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GC-MS Bioactive Compound Identification, *in vitro* Nutraceutical and Pharmacological Potential of Underutilized Leafy Vegetable *(Ipomoea batatas leaf)*

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Abstract

The search for dietary sources with potent biological activities has increasingly attracted considerable attention. Sweet potato leaves (SPL) are considered to be a leafy vegetable consumed by humans, which is not currently widely used, despite it possession drought tolerance, and the ability to grow in different climates and farming systems. This study evaluated the *in vitro* Nutraceutical and Pharmacological potential of underutilized leafy vegetable (Ipomoea batatas leaf). The phytochemicals, in vitro antioxidant and anti-inflammatory activities; mineral and vitamins compositions of the leaf were carried out using standard method. The bioactive compounds in *Ipomoea batatas* were identified using GC-MS. The results of the GC-MS profiling revealed n-Hexadecanoic acid (23.43%), Cyclotrisiloxane, hexamethyl (17.73%), and 16-Pregnenolone (11.30%) as the most abundant bioactive compounds in *Ipomoea batata* leaves. The mineral analysis showed that *Ipomoea batata* leaves contains favorable amount of macro elements such as Ca (915.40±9.50), K (2083.30±15.00), P (511.26±4.70), Mg (271.25±7.20), Na (9.98±1.06) and considerable amount of trace elements such as Fe (10.60±1.30), Mn (2.55±0.27), Zinc (2.70±0.11), and Cu (1.32±0.08) in which all were measured in miligram per 100g (mg/100). The vitamins such as of Vitamin A, vitamins C, vitamin E, niacin (B3), thiamine (B1), riboflavin (B2), pyridoxine (B6), vitamin D and vitamin K are present in trace amount in the leaves. The in vitro antioxidant activities of the extract showed a significant ($p<0.05$) dose dependent free radical scavenging activities. The anti-inflammatory analysis showed that the percent inhibition of hemolysis (20–100 µg/mL dry weight basis) was within the range of 4.1% to 18%. Percent inhibition of protein denaturation was within the range of 29.0–71.0%, Proteinase inhibitory activity was within the range of 16–30.0% and the lipoxygenase inhibition was within the range of 8.2–34.5%. This study provide validation for the usage of *Ipomoea batatas* leaf as new leafy vegetable with appreciable nutritional values and also provided credence to the bioactive compounds in leaf as potential novel drug candidate for pharmaceutical industries.

Keywords: Antioxidant, anti-inflammatory, Nutraceutical, Pharmacology, GC-MS profiling.

INTRODUCTION

The world is confronted with the challenges of climate change, terrorism, and poverty, among other factors, which hinder food production, food availability, as well as food and nutritional security. Globally, food insecurity and low supply in many areas are threatening the human population and survival in the areas where terrorism exist. Food and vegetable as an important commodity for survival is under threat, and if survival strategies are not devised, the catastrophe will be overbearing. A number of leafy vegetable species are becoming extinct from our agricultural and forest fields, while some others are declining in utilization (Mabhaudhi *et al.*, 2017).

In order to meet the global food demands, focus should be on promoting the utilization leafy vegetable which have been neglected but have the potential to enhance food and nutrition security especially in the developing countries of sub-Saharan Africa (Li *et al.*, 2018). The search for dietary sources

with potent biological activities has increasingly attracted considerable attention (Nguyen et al., 2018). There is a great deal of interest in using potent dietary antioxidants in foods and pharmaceuticals to prevent oxidative reactions and chronic degenerative diseases (Pham et al., 2020).

Sweet potato (Ipomoea batatas [L.] Lam.) is considered to be a major food crop worldwide, and it is widely produced and consumed in East Asia, Oceania, and Sub-Saharan Africa, with the highest production in China which comprises 76.07% of the world's production (Shekhar *et al.*, 2015). Sweet potato leaves (SPL) are considered to be a leafy vegetable consumed by humans, which is not currently widely used, despite it possession drought tolerance, and the ability to grow in different climates and farming systems (Taira *et al.*, 2013).

