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Different Flavonoid Profiles in *Xanthosoma sagittifolium* L. Schott Leaves (White and Red CV) During Growth under the Influence of Poultry Manure and NPK Fertilizers

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ABSTRACT

Extracts from white and red cultivar *Xanthosoma sagittifolium* leaves treated with poultry manure (0t ha^{-1} and 30t ha^{-1}) and NPK 20:10:10 fertilizers (0kg ha^{-1} and 150kg ha^{-1}) were subjected to flavonoid profile determination using HPLC-DAD-MS. Analysis of the spectra obtained, identified eight flavonoid compounds in all treatments. The results illustrated two newly identified flavone C- glycosides (2 isomers of apeginin) and six known flavone C- glycosides (6,8-Di-C-glucopyranosylapigenin, Isovitexin 6"-O-glucopyranoside, Apigenin 6-C-glucoside 8-C-arabinoside, vitexin, isovitexin and. 2"-O-Malonylvitexin). The intensity of the first 3 peaks after 6 months of growth showed that poultry manure treatments (30t ha^{-1}) enhanced flavone production in the white and red cultivar *Xanthosoma sagittifolium* plants as compared to the NPK fertilizer and control treatments. These results show that glycosides of apeginin can be used as chemotaxonomic markers of *Xanthosoma.*

INTRODUCTION

Flavonoids consist of a large group of polyphenolic compounds having a benzo-γ-pyrone structure and are ubiquitously present in plant fruits, leaves, grains, bark, roots, stems and flowers [1-2] and could also serve as chemotaxonomic marker compounds. Over 9000 flavonoids have been reported [3]. Flavonoids are frequently found as glycosylated or esterified forms, consisting of C6-C3-C6 rings, namely rings A and B linked by three carbon-ring C [2]. According to substitution pattern variations, flavonoids can thus be classified into different subclasses, providing an extremely diverse range of derivatives [4]. Due to their physical and biochemical properties, they are capable of participating in plants' interactions with other organisms (microorganisms or animals [2] and other plants [5]) and their reactions to environmental stresses [6]. Flavonoids protect plants from different biotic and abiotic stresses and act as unique UV-filter, function as signal molecules, allelopathic compounds [7] phytoalexins, detoxifying agents [8], antimicrobial defensive compounds [9]. The majority of their functions result from their strong antioxidative properties [10]. From the aforementioned functions, it could be suggested that flavonoids play significant roles in plant growth. Flavonoids are also believed to have various bioactive effects including anti-viral, anti-inflammatory, cardioprotective, anti-diabetic, anti-cancer, anti-aging, in humans *etc* [4]. Some flavones like vitexin and isovitexin are active components of many traditional Chinese medicines and were found in various medicinal plants. Vitexin (apigenin-8-C-glucoside) has recently received increased attention due to its wide range of pharmacological effects, including but not limited to anti-oxidant, anti-cancer, anti-inflammatory, anti-hyperalgesic, and neuroprotective effects. Isovitexin (apigenin-6-C-glucoside), an isomer of vitexin, generally purified together with vitexin, also exhibits diverse biological activities [11]. [12] Conducted oral glucose tolerance and antinociceptive activity tests with methanol extracts (containing apigenin and its sugar derivatives) from *Xanthosoma violaceum* aerial parts and concluded that bioactive components in these extracts like apigenin, vitexin and isovitexin could be used to lower blood sugar levels in diabetic patients and alleviate pain. [13] Also identified derivatives of apigenin in cocoyam (*Xanthosoma sagittifolium*) leaves.

Cocoyam (*Xanthosoma sagittifolium* L. Schott, Araceae) is a herbaceous plant cultivated in tropical and subtropical regions for its edible tubers and leaves. *X. sagittifolium* leaves contain significant antioxidant compounds which have anti-diabetic properties [14] and can also be used to treat gastrointestinal illnesses [15]. The increasing decline in soil fertility

levels and lack of soil management practices has significantly reduced *X. sagittifolium* production. *X. sagittifolium* responds very well to input of fertilizer whether organic or inorganic as reported by several workers [16-18]. Different manures (fish, pigeon and cow) and synthetic fertilizers (nitrogen) have been shown to influence some biochemical activities like phenolic constituents of plants [19]. The quantity of phenolic and polyphenolic compounds present in a given species of plant material varies with a number of factors such as cultivar, environmental conditions, cultural practices postharvest storage and processing [20]. It is in this logic that this investigation was carried out to evaluate the effects of poultry manure and NPK fertilisers on the profiles of flavonoids in the leaves of white and red cultivar *X. sagittifolium* plants during growth while highlighting the possible flavonoids which can serve as chemotaxonomic marker compounds for cocoyam (*X. sagittifolium*).

MATERIALS AND METHODS

Site location

A field trial was conducted to study the performance of *Xanthosoma sagittifolium* mini tuber seeds as influenced by poultry manure and NPK fertilizer during the 2017 cropping season on an experimental farm at Ngog Bibega, Mbankomo Sub-division, (Outskirsts of Yaoundé) Centre region, Cameroon, located at latitude 3°49′52.54″N and longitude 11°27′15.79″E and 714 m above sea level. The area is characterized as a humid rainforest zone and the soil is clay loam. The total annual rainfalls for 2017 was 1902.8 mm while the total rainfalls during the period of experimentation (April to December) for 2017 was 1775.6 mm.

