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In vitro Evaluation of Polyphenol Rich Fraction of *Ipomea batatas* (Mother delight) Leaf Extract as Anti-inflammatory Agents and its LCMS Profile

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Abstract

High intake of plant foods is associated with lower risk of chronic diseases as suggested by epidemiological evidence. This study aimed to investigate the anti-inflammatory potential of polyphenol rich fraction of Ipomoea batatas leaf extract and the compounds possibly responsible for the activity using LCMS. The anti-inflammatory studies were carried out in vitro by protein denaturation technique. The result shows significant different ($p<0.05$) percentage inhibition of denaturation 90.59% at 1000 µg/ml of the extract compared to the standard drug (declofenac) 95.12% at 1000 µg/ml. The LCMS profiling of the extract revealed the presence of many metabolites (polyphenols) such as chrogenic acid, myricetin, furocoumarinic acid, aromadendrin, naringenin, 4,5-dicaffeolquinic acid and abietinol among others. The presence of these metabolites could be the reason for the anti-inflammatory effect of the extract observed. This implies that the plant can be exploited for its medicinal, therapeutic properties and possibly used to reduce the risk of many chronic diseases.

Keyword: Anti-inflammatory activity, Protein denaturation technique, Mother delight, LCMS, Phytochemicals.

INTRODUCTION

Sweet potato, *Ipomoea batatas* (L.) Lam, is a perennial crop which belongs to the morning glory family or Convolvulaceae (Ayeleso *et al.*, 2016). It is majorly cultivated in tropical and subtropical areas, a popular staple food with a nutritional importance evidenced by increased cultivation and consumption. Sweet potato is mostly harvested for its tubers (Ayeleso *et al.*, 2016). In addition, about 95-98% of the leaves are discarded while the remaining 2-5% are used as animal food (Hue *et al.*, 2012). However, the leaves are also sometimes consumed as an alternative to other leafy vegetables (Ayeleso *et al.*, 2016). It is the sixth most important food crop in the world and it contains phytochemicals, which are important for human health (Ayeleso *et al.*, 2016). Other than their nutritional benefits such as a rich source of dietary fibre, antioxidants, vitamins, and minerals, sweet potato root tubers also contain no saturated fats or cholesterol. Islam (2014) reported that sweet potato leaves contain more polyphenols than any other commercial vegetables such as spinach, cabbage, and lettuce. He stated that,sweet potato leavescontain at least 15 anthocyanins and 6 polyphenolic compounds.Reports have indicated that the phytochemicals in sweet potato possess multiple actions, such as antioxidant, antimutagenic, anti-inflammatory, antimicrobial and anti-carcinogenesis and thus are important for several health-promoting functions in humans (Ayeleso *et al.*, 2016). Different sweet potato varieties are grown worldwide, generally characterized by the different flesh colors with varying phytochemical compositions. Different varieties of a plant may inherently differ in their nutritional values and in the bioactivities of phytochemicals present in the plants (Ayeleso *et al.*, 2016). The nutritional value and medicinal potentials of sweet potatoes are gaining the attention of so many research groups as the quest for natural remedies from plants and the understanding between diet and health increases worldwide. Sweet potato plant alongside being primarily a food resource, may as well be exploited for its medicinal properties due to its high nutritive and therapeutic properties (Ayeleso *et al.*, 2016).

Traditionally, the roots and leaves of *I. batatas* have been used in treating urinary infections, reducing fever, skin diseases, diabetes, curing boils and acnes (Kang *et al.*, 2014). A review on pharmacological studies on *I. batatas* indicated that it possess anti-diabetic, hypoglycemic, neuroprotective, antiproliferative, antioxidant, antiulcer, antitumor, anti-inflammatory, wound healing, antimutagenic and hepatoprotective properties (Kang *et al.*, 2014).

Inflammation is an orchestrated biological process, induced by microbial infection or tissue injury. A major inflammation trigger is the recognition of microbes by specific receptors of the innate immune system, which play a crucial role in the induction of early signals initiating and establishing the inflammatory setting (Nathan, 2002). The primary function of inflammation is resolving the infection and repairing the damage to achieve homeostasis equilibrium. Thus, the ideal inflammatory response is rapid, destructive, specific and self-limiting (Barton, 2008).