Sweet potato leaves are a good source of nutrients, enhancing dietary protein, amino acid intake, and growth performance (Hong and Lindberg, 2004). Furthermore, these major nutrients play a role in reducing the risks associated with certain diseases (Sun *et al.*, 2014). It was reported that SPL consumption can decrease the risks associated with cardiovascular disease due to the availability of complex carbohydrates, low-fat content, high dietary fiber (Islam, 2006).

Since numerous health-promoting phytochemicals are found in SPL, regular intake of the leaves provides various health benefits. Among them, polyphenol constituents show various physiological functions and promote human health (Islam, 2006). Leaves of sweet potato are rich in chlorogenic acid, a caffeoylquinic acid derivative, which is well-known for its health benefits, including protection against cancers, hypertensions, bacteria, diabetes, and heart disease. This study is designed to carry out *in vitro* Nutraceutical and Pharmacological potential of underutilized leafy vegetable (Ipomoea batatas leaf).

MATERIALS AND METHODS

Plant Collection

The leaf of *Ipomoea batatas* was collected from a garden in Owerri, Imo State. The plant was identified by a botanist at the Department of Biology and Environmental Microbiology Federal Polytechnic Nekede, Imo State. The fresh sample of *Ipomoea batatas* leaf was washed with distilled water and then allowed to get dried in a dust-free environment for ten days. The dried sample was blended using an electronic blender.

Preparation of Plant Extract

One thousand gram (1000g) of powdered leaves was macerated in 2.5L of 95% methanol at room temperature for 72h. It was continuously mixed and then filtered using a filter paper (Whatman size No.1). The filtrate was concentrated using a water bath at 45^0c. The extract of *Ipomoea batatas* leaf was refrigerated in air tight container.

Determination of Mineral and Vitamin Composition

This will be done using standard analytical methods as described by AOAC (2000)

Evaluation of In Vitro Antioxidant Activity

The reducing powers of the extract was carried out following the method of Moein *et al.*, (2012). Hydrogen peroxide (H_2O_2) Scavenging activity by the plant extract was determined by the method of Jie *et al.*, (2008). Nitric oxide radical scavenging activity was determined according to the method of Jun *et al.*, (2011).

Evaluation of In Vitro Anti-Inflammatory Activity

The heat-induced hemolysis was carried out as described by Okoli *et al.*, (2008), with some modifications. Protein denaturation assay was done according to the method of Mizushima and Kobayashi (1968). Proteinase inhibitory activity of the leaf extracts and lipoxygenase inhibition activity of the extracts of leafy vegetables were assayed according to the method described by Gunathilake *et al.*, (2018).

GC-MS (Gas Chromatography-Mass Spectrometry) Analysis

The analysis of bioactive compound from extract was carried out using Agilent Technologies Gas Chromatography systems 7890A coupled with Mass spectrometry 5975C model equipped with HP-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film). Helium gas was used as the carrier gas with flow rate of 1.5mL/min. The initial temperature was set at 70 for 0.5min to 280 °C with increasing rate of 12 °C/min and holding time of about 5min. Furthermore, One microliter was injected into 250°C inlet with a splitless mode. The detection of compounds involved an electron ionization system which involves high energy electrons (70 eV). The relative quantity of the compounds present in the extract was expressed as percentage based on peak area produced in the chromatogram.

Characterization of Compounds

Interpretation of mass spectra of GC-MS was conducted using the database of National Institute of Standard and Technology (NIST). The mass spectrum of the unknown compound was compared with that of the known components stored in the NIST-library. The name, molecular weight and structure of the components of the test materials were ascertained

Statistical Analysis

Statistical analysis of the data will be carried out with SPSS version 22.0 using One Way Analysis of Variance (ANOVA). The statistically analysed data was

reported as Mean+SEM. Significant difference will be accepted at 95% confidence level of probability ($P < 0.05$).

