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Food Value, Phytochemical Constituents and Physicochemical Properties of *Gladiolus Psittacinus* Bulb

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Abstract

The present study was designed to characterize the physicochemical properties, fatty acids and essential oils profile of *Gladiolus psittacinus* bulb. Physicochemical analyses were carried out following standard methods. Fatty acid compositions and essential oils were analysed by GC/MS. The saponification and Iodine values obtained were 165.50 mgKOH/g and 42.20 mg/g respectively. 1,8 Cineole (46.05%) dominated the essential oils, while fatty acids such as linoleic acid associated with lowering of fasting blood sugar was present in high concentration. Phosphatidylcholine 23.35 mg/100g was the most dominant phospholipid, while sitosterol 20.03 mg/100g was the highest occurring phytosterol in *Gladiolus psittacinus* bulb oil. Due to the presence of high concentration of essential fatty acids, essential oils, phytosterols and phospholipids in *Gladiolus psittacinus* bulb oil, it may be recommended as an important part of human diet, a potential medicinal food whose addition to diet will promote human well-being in the management and treatment of lipids related disorders such as atherosclerosis and cardiovascular diseases.

Keywords: Essential oils, Fatty acids, *Gladiolus psittacinus*, Phospholipids, Physicochemical properties, Phytosterols

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INTRODUCTION

Gladiolus psittacinus (Iridaccae) is an herbaceous plant commonly known as 'Maid of mist' and Baaka (Yoruba, southwestern Nigeria), belonging to Iridaceous family (Francois et al., 2013). It occurs virtually throughout the grasslands, savanna and woodlands of sub-Saharan Africa. It is the mostly distributed species of *Gladiolus*, throughout tropical Africa and into Western Arabia. Ethnomedicinally, it is used as a remedy for cold, dysentery, asthma, gonorrhea, mental disorder and intestinal parasite (Oyetayo et al., 2023). Ethanol extract of *Gladiolus psittacinus* bulb is used by traditional healers in south western Nigeria as an important recipe for the treatment of Diabetes mellitus (Karigidi et al., 2023).

Evaluation of the bulbs for phytochemical constituents revealed the presence of bioactive substances such as alkaloids, flavonoids, tannins, and saponins which have been shown to possess various antimicrobial properties (Munyemana et al., 2013). Several phenolic constituents have been identified to be responsible for myriads of bioactivities attributed to the *Gladiolus psittacinus* bulb. The bulb lowers blood glucose by releasing insulin from the residual beta cells, inhibition of glucagon, inhibition of gluconeogenesis in diabetic patients, act as an antimicrobial agent for the treatment of intestinal parasite and Gonorrhea. Oyetayo et al., (2023) reported that inclusion of *Gladiolus psittascinus* in diets of cognitive dysfunctioned experimental rats significantly decreased Acetylcholine estarase, butyrylcholine estarase and adenosine deaminase activities while Na^+/K^+ ATPase activity and Gamma-aminobutyric acid concentrations significantly increased following its use as a diet therapy for the treatment of scopolamine-induced cognitive dysfunctional rats. Thus, this study was carried out to assess the fatty acid composition, physicochemical properties and chemical composition *Gladiolus psittacinus* bulb oil.

MATERIAL AND METHOD

Sampling and sample treatment

Gladiolus psittacinus bulb were obtained from a local Market in Ado Ekiti, Ekiti State and were identified at the Herbarium of the Department of Plant Science and Biotechnology of Ekiti State University, Ado Ekiti. The bulbs were sliced and oven dried at 40^0C for 6 hours, the dried sample was powdered in a warring laboratory blender and stored in an air tight container at room temperature prior analysis.

Extraction of *Gladiolus psittacinus* bulb Oil

The *Gladiolus psittacinus* bulb oil was extracted using the soxhlet extraction procedure (Harwood and Moody, 1989). The bulb powder was packed into filter papers and tied neatly. They were placed in a thimble which was suspended above a round bottom flask containing the extraction solvent (n-Hexane) and below a condenser. The flask was heated to 50^0C. The soxhlet evaporated and moved up into the condenser where it was converted back to liquid which trickled into the extraction chamber through the sample and back into the boiling solvent. After 6hrs of this cycle, the boiling flask content was removed and placed in the rotary evaporator which separated the *Gladiolus psittacinus* bulb oil from the extracting solvent. The oil was afterwards collected into a clean bottle.

Physicochemical Analysis

The acid, saponification, iodine and refractive index values of the various oil samples were determined by AOCS (2005).

