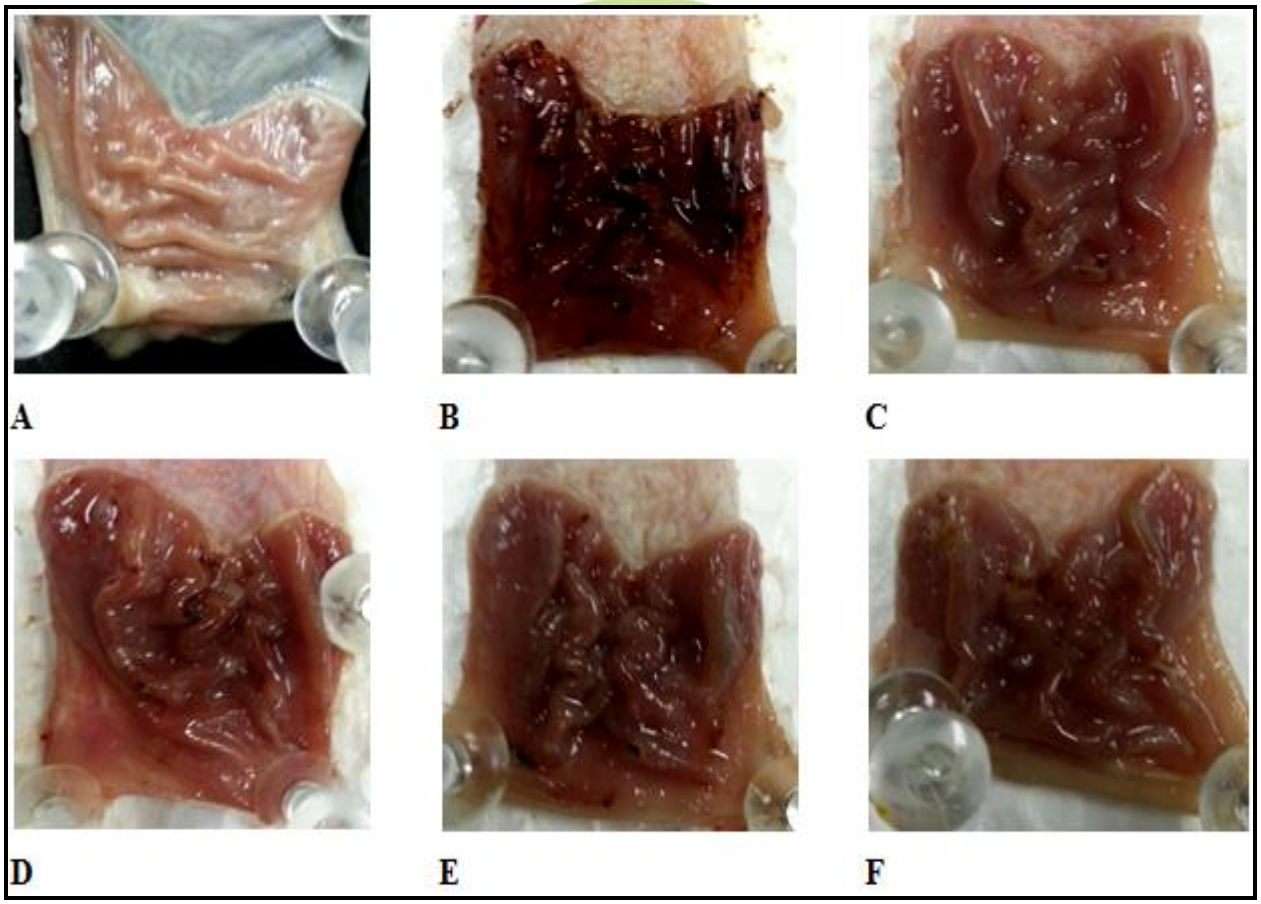

Figure 3: Gross evaluation of gastric mucosa of the rats in different groups.

Severe visible multiple hemorrhagic of gastric mucosa caused by stress in ulcer control animals (B). Mucosa lining with no ulcer formation (A). Positive control with lesser spots (C). The ethanolic crude rind extract against ethanol induced ulcer of gastric mucosa at 100, 250 and 500 mg/kg dose (D-F).