RESULTS AND DISCUSSION

Bioactive compounds in methanol extract of *Ipomoea batatas* leaf

The GC-MS profiling of methanol extract of *Ipomoea batatas* leaf revealed 34 bioactive compounds with n-Hexadecanoic acid (23.43%), Cyclotrisiloxane, hexamethyl (13.73%), 16-Pregnenolone (11.30%), 1-Docosene (6.51%), 3-(3,4-Dimethoxycinnamoyl)-4-hydroxy-6-methyl-2H-pyran-2-one (5.59%) as the five most abundant compounds. The Chromatogram of the GC-MS result was shown in figure 1. The different bioactive compounds in methanol extract of *Ipomoea batata* leaf are shown in table 1. The mineral and vitamin compositions of the extract are shown in the table 2 and 3 respectively. The results revealed appreciable amount of mineal and vitamin in *Ipomoea batata.*

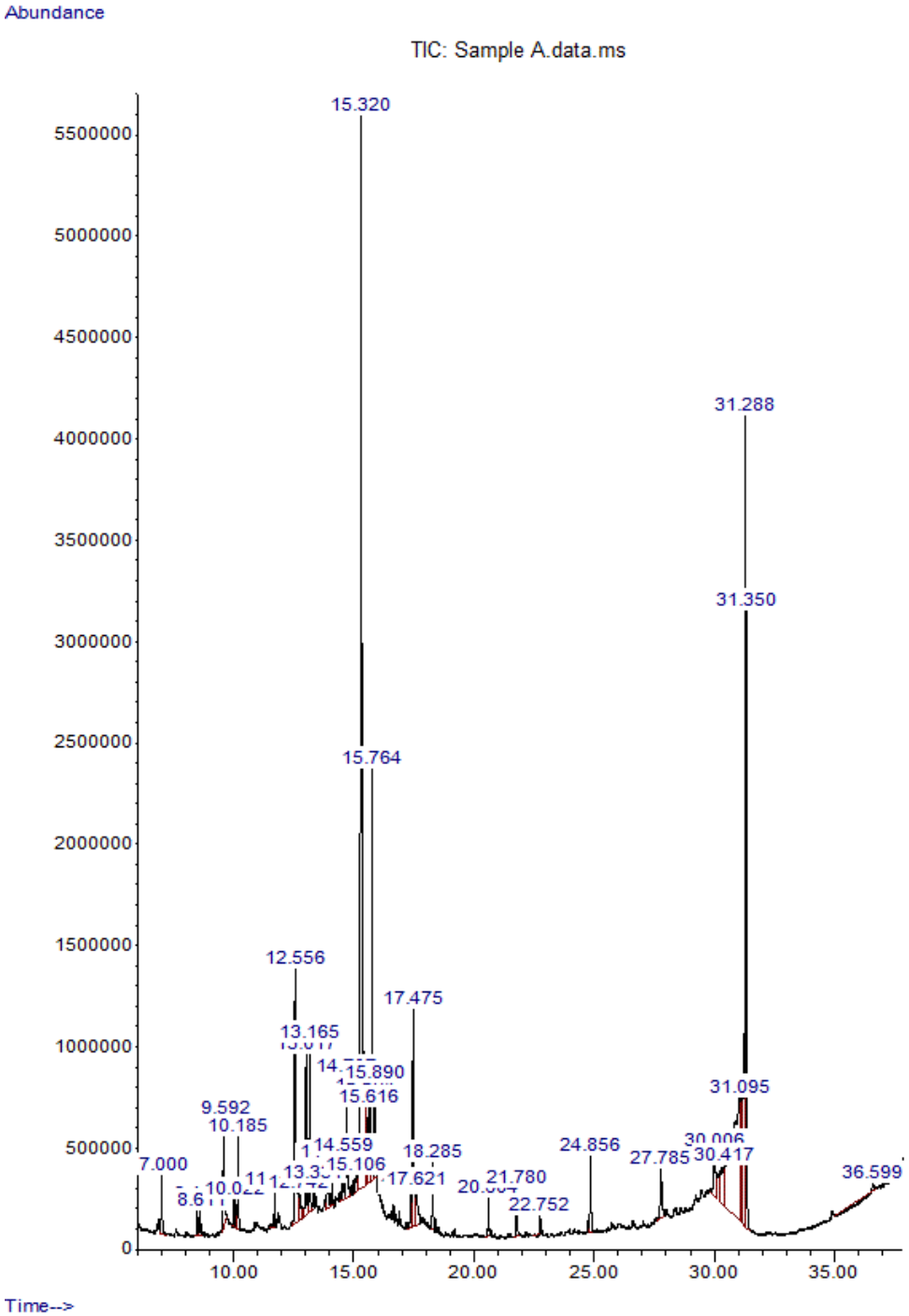

Figure 1: Chromatogram of methanol extract of *Ipomoea batata* leaf

Table 1: Phytocomponents identified in methanol extract of *Ipomoea batata* leaf by GC –MS