Materials

The planting material consisted of white and red cultivars of *X. sagittifolium* mini tuber seeds of mean weight 38g produced from acclimatised vitro plants under the shed in the plant physiology Laboratory of the Higher Teachers Training college (HTTC), University of Yaounde I. Yaounde, Cameroon [21-22]. The Poultry manure was obtained from Henri et Freres Poultry farm Yaoundé, Cameroon while the NPK fertilizer (20:10:10) was obtained from the fertilizer unit of the Centre Regional Delegation of Agriculture and Rural Development, Yaoundé, Cameroon.

Experimental design and treatments.

The experiment was a 4×2 factorial arrangements in a randomized complete block design and replicated three times. The site was ploughed, ridged and marked out into two main blocks, one for each cultivar. Each of these main blocks was further subdivided into 3 sub blocks which represent the three replicates. Each sub block was divided into three experimental plots, thus a total of nine plots were used for cultivar. Each gross plot measured 4m×3m (12 m^2) with a net plot of 2m×2m. The treatments comprised two rates each application of Poultry Manure (0, and 30 t ha-1) and NPK fertilizer (0, and 150 kg ha-1). A total of 9 treatment combinations and three replications were used. The Poultry manure was incorporated into the soils on the experimental plots in a single application based on the treatment combinations, at 2 weeks before planting to ease decomposition, while the NPK fertilizer was applied to the cocoyam stands according to treatment allocation at 4 weeks after planting (WAP) using the ring placement method. Each mini tuber was planted per hole at a depth of 15 cm and at a spacing of 0.5 m x 1.0m resulting to about twenty-five plants per plot and a total of 250 plants per cultivar. All plots were kept weed free by manual weeding. Five cocoyam plants were randomly selected from each of the net plots, tagged and the harvested leaves at 2 months and 6 months after planting were used for the determination of the flavonoid profiles.

Extraction of total phenolic compounds

The extraction of the content of total phenolic compounds were performed as described by [23] with modification. Total phenolic compounds were extracted twice using 80% methanol. One gram of fresh *X. sagittifolium* leaves was ground in 10 ml of 80% methanol at 4˚C. After 5 min of agitation, the ground material was centrifuged at 10,000 g for 5 min at 4˚C. The supernatant was collected, and the pellet was re-suspended in 5 ml of 80% methanol followed by agitation for 5 min. After the second centrifugation at 4˚C, the supernatant was collected and mixed with the previously collected supernatant to constitute the phenolic extract.

Qualitative determination of compound contents from *X. sagittifolium* leaves using HPLC-DAD-(HR) ESI-MS

Sample preparation

Aqueous preparation extracts were separately dissolved in HPLC grade methanol in a concentration of 5 mg/ml then filtrated through a syringe-filter-membrane. Aliquots of 5 µl were injected into the LC-DAD/MS Dionex Ultimate 3000 HPLC (Germany), used for performing the analyses.

HPLC-MS conditions

High resolution mass spectra were obtained with an OTOF Spectrometer (Bruker, Germany) equipped with a HRESI source and a UV-vis absorbance detector. The spectrometer was operated in positive mode (mass range: 100-1500, with a scan rate of 1.00 Hz) with automatic gain control to provide high-accuracy mass measurements within 2 ppm deviation using Na Formate as calibrant. Mass spectra were simultaneously acquired using electrospray ionization in the positive ionization mode. The following parameters were used for experiments: spray voltage of 4.5 kV, capillary temperature of 200°C. Nitrogen was used as sheath gas (10 l/min). The spectrometer was attached to an Ultimate 3000 (Thermo Fisher, USA) HPLC system consisting of LC-pump, UV traces were measured at 215, 218, 254, 280 and 330 nm and UV spectra-Diode Array Detector-(DAD) were recorded between 190 and 600 nm, autosampler (injection volume 5 µl) and column oven (35,0 °C). The separations were performed using a Synergi MAX-RP 100A (50x2 mm, 2.5µ particle size) with a H_2O (+0.1% HCOOH) (A)/acetonitrile (+0.1% HCOOH) (B) gradient (flow rate 500 µL/min). Samples were analyzed using a gradient program as follows: 95% A isocratic for 1.5min, linear gradient to 100 % B over 6 min, after 100% B isocratic for 2min, the system returned to its initial condition (90 % A) within 1 min and was equilibrated for 1 min.

Identification of peaks

Identification of all constituents was performed by HPLC-DAD-MS/MS analysis and by comparing the UV, MS spectra and MS/MS fragmentation of the peaks in the samples with those of data reported in the literature of Scifinder database.

RESULTS

Flavonoids were determined in the fluorescent region of the spectra. Four peaks with varying intensities were identified in the spectra of all treatments. In the first peak, two metabolites with a molecular formula of $C_{27}H_{30}O_{15}$ and an average molecular weight of 595.16g/mol were identified. 6,8-Di-C-glucopyranosylapigenin (Fig. 2A) was identified with a retention time of 2.63 min with 3 absorption wavelengths peaks (210nm, 270nm and 333nm) while Isovitexin 6"-O-glucopyranoside (Fig. 2B) had a retention time of 2.72 min with 3 absorption wavelength peaks (214nm, 270nm and 335nm) (Table 1).