Stress is a major ulcerogenic factor and stress ulceration arises from diffuse lesions of the mucosal layer of the stomach occurring due to stressful events, such as severe trauma, extensive burns, shock, anxiety, and depression. Clinical fact has been proven that stress-induced gastric mucosal damage is mediated through reactive oxygen species (ROS) such as hydroxyl radicals (·OH) which is a toxic ROS, as it only needs a millisecond to cause DNA damage[32]. Antioxidants have been reported to play a significant task in gastric mucosa protection against various necrosis agents[33]. Table 6 shows significant protection against stress-induced gastric ulcers in rats. There were mild spot to almost normal in positive control (0.66 ± 0.57), haemorrhage spots were very severe and multiple dark patches of blood, black in appearance in negative control (4.0 ± 0.0), compared to the treated groups; mild to moderate spots in 100mg/kg (2.33 ± 0.58), mild spots in 250 and 500mg/kg (1.66 ± 0.57; 1.33 ± 0.57).

CONCLUSION

In conclusion, the present result demonstrates that the ethanolic cassava rind extract possess gastro-protective effect which may be due to the presence of phenolic compounds and its free-radical scavenging and in-*vitro* antioxidant activities.

REFERENCES

1. Tonukari, N. J. (2004). Cassava and the future of starch. *Electronic journal of biotechnology*, 7(1), 1982-1985.
2. AA, O., & Odunfa, S. A. (2012). Enhancing the Production of Reducing Sugars from Cassava Peels by Pretreatment Methods. *International Journal of Science and Technology*, *2*(9).
3. Oyedemi, S. O., Bradley, G., Afolayan, A. J. (2010). In -vitro and -vivo antioxidant activities of aqueous extract of Strychnos henningsii Gilg. 4(February), 70–78.
4. Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, *269*(2), 337-341.
5. Aarti, R., Sushil, B., Tenpe, C. R., & Yeole, P. G. (2009). Free radical scavenging activity of polyherbal formulation. *Pharmacologyonline*, 3, 944–951.
6. Naskar, S., Islam, A., Mazumder, U. K., Saha, P., Haldar, P. K., & Gupta, M. (2009). In vitro and in vivo antioxidant potential of hydromethanolic extract of Phoenix dactylifera fruits. *Journal of Scientific Research*, 2(1), 144-157.
7. Ragini, V., Bharathi, K., Prasad, K. V. S. R. G., & Sumalatha, G. (2010). Antidiabetic and Antioxidant Activities of Decalepis hamiltonii Wright & Arn. *International Journal of Innovative Pharmaceutical Research, 1(1), 1–10*.
8. Bose S, Maji S, Chakraborty P. (2011). Comparative study of In vivo antioxidant property at different Ixora species. *Journal of Advanced Pharmarcy Education and Research*. 2, 90–113.
9. Sidahmed, H., Hashim, N. M., Amir, J., Abdulla, M. A., Hadi, A. H. A., Abdelwahab, S. I., & Mohan, S. (2013). Pyranocycloartobiloxanthone A, a novel gastroprotective compound from Artocarpus obtusus Jarret, against ethanol-induced acute gastric ulcer in vivo. *Phytomedicine*, *20*(10), 834-843.
10. Mishra, V., Agrawal, M., Onasanwo, S. A., (2013). Anti-sensory and cyto-protective effects of chebulinic acid isolated from the fruits of Terminalia chebula on gastric ulcers. *Phytomedicine*. 20, 506–511.
11. Ajeigbe, K. O., Oladejo, E. O., Emikpe, B. O., Asuk, A. A., & Olaleye, S. B. (2011). The Dual Modulatory Effect of Folic Acid Supplementation on Indomethacin Induced Gastropathy in the Rat. *European Journal of Biological Sciences*. 3(3), 86–93.
12. Konturek, P. C., Brzozowski, T., Burnat, G., Szlachcic, A., Koziel, J., Kwiecien, S., &

Harsch, I. A. (2010). Gastric ulcer healing and stress-lesions preventive properties of pioglitazone are attenuated in diabetic rats. *Journal of Physiology and Pharmacology*, *61*(4), 429.

