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Original Research Article

Phytochemical, Heavy Metal Analysis and HPLC Profiling of Jobelyn® – An Herbal Dietary Supplement

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ABSRTACT

Jobelyn®, an herbal dietary supplement derived from *Sorghum bicolor* sheath, is known for its anti-inflammatory, anti-cancer, anti-anaemic, and antioxidant properties due to its bioactive constituents. The therapeutic potential of Jobelyn® has aroused scientific interest. However, data on its proximate, phytochemical, and heavy metal profiles remain scarce. Such data are essential for the critical evaluation of its safety, efficacy, and potential health benefits. This research aims to perform a systematic evaluation of Jobelyn® through proximate assessment, phytochemical constituents, metal composition, and bioactive compounds. Standard procedures were used for qualitative and quantitative phytochemical evaluation, whereas metal analysis was performed using Inductively Coupled Plasma Optical Emission Spectroscopy, and High-Performance Liquid Chromatography was used for chemical profiling. Major phytochemicals found in Jobelyn® include Saponins (48.81 mg/100g), Tannins (45.34 mg/100g), Terpenes (40.58 mg/100g), and Phenols (32.33 mg/100g). Proximate analysis showed that Jobelyn® is rich in carbohydrates (81.3%), crude protein (4.7%), and fatty acids/oil (3.0%). The light and heavy metal evaluation revealed beneficial metals like Sodium (23.30 ppm), Potassium (9.14 ppm), and Iron (1.07 ppm), besides non-essential metals such as Lead (0.05 ppm), Arsenic (0.02 ppm), and Mercury (0.001 ppm). All of which are within the WHO permissible limit. Bioactive compounds like Quercetin (16.95 µg/mg), Formononetin (3.23 µg/mg), Luteolin (2.83 µg/mg), Caffeine (0.31 µg/mg), and Gallic acid (0.13 µg/mg) were identified and quantified by HPLC profiling. The novelty of this research lies in its comprehensive profiling of Jobelyn® as a polyphenol-rich supplement, with formononetin observed for the first time, giving a basis for its scientific repurposing and potential clinical application.

Keywords: Jobelyn®, *Sorghum Bicolor*, Phytochemical Screening, Proximate Analysis, and Heavy Metals.

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Introduction

Jobelyn® is an established herbal dietary supplement produced from the leaf extract of *Sorghum bicolor* (L.) Moench, a cereal crop native to Africa, is generally known for its ethnopharmacological impact. Produced, branded, and distributed by Health Forever Products Ltd., Lagos, Nigeria. Jobelyn® is formulated as a syrup for children and capsules for adults. It is accessible in pharmacies across Nigeria and is mainly promoted for its strong anti-inflammatory and antioxidant properties.[1] The sole phytoconstituent of Jobelyn®, *Sorghum bicolor leaf extract*, has profound therapeutic and cultural origins in African traditional medicine. Historically eminent for its resilience and dietetic value, *Sorghum bicolor* has long been used as a functional diet and a medicinal agent in traditional healthcare systems.[2] This ethnobotanical basis offers a sturdy rationale for its present-day use in current nutraceuticals like Jobelyn®. The therapeutic effect of Jobelyn® is ascribed to its rich polyphenolic profile, including anthocyanins, flavonoids, and phenolic acids.[3,4] These bioactive compounds are well-known and established antioxidants, which are strong scavengers of free radicals and reactive oxygen species (ROS) neutralisers, thus alleviating oxidative impairment, an established stimulant of cellular senescence and disease advancement.[5]

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Moreover, Jobelyn® demonstrates anti-inflammatory potential by regulating significant signalling pathways implicated in the inflammatory response, thereby inhibiting chronic inflammatory damage connected to various non-communicable maladies.[6]

Comparatively, Jobelyn® present several advantages over celluloid antioxidants and orthodox anti-inflammatory agents, including a decreased occurrence of side effects and improved cultural acceptance owing to its natural derivation and historical application. It is also outstanding amid existing herbal dietary formulations for its standardisation, bazaar presence, and incipient scientific validation.[1,3,4]

Health conditions such as cardiovascular diseases, arthritis, diabetes, and neurodegenerative syndromes, typified by exacerbated oxidative stress and chronic inflammation, are principally significant for Jobelyn®-centered interventions.[7,8,9,10] These potentials of Jobelyn® as documented in existing literature,[1,3,4] accentuate the need for its systematic scientific investigation.