PK	RT	Area%	PubChem CID	Molecular Formular	Library ID
1	7.000	0.88	12389	C14H30	Tetradecane
2	8.481	0.46	7311	C14H22O	2,4-Di-tert-butylphenol
3	8.611	0.35	12391	C15H32	Pentadecane
4	9.592	1.87	3893	C12H24O2	Dodecanoic acid
5	10.022	0.34	545757	C20H39ClO2	3-Chloropropionic acid, heptadecyl ester
6	25.985	2.25	11006	C16H34	Hexadecane
7	11.706	0.50	12398	C17H36	Heptadecane
8	23.298	5.69	11005	C14H28O2	Tetradecanoic acid
9	13.017	2.24	5365037	C20H40	9-Eicosene
10	13.165	2.18	11635	C18H38	Octadecane
11	13.331	0.49	66281	C18H37ClO2S	1-Octadecanesulphonyl chloride
12	13.920	0.94	10887	C17H34O	9-Heptadecanone
13	14.062	0.65	545303	C17H24O3	7,9-Di-tert-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione
14	14.559	0.74	12401	C19H40	Nonadecane
15	14.707	2.03	3026	C16H22O4	Dibutyl phthalate
16	15.106	0.47	550059	C23H30N2O5	Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy
17	30.824	23.43	985	C16H32O2	n-Hexadecanoic acid
18	15.616	1.70	109858	C48H99AuS3	tert-Hexadecanethiol
19	33.964	6.51	74138	C22H44	1-Docosene
20	17.367	0.61	6439696	C36H66O4	9,12-Octadecadienoic acid (Z,Z)
21	17.475	4.15	5282761	C18H34O2	cis-Vaccenic acid
22	17.621	0.97	5365672	C20H36O2	9,12-Octadecadienoic acid, ethyl ester
23	20.604	0.47	9554	C8HF15O2	Pentadecafluorooctanoic acid, octa decyl ester
24	21.780	0.72	33934	C24H38O4	Diisooctyl phthalate
25	22.752	0.16	5365022	C35H70	17-Pentatriacontene
26	24.856	1.13	638072	C30H50	Squalene
27	27.785	1.22	444679	C28H44O	Ergosterol
28	30.006	1.52	541084	C24H27NO7	N-Acetoacetyl-deacetylcolchicine
29	30.417	2.27	521562	C13H22OSi	Thymol, TMS derivative
30	31.052	13.73	172238	C6H18O5Si4	Cyclotrisiloxane, hexamethyl
31	31.095	1.82	77092	C10H28O4Si3	Silicic acid, diethyl bis(trimethylsilyl) ester
32	31.288	11.30	3035284	C21H32O2	16-Pregnenolone
33	31.350	5.59	135408774	C17H16O6	3-(3,4-Dimethoxycinnamoyl)-4-hydroxy-6-methyl-2H-pyran-2-one
34	36.599	-1.04	91733953	C13H20N2SSi	1,2-Benzisothiazol-3-amine, TBDMS derivative

Table 2: Mineral composition of Ipomoea batata leaf

Minerals	Amounts (mg/100g)
Ca	915.40±9.50
K	2083.30±15.00
P	511.26±4.70
Mg	271.25±7.20
Na	9.98±1.06
Fe	10.60±1.30
Mn	2.55±0.27
Zn	2.70±0.11
Cu	1.32±0.08

Values are Mean±SD of triplicate determination.