In the second peak, three metabolites with a molecular formula of $C_{26}H_{28}O_{14}$ and an average molecular weight of 565.15 $g.mol^{-1}$were recorded. The first metabolite on this peak Apigenin 6-C-glucoside 8-C-arabinoside (Schaftoside) (Fig. 3) had a retention time of 2.82 min with 3 absorption wavelength peaks (214nm, 270nm and 235nm). The second metabolite Apigenin-6-C-pentoside-8-C- hexoside (Isomer 1) (Fig. 3) with a retention time of 2.84 min and 3 absorption wavelength peaks (214nm, 270nm and 339nm) while the third metabolite Apigenin-6-C-pentoside-8-C- hexoside (Isomer 2) (Fig.3) recorded a retention time of 2.90 min. with 3 absorption wavelength peaks (214nm, 270nm and 335nm) (Table 1). The third peak recorded two metabolites having a molecular formula of $C_{21}H_{20}O_{10}$ and average molecular weight of 433.11 $g.mol^{-1}$. 8-C-Glucosylapiginin (vitexin) (Fig. 4A) had a retention time of 2.97min with 3 absorption wavelength peaks (210nm, 270nm and 339nm) while 6-C-Glucosylapiginin (isovitexin) (Fig. 4B) recorded a retention time of 3.06 min with 3 absorption wavelength peaks (210nm, 270nm and 335nm) (Table 1). One metabolite was identified at the fourth peak with a molecular formula of $C_{24}H_{22}O_{13}$ and average molecular weight 519.11$g.mol^{-1}$. 2"-O-Malonylvitexin (Fig. 5) recorded a retention time of 3.13 min with 3 absorption wavelength peaks (210nm, 270nm and 335nm) (Table 1).

Flavonoid profile results show that there was a general increase in peak intensities between 2 and 6 months of growth for all treatments in both, white and red cv of *X. sagittifolium* leaves (Table 2). Analysis of the spectra obtained from the white cv of *X. sagittifolium* leaves after 2 months of planting revealed that the intensities of the first 3 peaks between the retention times of 2.63 min and 3.06 min were more significant in poultry manure treatments (30 $t.ha^{-1}$) and NPK fertilizer treatments (150 $kg.ha^{-1)}$ as compared to the control treatments (0 $kg.ha^{-1}$) (Fig. 6 and Table 2). Anyway, the spectra from the red cv of *X. sagittifolium* leaves after 2 months of planting also showed that the intensities of the first 3 peaks between the retention

times of 2.63 min and 3.06 min were most significant in the NPK fertilizer treatments (150kg.ha$^{-1)}$ than in the control treatments (0 kg.ha^{-1}) and the poultry manure treatments (30 t.ha^{-1}) (Fig. 7 and Table 2).

Six months after planting the spectra obtained from both white and red cv of *X. sagittifolium* leaves illustrated that the first and second peaks with retention times between 2.63 min and 2.9 min were significantly reduced in intensity with the NPK fertilizer treatments (150 kg.ha^{-1}) as compared the control treatments (0 kg.ha^{-1}) and the poultry manure treatments (30 t.ha^{-1}). The third peak recorded the most significant intensity values with poultry manure treatments (30t ha^{-1}) when compared to the control treatments (0 kg.ha^{-1}) and NPK fertilizer treatments (150 kg.ha^{-1}) (Fig.8, Fig. 9 and Table 2).

Table No. 1: Spectra analysis

N°	Rt (min)	$[M+H]^+$			UV, λ_{max} (nm)	Formular	Metabolites
		Exp.	Calcd.	Δ(*ppm*)			
01	2.63	595.1690	595.1557	2.4	210 270 335	$C_{27}H_{30}O_{15}$	6,8-Di-C-glucopyranosylapigenin
02	2.72				214 270 335		Isovitexin 6"-O- glucopyranoside
03	2.82	565.1585	565.1552	2.1	214 270 335	$C_{26}H_{28}O_{14}$	Apigenin 6-C-glucoside 8-C-arabinoside
04	2.84				214 270 339		**Apigenin-6-C-pentoside-8-C- hexoside (Isomer 1)**
05	2.90				214 270 335		**Apigenin-6-C-pentoside-8-C- hexoside (Isomer 2)**
06	2.97	433.1152	433.1129	3.9	210 270 339	$C_{21}H_{20}O_{10}$	8-C-Glucosylapigenin (vitexin)
07	3.06				210 270 335		6-C-Glucosylapigenin (Isovitexin)
08	3.13	519.1169	519.1133	0.8	210 270 335	$C_{24}H_{22}O_{13}$	2"-O-Malonylvitexin

Table No. 2: Peak Flavonoid intensities in *X. sagittifolium* leaves

		Peak intensity (mAU) 2 months after planting			Peak intensity (mAU) 6 months after planting		
leaves	Peak (N^o)	Control	Poultry manure	NPK	Control	Poultry manure	NPK
X. sagittifolium (White cv)	**1**	0.8 x 10^5	1.1 x 10^5	1.0 x 10^5	1.0 x 10^5	1.1 x 10^5	0
	2	1.0 x 10^5	1.4 x 10^5	1.4 x 10^5	2.4 x 10^5	2.4 x 10^5	0
	3	1.3x 10^5	2.1 x 10^5	1.9 x 10^5	3.4 x 10^5	4.4 x 10^5	3.6 x 10^5
	4	0.4 x 10^5	0.55 x 10^5	0.5 x 10^5	1.0 x 10^5	1.5 x 10^5	1.6 x 10^5
X. sagittifolium (Red cv)	**1**	0.8 x 10^5	0.65 x 10^5	1.5 x 10^5	1.1 x 10^5	1.2 x 10^5	0
	2	1.05 x 10^5	0.7 x 10^5	2.5 x 10^5	2.3 x 10^5	2.4 x 10^5	0
	3	1.28x 10^5	1.45 x 10^5	3.8 x 10^5	3.4 x 10^5	4.4 x 10^5	3.68 x 10^5
	4	0.41 x 10^5	0.5 x 10^5	0.6 x 10^5	0.8 x 10^5	1.49 x 10^5	1.6 x 10^5