Polyphenols are plant's secondary metabolites in various foods (López-Fernández *et al.*, 2020). These natural compounds constitute a group of molecules divided according to their chemical structure (Belš˘cak Cvitanovi´c *et al.*, 2018, Rajauria, 2018). However, they can also be classified by their source of origin, natural distribution or biological function. In particular, according to their chemical structure, they can be classified into different groups as a function of the number of phenol rings contained and the structural elements that bind these rings (Rajauria, 2018). In recent years, numerous studies have shown that consuming polyphenols in the diet provides multiple health benefits. This is primarily due to the antioxidant properties that help to prevent various diseases associated with oxidative stress (López-Fernández *et al.*, 2020). Studies like those of Scalbert *et al.* (2005) and Seo *et al.* (2013) demonstrated that the antioxidant activity of plant polyphenols can retard the development of diseases such as cancer, cardiovascular and neurodegenerative diseases (Seo *et al.*, 2013).

Besides the health implications, there is a growing interest in using new natural additives in food industry (Munekata *et al.*, 2020). It is well known that oxidative reactions are the leading non-microbial cause of food quality deterioration (Domínguez, *et al.*, 2019). However, consumers are concerned about the diet–health relationship and demand healthy and natural foods, forcing manufacturers to limit the use of synthetic antioxidants in food formulation. Thus, using of polyphenol rich extracts as synthetic additives re-placers was an essential strategy for food manufacturers (Pateiro *et al.*, 2018).

On the other hand, several studies have reported that antioxidants play important roles in preventing ageing and age-related diseases (Sun, *et al.*, 2014). Due to the safety concerns associated with supplemental forms of antioxidants, consumers are paying more attention to fruits and vegetables as natural sources of antioxidants. Hence, these leaves can be utilized as a potential source of natural antioxidant. Therefore, this study aimed to investigate the anti-inflammatory potential of the polyphenols rich fraction of I. *batatas* leave extract and evaluate its LCMS profile.

MATERIALS AND METHODS

Plant Collection

Fresh Leaves from *Ipomoea batatas* (Mother delight) cultivars were collected from the Training farm of The Centre for Dryland Agriculture, Bayero University, Kano. And it was authenticated at the Habarium unit, where a reference number (Umuspo 2) was deposited.

Extraction of the plant leaves

The extraction was carried out as reported by Bello *et al.* (2022). The grounded sample was exhaustively extracted with 50:50v/v (ethanol-water) solvent using the percolation method. About 250 g of the grounded sample was weighed into an extraction bottle containing 400 mL ethanol-water (50:50v/v) and allowed to soak. The soaked sample was filtered into a different sample bottle. However, the extract was concentrated using a rotary evaporator (RE 300, Barloworld Scientific Limited), and subjected to dryness using evaporating basin for some days.

Evaluation of the *in vitro* anti-inflammatory activity

Both the fraction of the extract and the standard drug were subjected to *in vitro* anti-inflammatory activity by protein denaturation technique in various concentrations i.e. 62.5, 125, 250, 500 and 1000 µg/mL as described by Pawar and Chavan (2012), with some modifications. Briefly, the standard drug and fraction of the extract were dissolved in minimum quantity of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solution was less than 2%. Test solution (250 µL) containing different concentrations of the drug was mixed with 250 µl of 1mM albumin solution in phosphate buffer and incubated at 27° + 1° C in a water bath incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60° + 1° C in a water bath for 10 min. After cooling, the turbidity was measured at 660 nm using micro plate reader (multiskan sky). The percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate, and the average was taken. The diclofenac was used as positive control. The percentage inhibition of denaturation was calculated by using the following formula (Pawar and Chavan 2012).