The medicinal impact of Jobelyn® has stimulated increasing scientific curiosity; however, comprehensive information on its nutritional, phytochemical, and toxicological profile is scarce. Particularly, there is a paucity of detailed evidence of Jobelyn's proximate constituents, the qualitative and quantitative evaluation of secondary metabolites, the HPLC profile of major bioactive compounds such as quercetin, gallic acid, formononetin, luteolin, and caffeine, and its heavy metal content. Such records are essential for assessing the safety, efficacy, and potential health impact or benefits of the supplement.

Thus, this research seeks to perform a systematic evaluation of Jobelyn® through proximate assessment, qualitative and quantitative phytochemical examination, high-performance liquid chromatography (HPLC) profiling of selected bioactive compounds, and the evaluation of heavy metal concentrations using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Each method used in this study has been scientifically validated and extensively adopted for

evaluating herbal formulations, consequently safeguarding the dependability and significance of the data obtained.[11,12,13,14,15,16]
The significance of this study lies in its potential to validate the nutritional and pharmacological value of Jobelyn®, substantiate its health claims, and endorse its safe use within the context of an evidence-based herbal drug. In addition, the research addresses a gap in contemporary literature by incorporating nutritional, phytochemical, and toxicological evaluations in a single analytical framework. The novelty of this research lies in its comprehensive profiling of Jobelyn® as a polyphenol-rich supplement, giving a basis for its scientific repurposing and potential clinical and scientific application.

Materials and Methods

Sample collection
Jobelyn® capsules, 250 mg, were procured from Tonyson Pharmacy in Lagos, Nigeria, with the Batch number: J2365, Manufacturing date: 05/2022, and Expiry date: 04/2024.

Phytochemical analysis
Extraction of Jobelyn® sample for phytochemical screening
Extraction of Jobelyn® powder was done according to the method highlighted by Chibuye *et al*.,[17] Jobelyn® powder, 5 g was weighed each into three conical flasks and 250 mL of water, 99 % v/v ethanol (Analar®grade, with percentage purity of 98) and 70 % v/v aqueous ethanol (Analar® grade, with percentage purity of 98) was added. Maceration was done for 72 hours. The ethanol extract was then filtered and concentrated using a rotary evaporator (Stuart™, Cole-Parmer Ltd., Stone, ST15 OSA, UK) while the water and aqueous ethanol (70 % v/v) extracts were freeze-dried (Freeze dryer BK-FD10P Model, Biobase Biodustry (Shandong) Co., Ltd, China) for further phytochemical analysis.
Results were obtained using the equation:
% Yield = (Weight of dried extract/Weight of dried plant sample) × 100. …… equation 1

Qualitative phytochemical analysis
The extracts (water, 99 % v/v ethanol, and 70 % v/v aqueous ethanol) were separately screened for secondary metabolites or phytochemicals such as alkaloids, flavonoids, saponins, tannins, reducing sugars, cardiac glycosides, phenols, and terpenes using standard methods of .[11,12]

Quantitative phytochemical analysis
Quantitative phytochemical analysis, which encompasses the determination of the concentration/amount of the actual phytochemicals (alkaloids, flavonoids, saponins, tannins, and terpenes) present in the Jobelyn® capsule, was achieved through standard procedures.[11,12]

Proximate analysis of Jobelyn®
Proximate analysis of Jobelyn®**,** which involves the determination of moisture, ash, fatty acids, crude fibre, carbohydrate, nitrogen, and crude protein contents in the Jobelyn® capsule, was evaluated using standard methods.[13]

Heavy metal analysis using ICP-OES
Heavy metal screening involves three essential stages. These stages include acid digestion, filtration, and instrumentation utilizing Inductively coupled plasma-optical emission spectrometry (ICP-OES). Powdered Jobelyn® sample (1 g) was weighed into a 50 mL beaker and digested using aqua regia, 37% HCL and 65% HNO_3 in the ratio of 3:1 (30 mL to 10 mL), respectively. The resulting solution was heated at 105^0C for 3 hours on a heating mantle till complete digestion was observed.[12,14] Liberation of the heavy metals from their binding or bond state requires digestion.
The heavy metals screened in the powdered Jobelyn® sample are arsenic, chromium, copper, iron, lead, mercury, and zinc. The concentration of these metals in the solution was analyzed using Inductively coupled plasma-optical emission spectrometry (ICP-OES).[12]