Determination of Iodine Value

Fat solution of 20g of *Gladiolus psittacinus* bulb oil was dissolved in 100 ml of chloroform. 10ml of the oil solution was pipetted into a stopper bottle and 25ml of Iodine monochloride was added. The stopper bottle was shaken thoroughly and placed in the dark for one hour. A blank solution was prepared with the oil solution replaced with 10ml of water.

After an hour, the stopper bottles were rinsed with about 50ml of water and 10ml of potassium Iodide were added. The resulting solution was titrated with standard thiosulphate. When the solution turned pale straw, 1ml of starch solution was added and the titration continued until blue colouration formed with the starch solution disappeared. The titre values for the test and blank were used to calculate the iodine value

$$\text{Iodine Value} = (\text{Blank} - \text{Test}) \text{ X } 6.35 \quad (1)$$

Determination of Acid Value

About 10g of *Gladiolus psittacinus* bulb oil was weighed into a beaker. Fifty ml of fat solvent was pipetted into the oil and 1ml of phenolphathalein solution was added and mixed thoroughly. The solution was titrated with 0.1M of potassium hydroxide until faint pink colour persisted for 20 seconds. The titration was done in duplicate and the acid value was calculated.

$$\text{Acid Value} = (\text{titre value X } 5.6)/10\text{g (weight of the sample)} \quad (2)$$

Determination of Saponification Value

About 5g of *Gladiolus psittacinus* bulb oil was placed in a conical flask and 50ml 0.5M of alcoholic KOH was added to the oil. A blank was prepared by dispensing 50ml of 0.5M alcoholic KOH with blank solution into another conical flask. A reflux condenser was connected to each flask and was boiled for an hour. On cooling, the condenser was rinsed with little distilled water and was removed. One ml of phenolphthalein indicator was added into each flask and titrated against 0.5ml HCl until the pink colour disappeared. The titre value was taken and the saponification value was calculated thus

$$\text{Saponification value (mg/g)} = (\text{Blank titre value} - \text{sample titre value x } 28.05)/\text{Weight of the sample} \quad (3)$$

Phospholipids Analysis

About 0.01g of *Gladiolus psittacinus* bulb oil was added to test tubes. Any remaining solvent was removed by passing a stream of nitrogen gas over the oil. Then 0.40ml of chloroform was added, followed by the addition of 0.10ml of chromogenic solution. The tube was heated to 100^0 C on a water bath for 1min 20 seconds, cooled to room temperature, 4ml of hexane was added and the solvent and aqueous layers the hexane were recovered and concentrated to 1.0ml for analysis. Analysis was performed using gas chromatograph with a polar capillary column (30m x 0.25mm x 0.2micrometer). The oven programme was initially at 50^0C ramping at 10^0C/ min for 20min, held for 4min, a second ramping at 15^0C/ min for 4mins and held for 5minutes. The injection temperature was 250^0C, and the detector temperature 320^0C. As previously described, a split injection type was used having a split ratio of 20:1. Peaks were identified by comparison with known standards (Raheja et al., 1973).

Phytosterols Analysis

An aliquot of *Gladiolus psittacinus* bulb oil was added to screw-capped test tubes. The sample was saponified at 95^0C for 30mins, using 3ml of 10% KOH in ethanol, to which 0.20ml of benzene was added and 2ml of hexane was used in extraction of the non-saponifiable materials. Three extractions, each with 2ml of hexane, were carried out for 1hr, 30min and 30min respectively, to achieve complete extraction of the phytosterols. Hexane was concentrated to 1ml of gas for chromatographic analysis (AOAC, 1997).

Essential Oils Analysis

Essential oils extraction was carried out following the modified method of Jarubol (2009). About100g of pulverized sample was weighed into 1000ml round bottom flask. The flask with weighed sample, condenser and other gadgets were connected to complete the hydro-distillation arrangement using Clevenger-type apparatus. The crushed sample in the flask was entirely covered with deionized water suspension and placed on the heating mantle. The water was allowed to boil in the flask and the essential oil carried over to the condenser along with the steam. The essential oil and steam were separated below the condenser through a separator. It was then dried over anhydrous sodium sulphate and stored in a 2ml sealed Agilent vial protected from light at 4^0C before chromatographic analysis. The oils were analyzed on an HP6890 GC, powered by HP chemstation Rev.A09.0 (1206) software. Flame ionization detector (FID) fitted with fused silica capillary column with dimension 30m x 0.23mm x 0.25micrometer was used. The oven temperature was programmed from 40^0 – 200^0C at 5^0C / min and run at 200^0C for two minutes. Split injection temperature of 150^0C with split ratio 20:1 was used. The detector temperature was 300^0C and the carrier gas was hydrogen at flow rate 1.0ml/minute. Hydrogen pressure was 22psi with compressed air of 28psi.