$$\% \, Inhibition = 100 \times [\frac{Vt}{Vc}] - 1$$

Where,
Vt = Mean absorbance of test sample.
Vc = Mean absorbance of control

The LCMS profiling of the *Ipomoea batatas* leaves extract

The polyphenol rich fraction of *Ipomoea batatas* (Mother delight) leaf extracts were analyzed using liquid chromatography (LC) and mass spectrophotometer (MS) as described by (Piovesana *et al.*, 2019) with some modifications. The extracted sample was reconstituted in methanol and filtered through a polytetrafluoroethylene (PTFE) membrane filter of 0.45 µm size. After filtration, the filtrate (10.0 µl) was injected into the LC system and allowed to separate on Sunfire C18 5.0µm 4.6mm x 150 mm column. The run was carried out at a flow rate of 1.0 mL/min, with sample and column temperature at 25ºC. The mobile phase consists of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) with a gradient as shown in Table 1:

Table 1: Solvent Gradient

Time	0.1% formic acid in water (%A)	0.1% formic acid in Acetonitrile (%B)
0	95	5
1	95	5
13	5	95
15	5	95
17	95	5
19	95	5
20	95	5

From the ratio of A/B 95:5 this ratio was maintained for a further 1 min, then A/B 5:95 for 13min, to 15min. Then A/B 95:5 to 17min, 19min and finally 20min. The PDA detector was set at 210-400nm with resolution of 1.2nm and a sampling rate at 10 points/sec. The mass spectra were acquired with a scan range from m/z 100 – 1250 after ensuring the following settings: ESI source in positive and negative ion modes; capillary voltage 0.8kv (positive) and 0.8kv (negative); probe temperature 600ºC; flow rate 10 mL/min; nebulizer gas, 45 psi. MS set in automatic mode applying fragmentation voltage of 125 V. The data was processed with Empower 3 software. The compounds were identified based on fragmentation pattern, Base m/z and the spectrum interpretation was performed using a spectrum database for organic compounds in SDBS application as described by Hanafi *et al.*, (2018).

Statistical analysis

The data of the average percentage inhibition of inflammation by the extract used was analysed using One-Way ANOVA. The results were presented as the means ± standard deviation. Significance level for the differences was set at $p<0.05$.

RESULTS

The *in vitro* anti-inflammatory activity was determined by protein denaturation technique in various concentrations, i.e. 62.5, 125, 250, 500 and 1000 µg/mL of both the polyphenol rich fraction of *Ipomoea batatas* extracts, as well as standard drug as reported by Pawar and Chavan (2012), the percentage inhibition of both the extract and standard drug were presented in Table 2.

Table 2: The inhibition effect of different concentration of the extract and the standard drug

Concentration (µg/mL)	% Inhibition	
	Std drug	Extract
62.50	28.32	11.62
125.00	39.09	27.39
250.00	52.32	48.70
500.00	85.44	74.64
1000.00	95.12	90.59

Similarly, the polyphenol rich fraction of *Ipomoea batatas* leaf extract was subjected to LCMS analysis. The total ion chromatogram and tentative compounds identified were presented in Figure 1 and Table 3, respectively.

Many phenolic metabolites (polyphenols and others) were present such as Chlorogenic acid, Myricetin, furocoumarinnic acid, Aromadendrin, Naringenin, 4, 5 - Dicaffeolquinic acid and Abietinol, among others. The molecular fragmentation patterns of the identified metabolite were presented in figures 2-8 and are reported to have pharmacological properties.