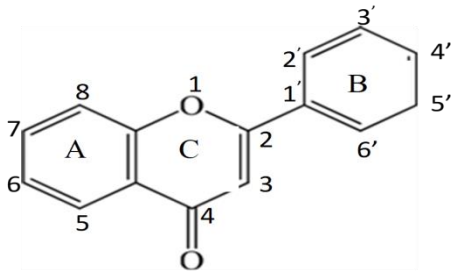

Fig No. 1: Basic structure of flavones (Rana and Gulliya, 2019)

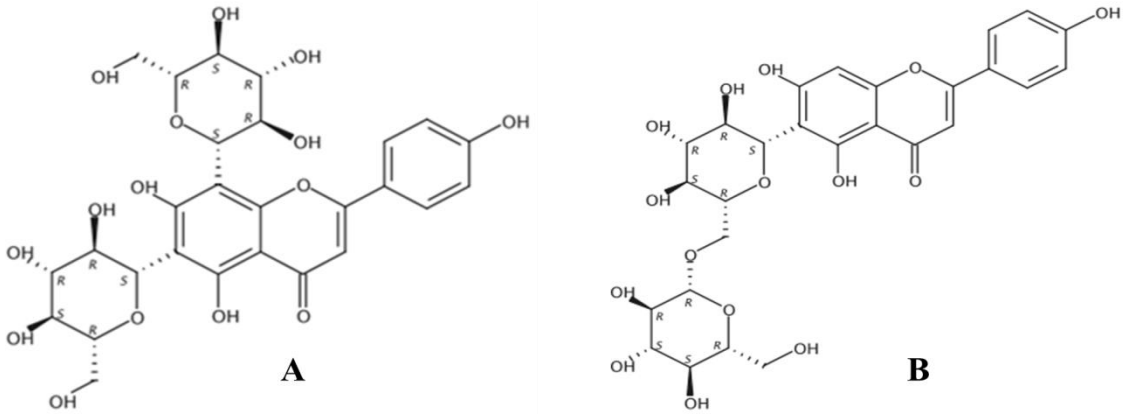

Fig No. 2: Flavone metabolite structures with same molecular formula ($C_{27}H_{30}O_{15}$) at 2.36 and 2.72 (min) retention times. 6,8-Di-C-glucopyranosylapigenin (A) and Isovitexin 6''-O-glucopyranoside (B).

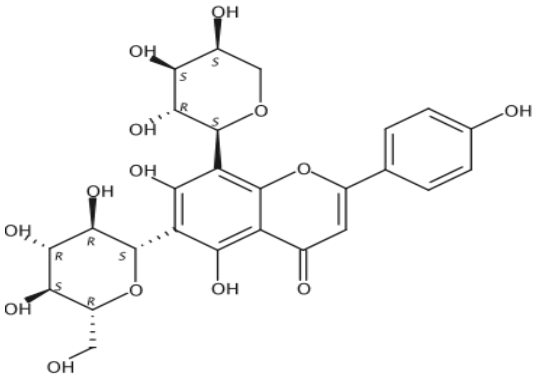

Fig No. 3: Isomer structures of three metabolite flavones with same molecular formular ($C_{26}H_{28}O_{14}$) at 2.82, 2.84 and 2.90 (min) retention times. Apigenin 6-C-glucoside 8-C-arabinoside or Apigenin-6-C-pentoside-8-C- hexoside (Isomer 1) or Apigenin-6-C-pentoside-8-C- hexoside (Isomer 2).

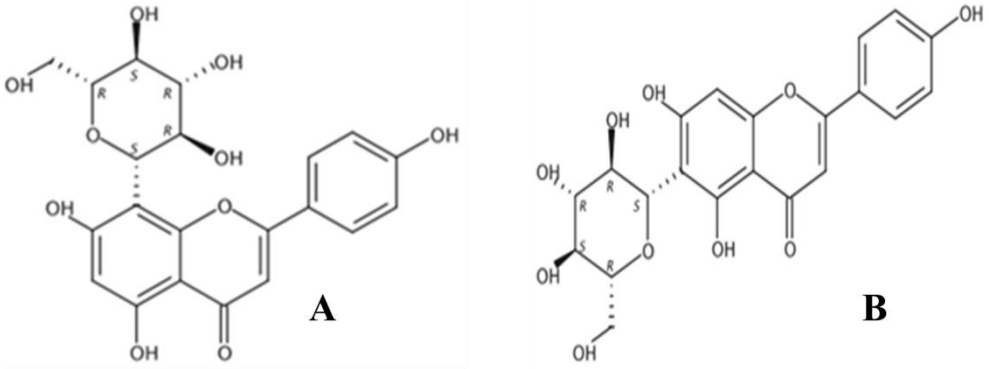

Fig. No. 4: Flavone metabolite structures with same molecular formular ($C_{21}H_{20}O_{10}$) at 2.97 and 3.06 (min) retention times. 8-C-Glucosylapigenin (vitexin) (A) and 6-C-Glucosylapigenin (Isovitexin) (B).

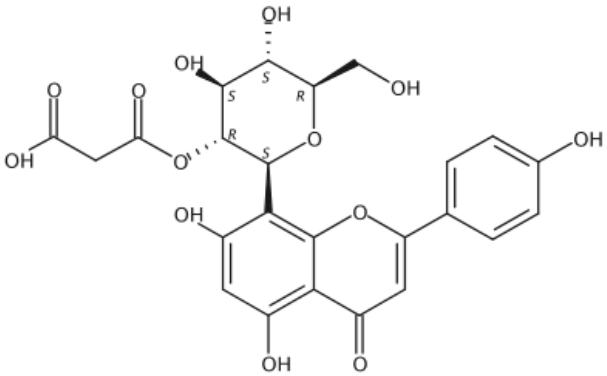

Fig. No. 5: Flavone metabolite structure of 2''-O-Malonylvitexin with $C_{24}H_{22}O_{13}$ molecular formular at 3.13 (min) retention time.