Fatty Acids Analysis

Crude oil of *Gladiolus psittacinus* bulb oil was made water free by filtering through anhydrous sodium sulphate salt. Hexane was removed from the oil per hexane mixture using rotator evaporator. Fatty acid profile, saturated, mono and poly unsaturated analysis were carried out following the modified AOAC (1997) methods. About 50mg of *Gladiolus psittacinus* bulb oil was saponified for 5mins at 95^0C with 3.4ml of the 0.5M KOH in dry methanol. The mixture was neutralized by using 0.7M HCl. About 3ml of the 14% boron triflouride in methanol was added. The mixture was heated for 5mins at 90^0C to achieve the complete methylation process. The fatty acid methyl esters were thrice extracted from the mixture with redistilled n-hexane.The fatty acid content was concentrated to 1ml for the gas chromatography analysis and 1ml was injected into the injection port of the GC equipment. Flame ionization detector (FID) fitted with fused silica capillary column with dimension 30m x 0.23mm x 0.25micrometer was used. The oven temperature was programmed from 40^0 – 200^0C at 5^0C/ min and run at 200^0C for two minutes. Split injection temperature of 150^0C with split ratio 20:1. The detector temperature was 300^0C and the carrier gas was hydrogen at flow rate 1.0ml / min.

RESULTS AND DISCUSSION

Table 1 shows the physiochemical properties of *Gladiolus psittacinus* bulb oil. The bulb oil is a rich source of plant oil with concentration as high as 21.6%. This concentration was relatively high compared with those obtained for various plant foods such as the bulb (0.08-0.03%) and aerial part (0.16-0.25%) of *Allium sativum* (Nazzaro et al., 2022).Acid value, an indication of the concentration of free fatty acids present in oils is an important parameter to assess food quality. The higher the acid value, the lower the possibility of oil to be used for cooking. High acid value shows that the oil triglycerides are converted to fatty acids and glycerol which can lead to oil rancidity. The acid value (mg/KOH/g) of the *Gladiolus psittacinus* bulb oil, 3.0 mg/KOH/g is lower than that of Neem seed oil (Hamadou et al., 2020). The low concentration of fatty acids in the bulb oil is an indication that the oil is predominately composed of triacylglycerol. Iodine value is a measure of degree of unsaturation of fat. The Iodine value of *Gladiolus psittacinus* bulb oil was 42.20 mg/g indicating the oil as a non-drying oil which could be useful as a lubricant. The low Iodine value of *Gladiolus psittacinus* bulb oil also suggests greater storage ability of the oil. Saponification value is another important parameter used for the characterization and assessment of the quality of edible fats and oils. Furthermore, it gives information about the average molecular weight of all constituting fatty acids. The higher the saponification value, the lower the molecular weight of all fatty acids. Saponification value of *Gladiolus psittacinus* bulb oil, 195 mg/KOH/g compared favourably to Neem seed oil of 199.81 mg/KOH/g (Hamadou et al., 2020).

Table 2 shows the phospholipids composition (mg/100g) of *Gladiolus psittacinus* bulb oil. Phosphatidylcholine (lecithin) the most abundant phospholipid in *Gladiolus psittacinus* bulb oil (23.35 mg/100g) is a key building block of membrane bilayers. It is also the principal phospholipid circulating in the plasma, where it is an integral component of lipoproteins, especially the HDL. It has been reported to enhance neuronal differentiation and lessen neuronal alterations caused by inflammation (Magaquian et al., 2021). Up to 30 grams of lecithin per day is considered safe if taken as a supplement. However, higher doses may result in anorexia, sweating, increased salivation, hepatitis and gastrointestinal distress, such as nausea and diarrhea (Annonymous, 2024). Phosphatidylserine was the second most abundant phospholipids in *Gladiolus psittacinus* bulb oil with concentration as high as 15.75 mg/100g, phosphatidylserine aids in the transmitting messages between brain nerve cells. It helps in blood clotting, coats and shields brain cells, and may be crucial for maintaining memory and aiding in neurotransmitter release, synaptic transmission, and neural signaling. Phosphatidylserine forms part of the cerebral cortex and aids cognitive functions (Eun et al., 2022). The consumption of phosphatidylserine may reduce the risk of cognitive dysfunction, enhancement of mood in young people during mental stress.