13. Akuodor, G. C., Akpan, J. L., Ezeunala Mercy, N., Ajoku Gloria, A., Essien, A. D., Megwas, A. U., & Osunkwo, U. A. (2012). Evaluation of anti-ulcer and antimicrobial effects of Verbena hastata leaf extract. *Afr. J. Pharm. Pharmacol*, *6*(11), 778-782.
14. Dai, J., & Mumper, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, *15*(10), 7313-7352.
15. Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, *269*(2), 337-341.
16. Zheng, W., & Wang, S. Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry*, *49*(11), 5165-5170.
17. Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S., & Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry*, *47*(10), 3954-3962.
18. Škerget, M., Kotnik, P., Hadolin, M., Hraš, A. R., Simonič, M., & Knez, Ž. (2005). Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities, *Food Chemistry*, *89*(2), 191-198.
19. Sowndhararajan, K., & Kang, S. C. (2013). Protective effect of ethyl acetate fraction of Acacia ferruginea DC. against ethanol-induced gastric ulcer in rats. *Journal of Ethnopharmacology*, *148*(1), 175-181.
20. Tirzitis, G., & Bartosz, G. (2010). Determination of antiradical and antioxidant activity: basic principles and new insights. *Acta Biochim Pol*, *57*, 139-42.
21. Parmer, S. M. (2012). Preliminary investigation of Herniaria incana Lam. Determination of the total flavonoid content, antioxidant properties and free radical scavenging capacity.
22. Rana, M. G., Katbamna, R. V., Padhya, A. A., Dudhrejiya, A. D., Jivani, N. P., & Sheth, N. R. (2010). In vitro antioxidant and free radical scavenging studies of alcoholic extract of Medicago sativa l. *Rom. J. Biol.–plant biol*, *55*(1), 15-22.
23. Patel, D. S., Shah, P. B., Managoli, N. B. (2012). Evaluation of In-vitro Anti-oxidant and Free Radical Scavenging activities of Withania somnifera and Aloe vera. *Asian Journal Pharm Tech*. 2(4), 143–147.
24. Govindan, P., & Muthukrishnan, S. (2013). Evaluation of total phenolic content and free radical scavenging activity of *Boerhavia erecta*. *Journal of Acute Medicine*, *3*(3), 103-109.
25. Yi, B., Hu, L., Mei, W., Zhou, K., Wang, H., Luo, Y., & Dai, H. (2011). Antioxidant phenolic compounds of cassava (Manihot esculenta) from Hainan. *Molecules*, *16*(12), 10157-10167.
26. Keser, S., Celik, S., Turkoglu, S., Yilmaz, O., & Turkoglu, I. (2012). Hydrogen peroxide radical scavenging and total antioxidant activity of Hawthorn. *Chemistry Journal*, *2*(1), 9-12.
27. Ham, M., & Kaunitz, J. D. (2007). Gastroduodenal defense. *Current Opinion in Gastroenterology*, *23*(6), 607-616.
28. Luiz-Ferreira, A., Almeida, A. C. A. D., Cola, M., Barbastefano, V., Almeida, A. B. A. D., Batista, L. M., & Brito, A. R. M. S. (2010). Mechanisms of the gastric antiulcerogenic activity of Anacardium humile St. Hil on ethanol-induced acute gastric mucosal injury in rats. *Molecules*, *15*(10), 7153-7166.

29. Arumugam, S., Selvaraj, S. V., Velayutham, S., Natesan, S. K., & Palaniswamy, K. (2011). Evaluation of anti-ulcer activity of Samanea saman (Jacq) merr bark on ethanol and stress induced gastric lesions in albino rats. *Indian Journal of Pharmacology*, *43*(5), 586.

30. Megala, J., & Geetha, A. (2012). Antiulcerogenic activity of hydroalcoholic fruit extract of *Pithecellobium dulce* in different experimental ulcer models in rats. *Journal of Ethnopharmacology*, *142*(2), 415-421.

31. Andreo, M. A., Ballesteros, K. V. R., Hiruma-Lima, C. A., Machado da Rocha, L. R., Souza Brito, A. R. M., & Vilegas, W. (2006). Effect of *Mouriri pusa* extracts on experimentally induced gastric lesions in rodents: Role of endogenous sulfhydryls compounds and nitric oxide in gastroprotection. *Journal of Ethnopharmacology*, *107*(3), 431-441.

32. Liu, X., Chen, Z., Mao, N., & Xie, Y. (2012). The protective of hydrogen on stress-induced gastric ulceration. *International Immunopharmacology*, *13*(2), 197-203.

33. Das, A. K., Bigoniya, P., Verma, N. K., & Rana, A. C. (2012). Gastroprotective effect of *Achyranthes aspera* Linn. leaf on rats. *Asian Pacific Journal of Tropical Medicine*, *5*(3), 197-201.