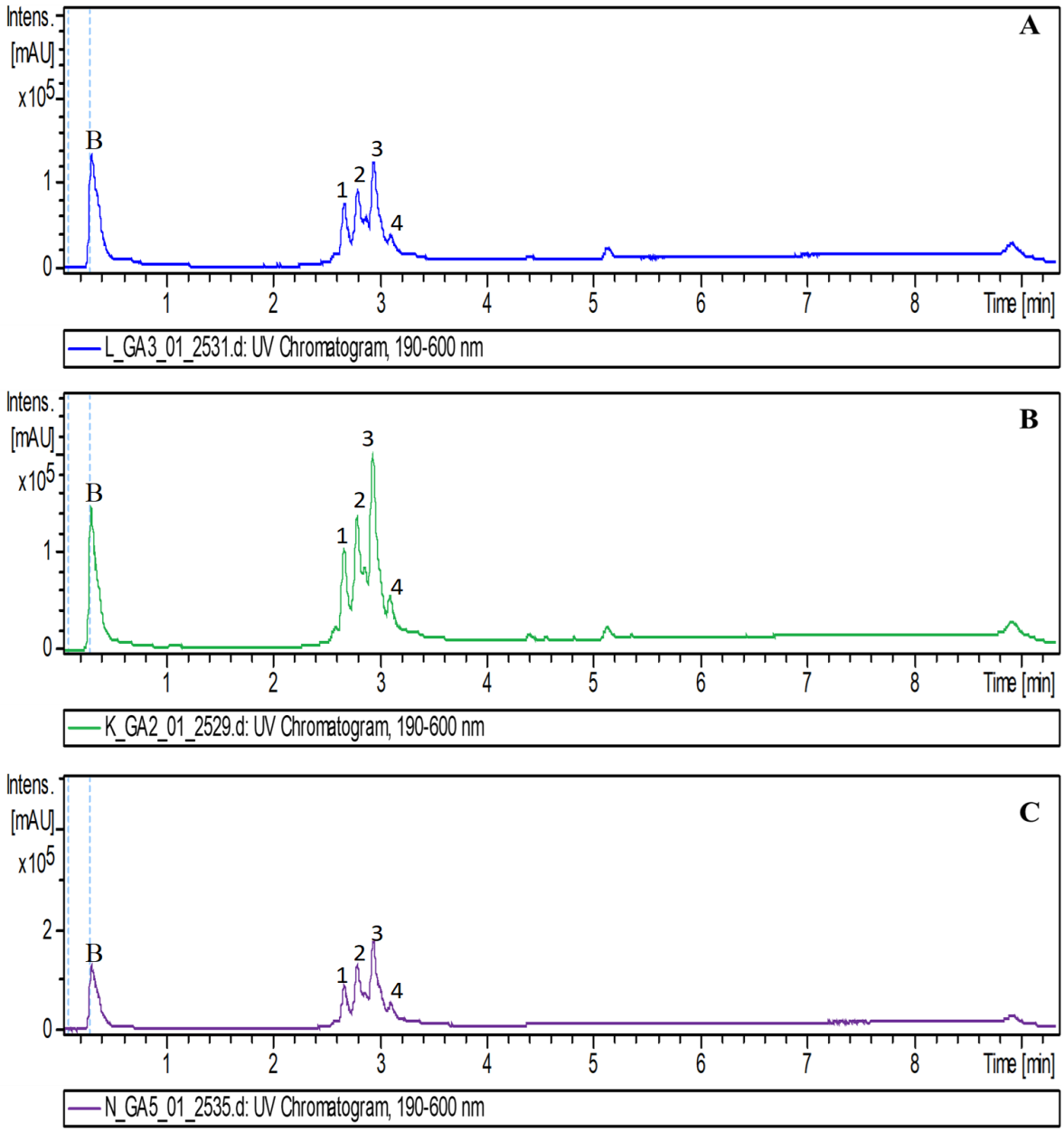

Fig. No. 6: Flavonoid profiles from white cv leaves two months after planting. Control (A), poultry manure (30t ha^{-1}) (B) and NPK fertilizer (150kg ha^{-1}) (C). Blank (B) and flavonoid peaks (1,2,3 and 4).