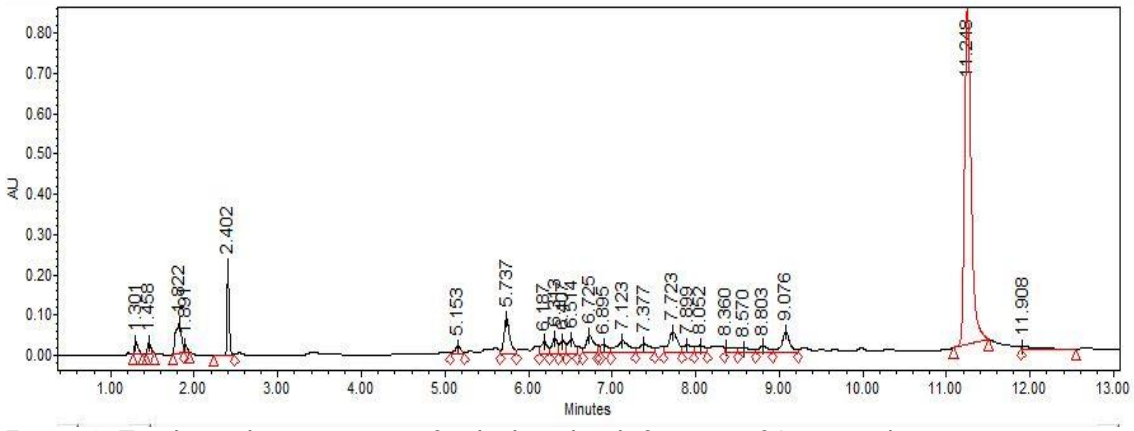

Figure 1: Total ion chromatogram of polyphenol rich fraction of *Ipomoea batatas* extract

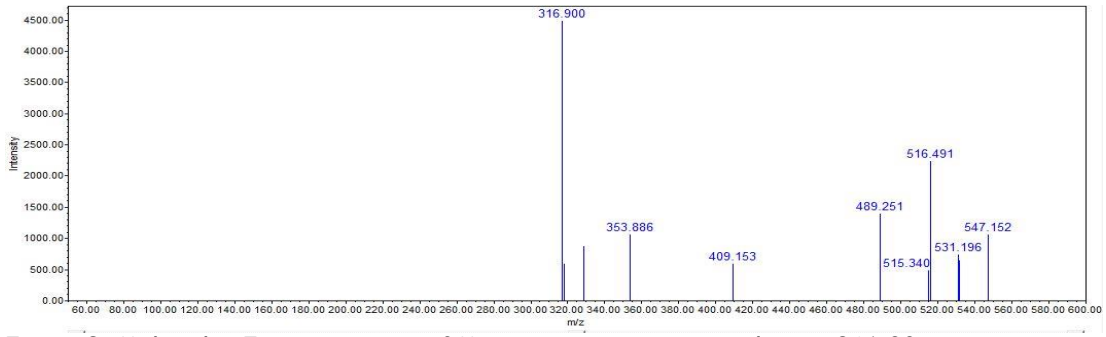

Figure 2: Molecular Fragmentation of Myricetin at positive mode (mz 316.90)

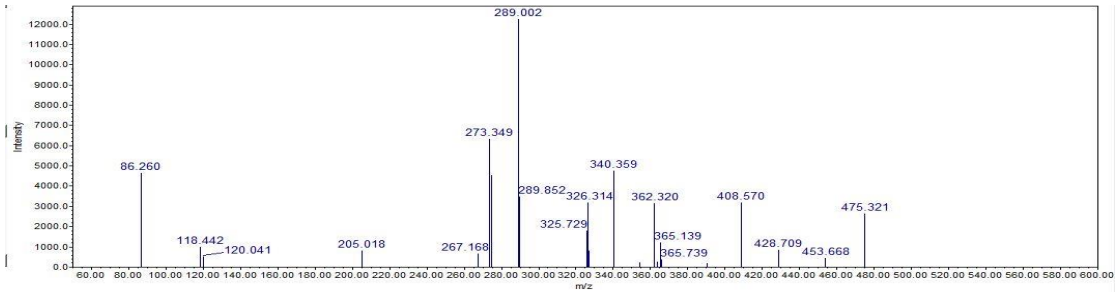

Figure 3: Molecular Fragmentation of Abietinol (Diterpenoids) at positive mode (mz 289.002)

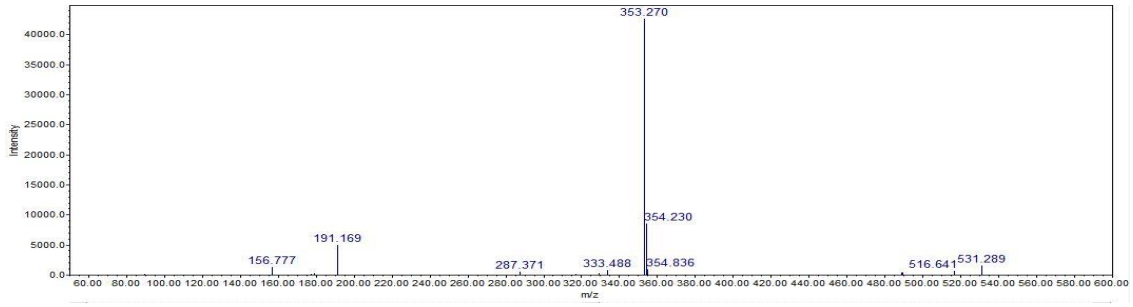

Figure 4: Molecular Fragmentation of Chlorogenic acid at negative mode (mz 353.270)