Table 3: Vitamin compositions of Ipomoea batata leaf

Vitamins	Amounts (mg/100g)
C	0.10±0.01
B_1	0.04±0.00
B_2	0.18±0.01
B_3	0.98±0.02
B_6	0.11±0.00
A (beta carotenoids)	0.17±0.01
E	0.03±0.00
D	0.02±0.00
K	0.02±0.00

Values are Mean±SD of triplicate determination

Antioxidant and anti-inflammatory activities

The antioxidant and anti-inflammatory activities of methanol extract of *Ipomoea batatas* leaf were shown in figure 2 to 6. The reducing power potential of the extract are represented in absorbance. The percentage nitric oxide inhibition by the extract ranged from 15.35-52.28% compared to ascorbic acid (39.45-79.13%) which served as the control. The percentage inhibition of hydrogen peroxide by the extract ranged from 11.6-58.27% compared to the standard control (ascorbic acid) which ranged from 32.34-75.63%. The anti-inflammatory analysis showed that the percent inhibition of hemolysis (20–100 µg/mL dry weight basis) was within the range of 4.1% to 18%. Percent inhibition of protein denaturation was within the range of 29.0–71.0%, Proteinase inhibitory activity was within the range of 16–30.0% and the lipoxygenase inhibition was within the range of 8.2–34.5%.

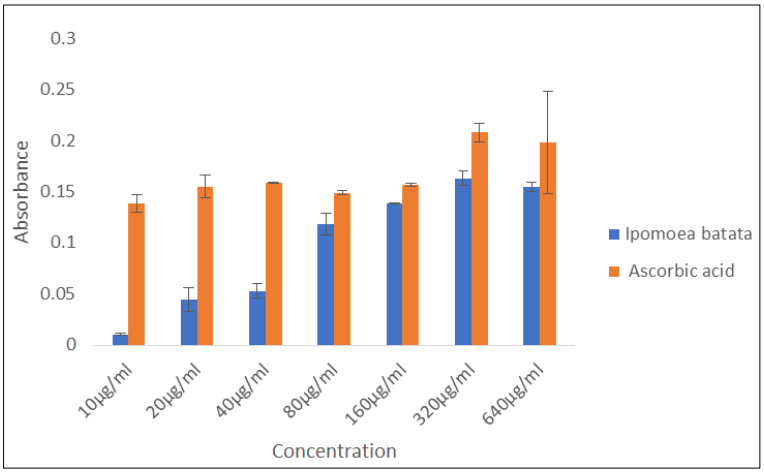

Figure 2: Reducing power of methanol fraction of Ipomoea batata leaf

Values are represented as mean ± SEM (n=3). Standard ascorbic acid served as the positive control

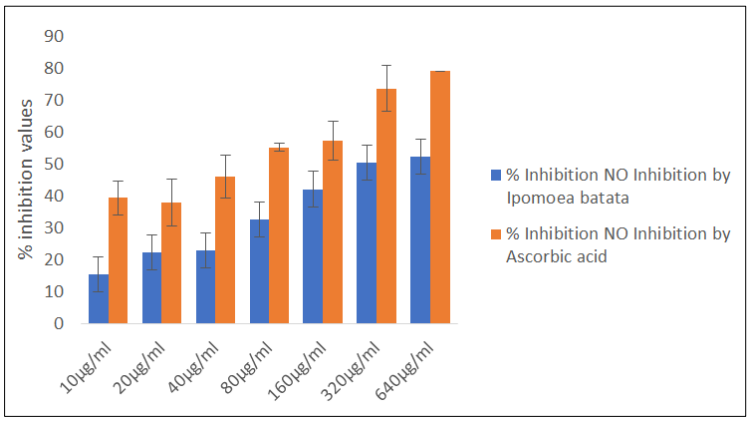

Figure 3: Nitric oxide (NO) scavenging activity of N-hexane fraction of *Ipomoea batata* leaf

Values are represented as mean ± SEM (n=3). Standard ascorbic acid served as the positive control