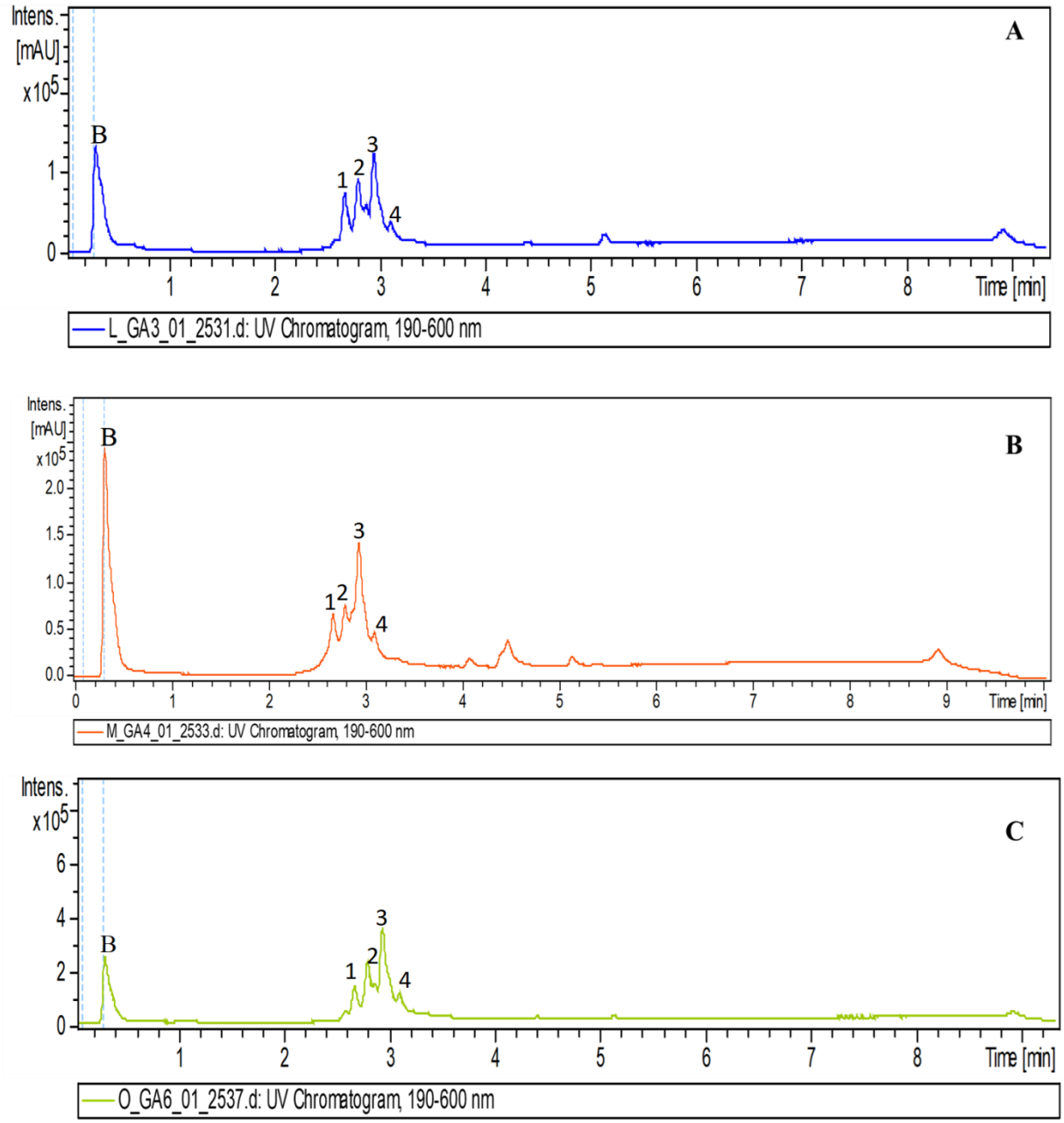

Fig. No. 7: Flavonoid profiles from red cv leaves 2 months after planting. Control (A), poultry manure (30t ha^{-1}) (B) and NPK fertilizer (150kg ha^{-1}) (C). Blank (B) and flavonoid peaks (1,2,3 and 4).