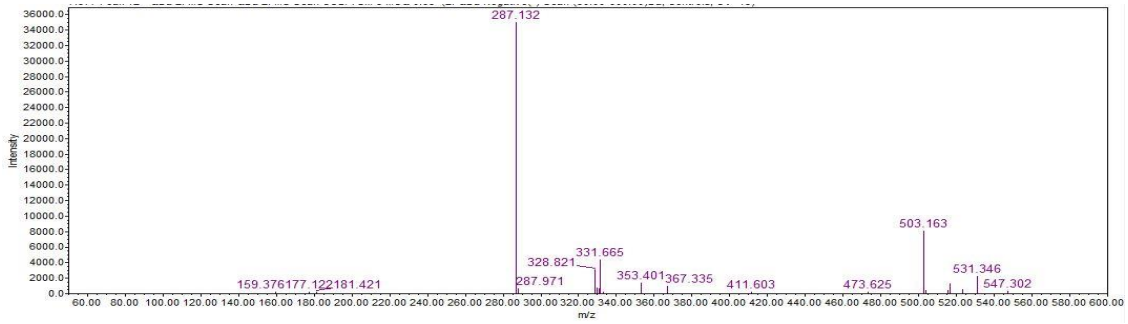

Figure 5: Molecular Fragmentation of Aromadendrin (flavononols) acid at negative mode (mz 287.132)

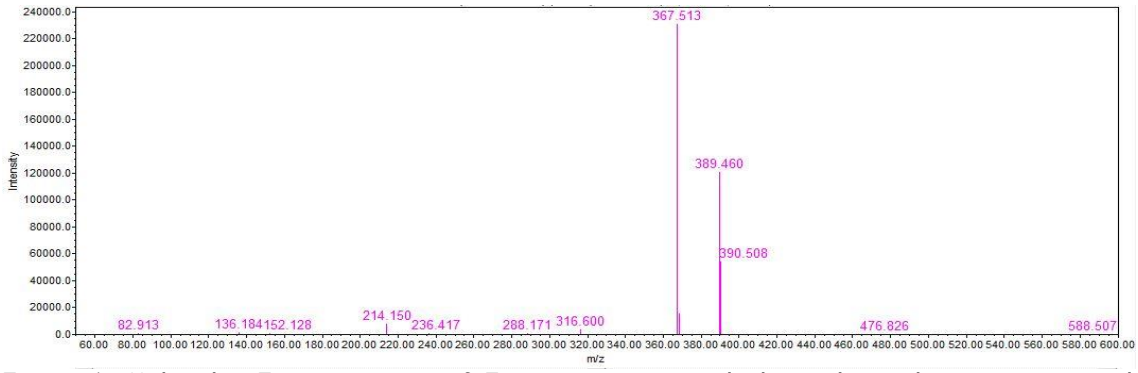

Figure 6: Molecular Fragmentation of Furocoumarinic acid glucoside acid at positive mode (mz 367.513)