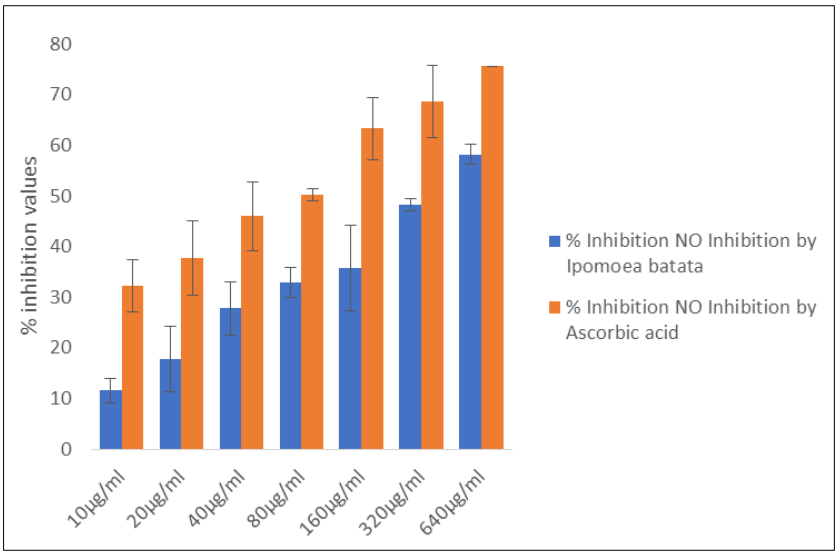

Figure 4: Hydrogen peroxide (H_2O_2) scavenging activity of methanol fraction of *Ipomoea batata* leaf
Values are represented as mean ± SEM (n=3). Standard ascorbic acid served as the positive control

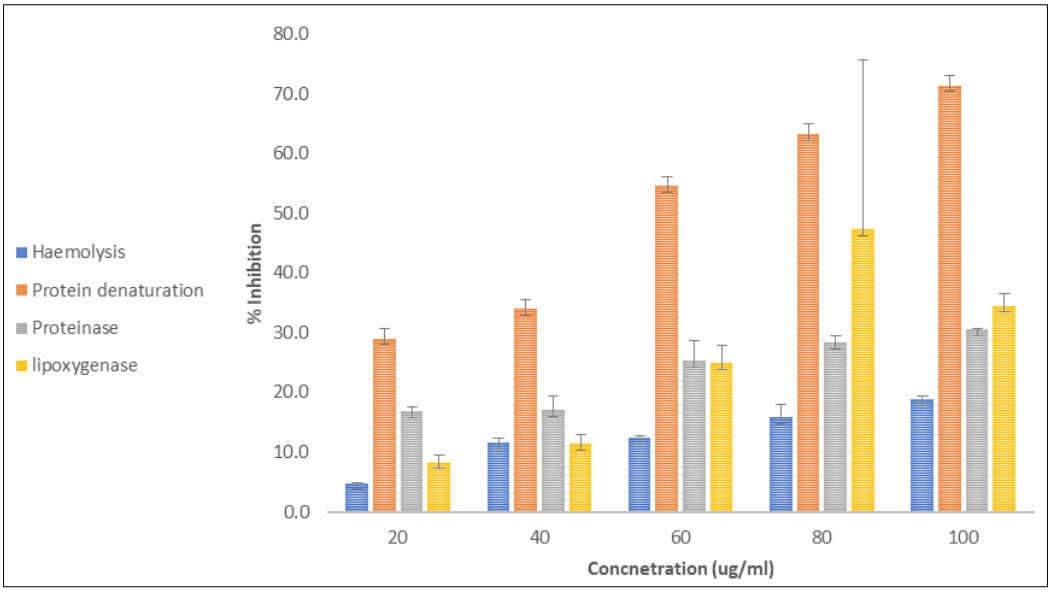

Figure 6: *In-vitro* anti-inflammatory effect of the methanol extract of Ipomoea batatas leaf

DISCUSSION

In recent years, the search for phytochemicals possessing anti-inflammatory and antioxidant properties has been on the rise due to their potential use in the therapy of various chronic and infectious diseases. Epidemiology and experimental studies have implicated oxidative cellular damage arising from an imbalance between free radical generating and scavenging systems as the primary cause of cardiovascular, diseases, cancer, aging (Halliwell, 2004). The present study reveals 34 bioactive compounds in the methanol extract of *Ipomoea batatas* leaf with n-hexadecanoic acid, Cyclotrisiloxane, hexamethyl and 16-Pregnenolone as the most abundant. Guerrero *et al*. (2017) reported that n-hexadecanoic possess antioxidant, Pesticide, Flavor, 5-Alpha reductase-inhibitor, antifibrinolytic, and Nematicide potentials. Ismail *et al*. (2020) reported that cyclotrisiloxane, hexamethyl possess some antimicrobial and antioxidant properties. Murugan *et al.* (2019) reported that pregnenolone exerts anti-inflammatory effect and maintain immune homeostasis in various inflammatory conditions. Pregnenolone and its metabolic derivatives have also been shown to have beneficial effects in the brain, including enhancing memory and learning, reversing depressive disorders, and modulating cognitive functions (Murugan *et al.*, 2019).