Gladiolus psittacinus bulb oil contains 7.81 mg/100g of phosphatidylinositol. It is a minor component in the cytosolic site of eukaryotic cell membrane. Inositol can be phosphorylated to form phosphatidylinositol phosphate (PIP), Phosphatidylinositol biphosphate (PIP_2) and phosphatidylinositol triphosphate (PIP_3) which are collectively called phosphoinositides. They control a wide range of biological processes, including vesicular endocytosis, membrane identification and fusion of membrane vesicles (Jill et al., 2022).

PIP_2, a precursor for PIP_3 synthesis, has been reported to play a major role in cytoskeletal linkage, regulation of ion channels, and intracellular trafficking. (Mandal, 2020). Phosphatidylethanolamine (Cephaline) with a concentration of 6.64 mg/100g in *Gladiolus psittacinus* bulb oil is found in all living cells, although in human physiology it is found particularly in nervous tissue such as the white grey matter.

Clinically, significant reduction in LDL-cholesterol values have been linked to diets enriched with plant sterols, and its supplementation into food is a treatment strategy to manage familial hypercholesterolemia (Barkas et al., 2023). In view of this, phytosterols (mg/100g) composition of *Gladiolus psittacinus* bulb oil were analyzed as shown on table 3. The most abundant phytosterols in *Gladiolus psittacinus* bulb oil, sitosterol, showed a concentration of 21.06 mg/100g. Phytosterols compete with cholesterol for absorption in the small intestine. When consumed, they occupy the same micelle sites as cholesterol, reducing the amount of cholesterol that can be absorbed into the bloodstream. This, in turn, leads to lower LDL (bad) cholesterol levels, a major factor in the development of coronary heart diseases. Campesterol, (12.75mg/100g) was the second most abundant phytosterol in *Gladiolus psittacinus* bulb oil which is comparable with the previous reports of 11.00 mg/100g by Li et al., (2022) in *Arabidopsis thaliana* plant. Campsterol (12.75) has shown promise in modulating inflammatory responses. Sarwat et al., (2024) demonstrated that Campesterol derivatives improved nociception behavior and exhibited anti-inflammatory and antioxidant effects by reducing the thickness of paws and inhibiting the release of inflammatory mediators such as IL-1β and TNF-α.

Table 4 shows the fatty acid profile of *Gladiolus psittacinus* bulb oil. The dominant fatty acid in *Gladiolus psittacinus* bulb was linoleic acid (41.34%). This concentration is considered high compared to 31.47% reported for the seed flour of *Luffa cylindrica* (Oyetayo and Ojo, 2012). Linoleic is a polyunsaturated fatty acid which is beneficial to health High linoleic concentration has been associated with a low risk of developing coronary heart diseases. Oleic acid (20.15%) was the next high occurring fatty acid in bulb oil. Both fatty acids are unsaturated which exhibit fewer tendencies towards the development of heart related disorders. The oleic/linoleic acid ratio (O/L) largely influence oxidative stability and hence shelf life (Sahin, *et al.,* 2022). O/L ratio of 1.00 and above is associated with high stability and potentiality of oil for deep frying. The O/L ratio of *Gladiolus psittacinus* bulb oil, 0.65 (approximately 1.0) shows its potential as a stable oil. Oleic acid influences membrane fluidity and integrity. Its incorporation into membranes affects cellular signaling and various cellular functions and plays a role in regulating cell proliferation and differentiation, particularly in skin health and wound healing (Xu *et al.,* 2015). *Gladiolus psittacinus* bulb oil was found to contain relatively low concentration of saturated fatty acid except for palmitic acid (21.68%) which is the most concentrated saturated fatty acid compared with 25.48% fatty acid composition of the seeds of *Costus afer* (Chioma *et al.,* 2020). Margaric (0.01%) and Behenic acid (0.08±%) concentrations were low. Behenic acid-rich structured lipids have demonstrated promise in the context of obesity and metabolic health for reducing weight gain and enhancing glucose and lipid balance (Reginaldo *et al.,* 2020). The total unsaturated fatty acid (69.90%) of *Gladiolus psittacinus* bulb oil was higher than the saturated fatty acid concentration (30.10%). Since diets with higher concentration of plant based unsaturated fats have been established to reduce the risk of cardiovascular diseases, *Gladiolus psittacinus* bulb presents a potential source of healthy oil in the prevention of cardiovascular disorders.