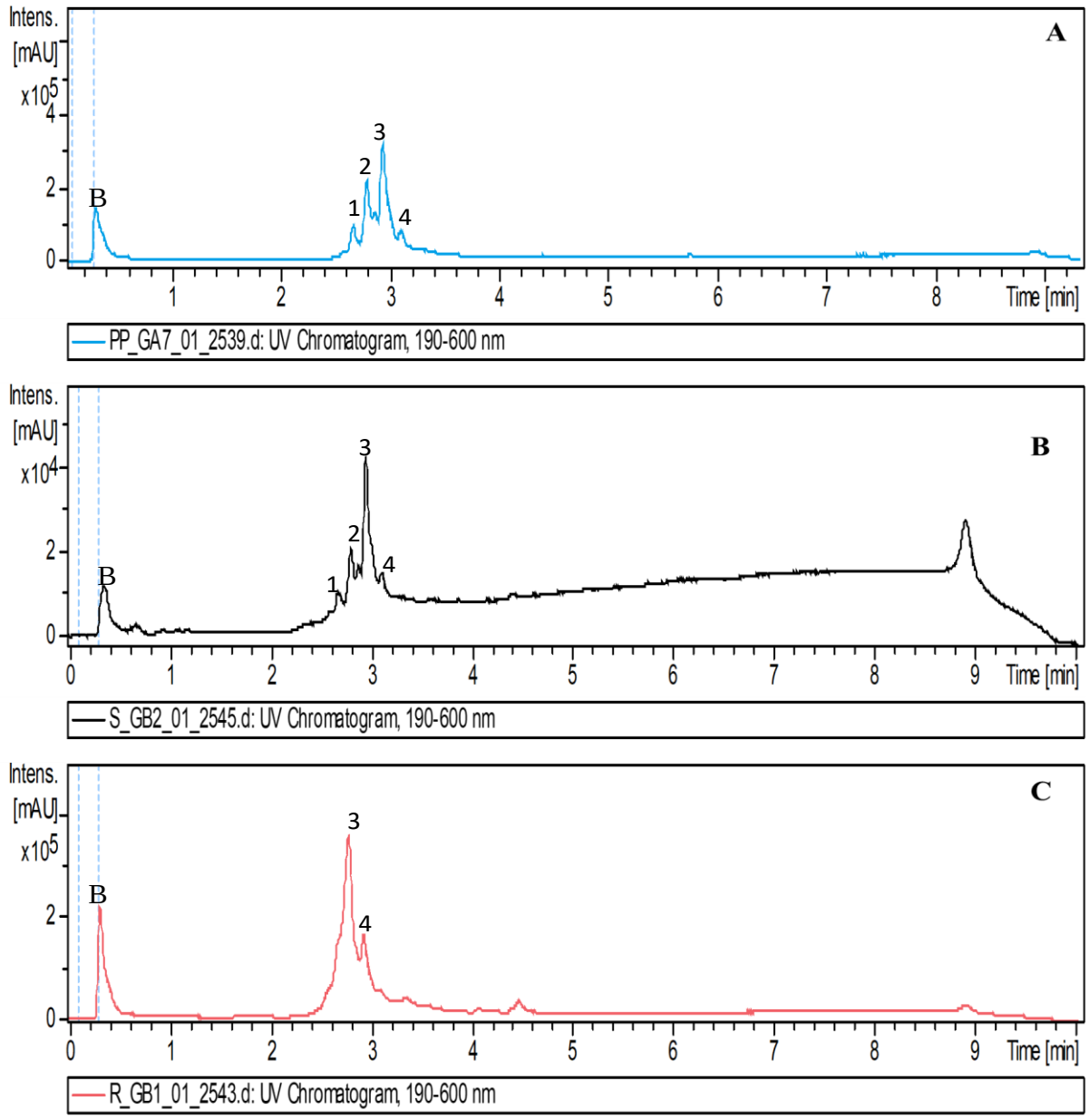

Fig. No. 8: Flavonoid profiles from white cv leaves 6 months after planting. Control (A), poultry manure (30t ha^{-1}) (B) and NPK fertilizer (150kg ha^{-1}) (C). Blank (B) and flavonoid peaks (1,2,3 and 4).

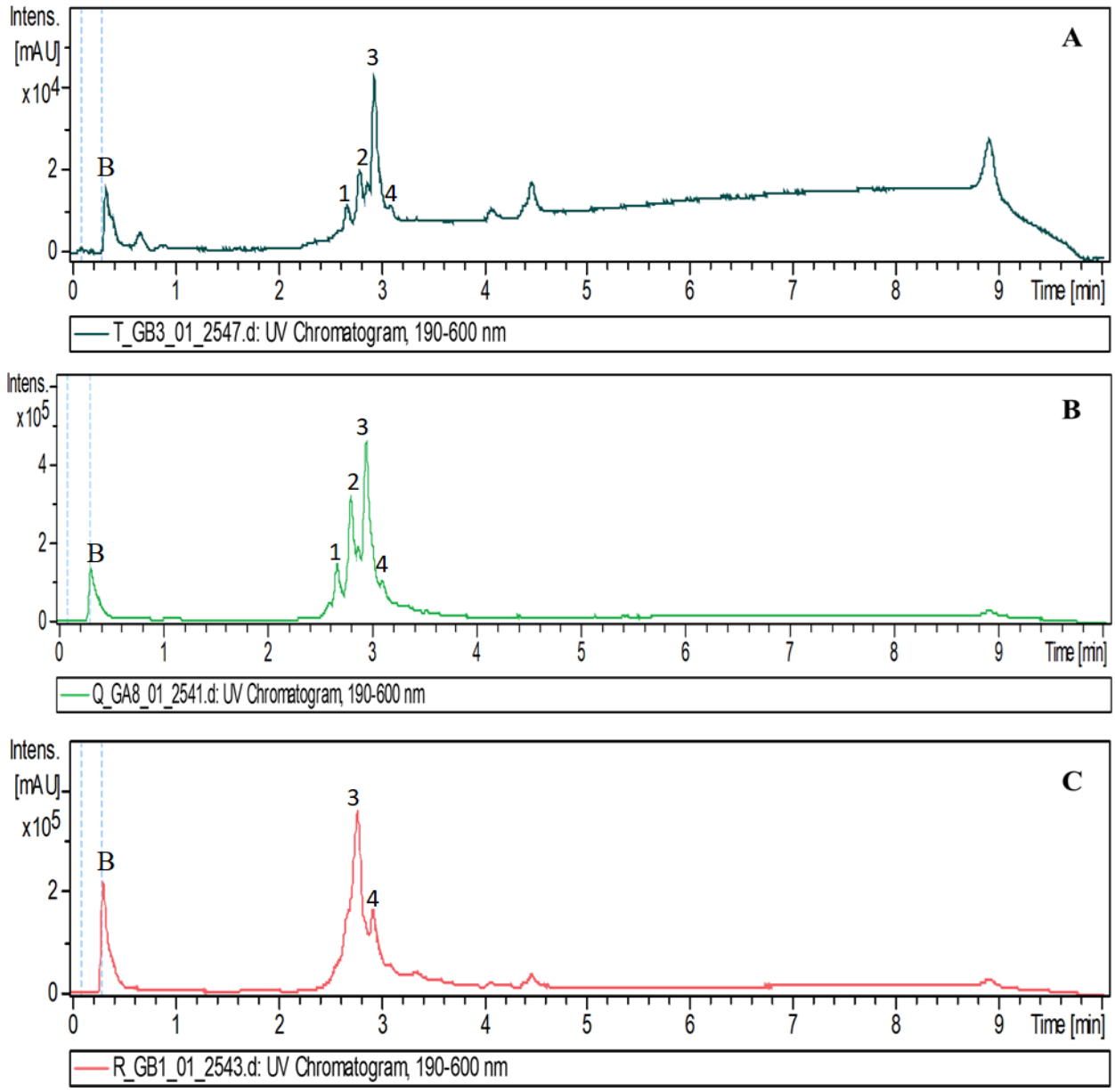

Fig. No. 9: Flavonoid profiles from red cv leaves 6 months after planting. Control (A), poultry manure (30t ha^{-1}) (B) and NPK fertilizer (150kg ha^{-1}) (C). Blank (B) and flavonoid peaks (1,2,3 and 4).