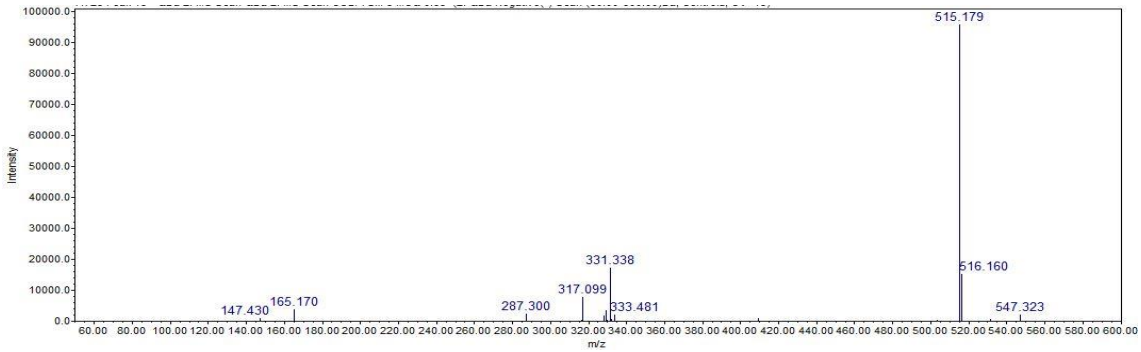

Figure 7: Molecular Fragmentation of 4,5-Dicaffeolquinic acid acid at negative mode (mz 515.179)

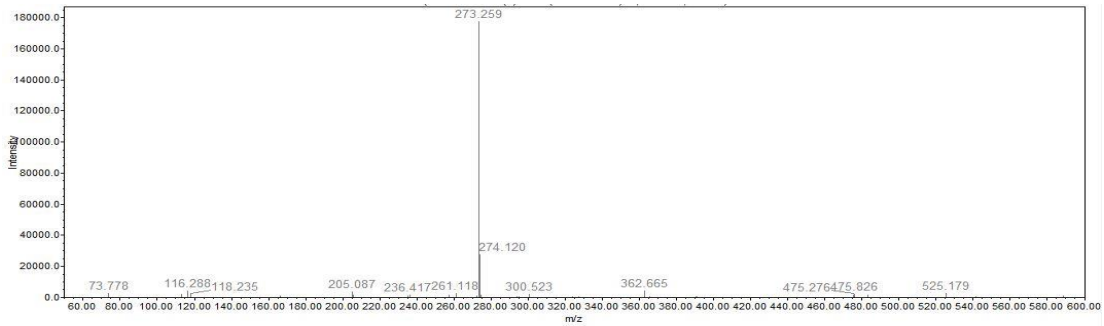

Figure 8: Molecular Fragmentation of Naringenin at positive mode (mz 273.259)

However, some of the metabolites (compounds) were identified at negative mode while others at positive mode as presented in Table 3. Similarly, their precursor and product ions also presented.

Table 3: Tentative compounds identified from poly-phenol rich fraction of *Ipomoea batatas* leaf extract

Analyte	Analyser/Ionization mode	Precursor Ion (m/z)	Product ion m/z	MW (g/mol)
Chlorogenic acid	QqQ/ESI (-)	353.270	156.777, 191.169, 287.371, 333.488.	354
Myricetin	QqQ/ESI (-)	316.90	316.900, 353.886, 409.153, 489.251.	317
Naringenin	QqQ/ESI (+)	273.359	73.778, 116.288, 118.235, 205.087, 236.417, 261.118.	272
4,5-Dicaffeolquinic acid	QqQ/ESI ((-)	515.179	147.430, 165.170, 287.300, 317.099, 331.338, 333.481.	514
Furocoumarinic acid glucoside	QqQ/ESI (+)	367.513	82.913, 136.184, 152.128, 214.417, 288.171, 316.600.	366
Aromadendrin (flavononols)	QqQ/ESI ((-)	287.132	159.376, 177.122, 181.421.	288
Abietinol (Diterpenoids	QqQ/ESI (+)	289.002	86.260, 118.442, 120.041, 205.018, 267.168 273.349.	288

DISCUSSION

From these results, the extract shows significant inhibition of the inflammation as compared to the blank control. This could be a result of experimental proved in human subjects that suggested a direct role for plant foods in tackling the inflammatory response *in vivo* (Serafini *et al.*, 2010).