Essential oils are secondary metabolites composed of mixture of different compounds including terpenic hydrocarbons like ketones, epoxides, aldehydes and esters (Stephane and Jules 2020). They give aroma and flavor to plants and are synthesized to attract pollinators, disperse seeds, and protect against pests and predators (Mugao *et al.*, 2020). The aroma is frequently employed in cosmetics and aromatherapy fields (Agrawal *et al.*, 2024). They are also popular as food additives due to their antimicrobial and antioxidant capabilities (Manso *et al.* 2014). The composition of essential oils isolated by the hydro distillation of *Gladiolus psittacinus* bulb are presented in table 5. A total of 19 essential oils were identified from *Gladiolus psittacinus* bulb. The most abundant essential oil was 1,8-Cineole (46.05%). Also referred to as eucalyptol, its concentration is comparable to 54.29% earlier reported for *E. maculate* leaf (Almas *et al.*, 2021). It has demonstrated a wide range of pharmacological qualities, such as anti-inflammatory and antioxidant effects, primarily through the regulation of NF-κB and Nrf2 and has been used in treatment of cardiovascular and respiratory disorders (Cai *et al.*, 2021). Beta-pinene, the second most abundant essential oil in *Gladiolus psittacinus* bulb oil (15.13%) compares favorably with 12.79% obtained for *Citrus medica* earlier reported (Weixuan *et al.*, 2023). *Gladiolus psittacinus* bulb oil contains limonene a cyclic terpene with an orange fruit-like aroma, a useful fragrance applicable in cosmetics. It has been reported to posses' effective anti-inflammatory activity in the prevention of respiratory system injuries (Santana *et al.*, 2020). Linalool also reported for its anti-inflammatory activity (Kim *et al.*, 2019) was the third highest concentrating essential oil present in the bulb oil.

Table 1. Fat Composition and Physicochemical of *Gladiolus psittacinus* bulb

Parameter	Value
% Crude fat	21.60
Acid value (MgKOH/g)	3.00
Iodine Value (Mg/g)	42.20
Saponification value (MgKOH/g)	195.00
Refractive index@40^0C	1.49

Table 2. Phospholipids Composition (mg/100g) of *Gladiolus psittacinus* bulb oil

Phospholipids	Concentration (mg/100g)
Phosphatidylethanolamine	5.84
Phosphatidylcholine	23.35
Phosphatidylserine	15.75
Lysophosphatidylcholine	2.42
Phosphatidylinositol	7.81
Phosphatidic acid	4.46

Table 3. Phytosterols Composition (mg/100g) of *Gladiolus psittacinus* bulb oil

Phytosterols	Concentration (mg/100g)
Campestrol	12.74
Stigmasterol	6.66
Savenasterol	2.23
Sitosterol	20.03

Table 4. Fatty Acids Composition % of *Gladiolus psittacinus* bulb oil

Fatty Acid	Concentration (%)
Palmitic acid	21.68
Margaric acid	0.01
Stearic acid	7.64
Arachidic acid	0.51
Behenic acid	0.08
Lignoceric acid	0.18
Total saturated fatty acid	30.10
Monounsaturated fatty acids	
Palmitoleic acid	0.10
Oleic acid	20.15
Erucic acid	0.10
Total monounsaturated fatty acid	20.35
Polyunsaturated fatty acid	
Linoleic acid	46.75
Arachidonic acid	0.05
Linolenic acid	2.76
Total polyunsaturated fatty acid	49.55
TOTAL UNSATURATED FATS (MUFA + PUFA)	69.90
Oleic/linoleic acid ratio	0.43

Table 5. Essential oil Composition (%) of *Gladiolus psittacinus* bulb oil

Essential oils	Concentration (%)
α -pienene	4.39
β-pinene	15.13
Limonene	0.54
Cis ocimene	1.64
Pinene-2-ol	1.20
α -troujere	0.70
ɣ-terpinene	0.05
Geranial	0.01
Linalool	6.27
1,8cinole	46.05
Citronellal	0.04
α –terpineol	11.74
Terpinen-4-ol	3.12
Citronellol	0.04
α--terpinenly acetate	4.34
Neryl acetate	0.02
Geranyl acetate	4.65
Humulene α- caryophylene	0.04

CONCLUSION

The present investigation reveals *Gladiolus psittacinus* bulb oil as a novel source of beneficial unsaturated fatty acids, phospholipids and essential oils. The bulb oil is rich in fatty acids such as linoleic and oleic acids which are unsaturated fats beneficial for lowering blood cholesterol levels. The phospholipids contain high levels of phosphatidylcholine and phosphatidylserine and the essential oils are rich in 1,8 Cineole and beta-pinene which have high nutritional, pharmacological and health benefits and as such, dietary supplementation and consumption of *Gladiolus psittacinus* bulb oil could be beneficial to human health. However, further studies to evaluate the possible *in vivo* toxicological potentials of the oil is expedient.

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