DISCUSSION

Recent advances in the pre-treatment procedures, separation techniques and spectrometry methods are used for qualitative and quantitative analysis of phenolic compounds. The online coupling of liquid chromatography with mass spectrometry (LC-MS) has become a useful tool in the metabolic profiling of plant samples [24]. The objective of this work was to

determine the effects of poultry manure and NPK fertilizers on the flavonoid profiles of white and red cv *X. sagittifolium* leaves during growth, using HPLC-DAD-MS. Eight flavonoid compounds with different retention times were identified on 4 peaks with varying intensities for all treatments. This implied that neither poultry manure nor NPK fertilizer influenced the synthesis of new flavonoid metabolites in *X. sagittifolium* leaves which were not found in control plants but stimulated the intensity of existing flavonoids. Similarly, [25] investigated the influence of inorganic fertilizer application on the flavonoid, phenol and steroid content in the leaves of *Ocimum gratissimum* and *Gongronema latifolium* and revealed that inorganic fertilizers treatments did not affect the presence of flavonoids, phenols and steroids in the leaves of these plants since these phytochemicals were present in both treated and untreated plants but affected their concentrations. The results show 8 flavonoid compounds identified amongst which 2 new specific flavone C- glycosides (isomers of apeginin) in *X. sagittifolium* and 6 known flavone C- glycosides (6,8-Di-C-glucopyranosylapigenin, Isovitexin 6"-O-glucopyranoside, apigenin 6-C-glucoside 8-C-arabinoside, vitexin, isovitexin and. 2"-O-Malonylvitexin. These 2 new flavone C- glycosides (isomers of apigenin) could play important roles in the growth and development of plants. These new flavone c- glycosides can also be isolated and used in the pharmacological industry to produce drugs against cancer, diabetes and inflammations. The 6 known flavone C- glycosides were also identified by [26]. They determined the polyphenol profile of *Xanthosoma violaceum* leaves and isolated a new flavone C-glycoside, apigenin 6-C-β-D-glucopyranosyl-8-C-β-D-apiofuranoside, as well as known flavone C-glycosides, including vitexin, isovitexin, isovitexin 4'-O-Orhamnopyranoside, apigenin 6-C-[β-D-glucopyranosyl-(1-6)-β-D-glucopyranoside], and apigenin 6,8-di-C-β-D-glucopyranosid. Nevertheless, [13] also identified apigenin-pentosyl-hexoside, apigenin-hexoside and apigeninrutinoside isomer in white cv *Xanthosoma sagittifolium* leaves while studying the effects of arbuscular mycorrhiza fungi on stimulation of nutrient content and induction of biochemical defense response in *Xanthosoma sagittifolium* plants against root rot disease caused by *Pythium myriotylum.* These results suggest that glycosides of apigenin can be used as chemotaxonomic markers for the genus *Xanthosoma.*

Results from spectra analysis at 2 months of growth depicted that poultry manure treatments (30 $t.ha^{-1}$) and NPK fertilizer treatments (150 $kg.ha^{-1}$) recorded significant intensities for the first 3 peaks in white cv *X. sagittifolium* leaves while NPK fertilizer treatments (150 $kg.ha^{-1}$) showed the most significant intensity values of the first 3 peaks in the red cv *X. sagittifolium*

leaves as compared to the control treatments. At 6 months of growth NPK fertilizer treatments (150 $kg.ha^{-1}$) recorded significantly very reduced intensity values for the first and second peaks for both white cv and red cv *X. sagittifolium* leaves. These results agree with those obtained by [27] who compared the effect of organic (vegetable waste, cattle dung) and inorganic fertilizer (NPK) on phytochemicals in *Solanum nigrum* and concluded that organic fertilizer treated plants have higher antioxidant activity than the inorganic fertilizer treated plants. Concordantly, [28] also studied the impact of organic and inorganic fertilizers application on the phytochemical and antioxidant activity of Kacip Fatimah (*Labisia pumila* Benth) and illustrated that the use chicken dung enhances the production of total phenolics, flavonoids, ascorbic acid, saponin and glutathione content in *L. pumila*, compared to the use of inorganic fertilizer. In addition, [29] evaluated the impact of excessive nitrogen fertilization on the biochemical quality, phenolic compounds, and antioxidant power of *Sesamum indicum* L seeds and concluded that total phenolic, flavonoid content, and antioxidant activity showed a significant decrease.

CONCLUSION

The present study clearly identified flavonoid profiles in the white and red cultivars of *Xanthosoma sagittifolium* leaves under different treatments of poultry manure and NPK fertilizers. All the compounds identified were flavone C-glycosides of apiginin, which could serve as chemotaxonomic markers of *Xanthosoma.* It was also observed that poultry manure treatments (30 $t.ha^{-1}$) enhanced the production of these polyphenolic compounds after growth in the white and red cv plants. Recent literature has demonstrated that some of the identified compounds like vitexin and isovitexin have promising pharmacological effects against type II diabetes, gastric ulcers, cancer and other illnesses. Therefore, *Xanthosoma sagittifolium* leaves could be a useful source for the isolation and purification of these compounds, for use in the pharmacological industry.

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CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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