Based on the LCMS analysis conducted, many compounds such as flavonoids are among the compounds identified in the present study. They are a subclass of polyphenols which are majorly distributed in the plant kingdom and are characterized by two or more aromatics rings. Also found in fruits, vegetables, legumes, herbs, spices, stems, flowers, tea, and red wine. (Kang 2014). Many studies have repeatedly proven that different flavonoid molecules exhibit anti-inflammatory functions. Thus, the anti-inflammatory activities of flavonols (quercetin, rutin and morin) and flavanones (hesperetin and hesperidin) were investigated in acute and chronic inflammation animal models (Kang 2014). Ayeleso *et al.*, (2016) reported the effect of polyphenols and further demonstrated the potential of polyphenols to modulate various parts of the inflammatory process in an *in-vitro* studies conducted recently. Many cellular action mechanisms have been proposed to explain *in vivo* anti-inflammatory activity of flavonoids (Ayeleso *et al.*, 2016). Furthermore, some flavonoids modulate the enzyme activities of arachidonic acid (AA) metabolizing enzymes such as phospholipase A2 (PLA2), COX, lipoxygenase (LOX) and nitric oxide synthase (NOS), the nitric oxide (NO) producing enzyme. Inhibition of such enzymes decreases the production of AA, prostaglandins (PG), leukotrienes (LT), and NO, which are crucial mediators of inflammation. However, the

inhibitory properties of flavonoids are one of the important cellular mechanisms of anti-inflammation (Ayeleso *et al.*, 2016).

According to several epidemiological and experimental studies, natural or synthetic products having anti-inflammatory potentials have proven to have a strong preventive effect on the development of atherosclerosis (Bhatt, 2008), traditionally, the roots and leaves of *I. batatas* have been used in treating several ailments. This could be the reason for the activity of polyphenol rich fraction of *Ipomea batatas* leaf extract observed. Therefore, current natural drugs are selected as a novel therapeutic strategy for managing inflammatory diseases, such as polyphenols used in the management of various ailments and commonly found in fruits, vegetables and herbal medicines (Alam *et al.*, 2020).

Chlorogenic acid was among the compound identified by LCMS profiling; however, the presence of this vital metabolite could be the reason for the activity observed. Bisht *et al.*, (2020) reported that the anti-inflammatory efficacy of curcumin has been well-established and recognized as an asset to reducing chronic disease risk. The potentiality of this property has been demonstrated by coupling with other bioactive. Consequently, the inflammatory-lowering effect of curcumin in THP-1 cell resulted from coupling with chlorogenic acid (Bisht *et al.*, 2020). Therefore the findings provide evidence of a complementary and synergistic reduction in mRNA level of NF-kB, COX-2 and increases in the expression of iNOS mRNA following concomitant treatment of cells with curcumin and chlorogenic acid (Bisht *et al.*, 2020).

On the other hand, naringenin was among the phytoconstituent identified. Excessive inflammation plays an essential role in initiating bone, liver, kidney and ovary damage and deterioration of their function (Orsolic *et al*, 2014; Bonaccoesi *et al.*, 2018), which could be modified by treatment with chrysin and naringenin. Recent data from literature suggested that chrysin and naringenin may reduce tissue damage through the improved oxidative status of specific tissues, attenuated production of pro-inflammatory cytokines. Furthermore, it seems that chrysin and naringenin may increase calcium absorption, delay the loss of bone mass, ameliorate interaction between the immune system and bone, and prevent the occurrence of oesteoporosis (Orsolic *et al.*, 2022). Similarly, Jin *et al.*, (2022) investigated another mechanism of naringenin inhibition potential. They reported that it involved a series of control processes, including transcription, post-transcription and post-translation, and the corresponding protein levels were dramatically reduced by naringenin treatment. The presence of 4,5-dicaffeolquinic acid was observed in this study, and its anti-inflammatory, anti-hepatotoxic and neuroprotective potentials were reported by Jang *et al.*, (2022). Additionally, Kim *et al.*, (2011) demonstrated that dicaffeolquinic acids have higher antioxidant properties, which are associated with the presence of catechol groups. Since dicaffeolquinic acids contained two catechol groups, they were found to be more powerful antioxidants (Phahlane *et al.*, 2022).

CONCLUSION

Based on the results obtained from this present study, it shows that polyphenol rich fractions of *Ipomea batatas* leaf extract has higher percentage inhibition of protein denaturation. Similarly, many polyphenolic compounds were identified by LCMS profiling. Therefore, *Ipomea batatas* leaf extract contains polyphenols with highly anti-inflammatory properties; thus, it could be useful in the inflammation management or drug design for chronic diseases.

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