Sweet potato leave can be processed to create goods with high nutraceutical values that offer wholesome food and enhance human health (Nguyen *et al.*, 2021) because of its high antioxidant potential, high mineral contents, moderate vitamin composition and anti-inflammatory potential. The present study showed that *Ipomoeo batatas* leaf has good amount of potassium which suggest usefulness in the management

hypertensive (Awol, 2014). Calcium is an essential mineral for life and a crucial part of a balanced diet. It is crucial for maintaining healthy bones and teeth throughout life and for developing strong, thick bones. Ipomoea batatas leaves have a have appreciable mount of calcium. The level of calcium in *Ipomoea batatas* leaves is an indication that the leaf may be helpful in the development and maintenance strong, robust, and healthy bones and teeth (Awol, 2014).

Vitamins are crucial for the normal growth and development of the human body. The study showed varying levels of vitamins in the samples. The moderate amount of vitamin E and C which serves as antioxidant also justify the radical scavenging potential seen in the antioxidant assay. These vitamins help in the maintenance of epithelial cell activities, vitamin A is essential for healthy immunological, growth, and gene expression (Lukaski, 2004). Vitamin C aids in the reduction of folic acid intermediates, the transport and uptake of non-heme iron at the mucosa, and the creation of cortisol (Tang *et al*., 2021). Red blood cell generation and appropriate muscle function depend on vitamin E, a potent antioxidant that helps prevent cell damage from free radicals. Vitamin E is also essential for healthy skin, eyes, and bones (Lukaski, 2004).

Although nitric oxide plays a crucial role in inflammatory processes, when levels are too high, they are directly harmful to tissues, leading to vascular damage and other illnesses. The activity of hydrogen peroxide (HP) as an active oxygen species has been reported to come mainly from its potential to produce the highly reactive hydroxyl radical through the Fenton reaction. Therefore, inhibition of H_2O_2 formation will prevent the further generation of radicals. The result showed some significant ($P<0.05$) hydrogen peroxide scavenging activity of the extract in dose dependent manner.

Membrane proteins may be able to control the volume and water content of cells. Damage to the membrane will impact this function by interfering with the passage of sodium and potassium ions (Okoli *et al*., 2008). Inhibiting red blood cell hemolysis may provide light on the inflammatory process since the red blood cell membrane resembles the lysosomal membrane (Umapathy *et al*., 2010). The lysis and subsequent release of the cytoplasmic contents of these cells may be delayed or inhibited by stabilizing their membranes, which reduces tissue damage and, consequently, the inflammatory response (Okoli *et al*., 2008). Therefore, in order to stop the spread of inflammation, chemicals that significantly protect cell membranes from harmful molecules are crucial. The current study on membrane stability revealed that the extract prevented the lysis of red blood cells brought on by heat. In situations like arthritis, an inflammatory process results in the denaturation of protein molecules, which is well-documented in the literature (Umapathy *et al*., 2010). The prevention of protein denaturation is one of the primary mechanisms of action of NSAIDs, as described by Mizushima. (Govindappa *et al*., 2011). Proteinase inhibitors reportedly offered a large amount of protection (Gunathilake *et al*., 2018). Various recent research have indicated that flavonoids contributed considerably to the antioxidant and anti-inflammatory effects of many plants. Therefore, these leaves' anti-inflammatory properties may be influenced by the bioactive compounds they contain. The cascade process of arachidonic acid metabolism is hypothesized to be blocked or interfered with by plant extracts decreasing lipoxygenase activity. Additionally, they may act as scavengers of various reactive free radicals created during arachidonic acid metabolism (Gunathilake *et al*., 2018).

CONCLUSION

This study showed that Ipomoea batatas leaf possess vital bioactive compounds that could play significant roles in promoting health and preventing disorders associated with oxidative stress and inflammation.

Conflict of Interest

The authors declare that no conflict of interest exists with respect to this work.

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