Analysis of Caffeine Present in Jobelyn®
Caffeine content in Jobelyn® was evaluated using distilled water according to the method of Nhan and Phu.[15] with slight modification. Jobelyn® sample (0.3 g) was weighed and transferred into 250 mL conical flasks. Distilled water, 200 mL, was added and placed in a water bath at 100ºC. Extraction was carried out for 30 minutes. Then the resulting solution was allowed to cool, made up to the 250 mL mark and filtered. The filtrate, 1 mL, was pipetted into a clean 10 mL volumetric flask and made up to the mark with HPLC water. A microfilter (0.2 µm) was used to filter the prepared sample solution and transferred into HPLC vials for analysis.
A stock solution of caffeine (100 µg/mL) was prepared by 1 in 10 dilution of a 1000 µg/mL caffeine solution, obtained by weighing pure caffeine (10 mg) into a 10 mL volumetric flask and making up to the mark with 70% v/v methanol.
Working standards (10, 20, 40, 80 and 100 µg/mL) were prepared by serial dilution of the stock solution (100 µg/mL) with HPLC-grade water. A plot of peak areas versus concentration of the standards was done as an external calibration curve. The regression line obtained from the calibration was used to evaluate the amount of caffeine in the Jobelyn® water extract.
Chromatographic system: The liquid chromatograph is equipped with an Agilent 1200 series Zorbax column (SB: C18, 4.6mm x 150 mm, 5 µm), it has a UV detector and has a wavelength of 275 nm with a flow rate of 1.6mL/min. Mobile phase: Methanol: Water (40:60), both are HPLC grade.[16]

Preparation of mobile phase for the HPLC profiling of other bioactives
The isocratic method was used to prepare the mobile phase. Deionized water (220 mL) was measured into a graduated cylinder; 75 mL of acetonitrile, 200 mL of methanol, and 5 mL of glacial acetic acid were added to prepare a 500 mL mobile phase. The resulting solution was sonicated for 15 minutes for proper mixing of the solvents.

Preparation of Jobelyn® sample for HPLC profiling
Jobelyn® powder, 250 mg, was weighed into the 25 mL volumetric flask and made up to volume with 70% v/v methanol. Stock solution (1 mg/mL) prepared from the resultant solution (10 mg/mL) by a 1:10 dilution was filtered using a syringe filter (0.45 µm) to remove insoluble contents that may interfere with HPLC analysis.

Preparation of Mixed Standard
Standards were prepared according to the method of Abid *et al*.,[17] with slight modification. Luteolin, gallic acid, quercetin, and formononetin standard, 5 mg, were weighed into separate 5 mL sample bottles, and 5 mL of methanol was added to obtain a solution having a concentration of 1 mg/mL. Each concentration of the standards prepared was filled into HPLC vials for analysis.
Chromatographic system: The liquid chromatograph is equipped with an Agilent Zorbax column (SB: C8, 4.6 nm x 150 mm, 5 µm) and has a wavelength of 257 nm with a flow rate of 1 mL per minute. Mobile phase: Methanol: Acetonitrile: water in the ratio of 40:15:45, respectively, and 1% glacial acetic acid.

Statistical analysis
The results are presented as the mean of three replicates (n = 3) done by Excel 2010 achieved by computing the mean and standard deviation ± SD for each parameter studied.

Results and Discussion

Percentage Yield of Jobelyn®
The percentage yield of Jobelyn® that was obtained in three different extracts, as highlighted in Table 1 below, ranged between 3.13 – 6.75 %. The highest yield (6.75 %) was obtained from the 70 %v/v aqueous ethanol extract.

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Table 1: Percentage yield of Jobelyn® extracts**.**

PERCENTAGE YIELD OF JOBELYN® EXTRACT			
Weight of Jobelyn®	% Yield Water extract)	% Yield (99% v/v Ethanol extract)	% Yield (70% v/v aqueous ethanol extract)
5 g	3.13	4.82	6.75

Qualitative and quantitative phytochemical screening

The result of qualitative phytochemical analysis that was carried out on Jobelyn® in three different solvent media (i.e., ethanol, water, and aqueous ethanol) is shown in Table 2, indicating the phytochemicals present in Jobelyn®. Aqueous ethanol (70 %v/v) extracted more of the secondary metabolites or phytochemicals present in Jobelyn®, followed by absolute ethanol (99 % v/v). However, water extracted less of the phytochemicals present in the Jobelyn® sample.

Table 2: Qualitative Phytochemical Analysis of Jobelyn®

S/N	Phytochemical	99 %v/v Ethanol	Water	(70% v/v Aqueous Ethanol
1	Alkaloids	+	+	+
2	Saponins	+	+	+
3	Tannins	+	+	+
4	Phlobatanins	+	+	+
5	Cardiac Glycosides	+	+	+
6	Phenols	+	+	+
7	Flavonoids	+	+	+
8	Reducing Sugar	+	+	+
9	Terpenoids	+	+	+

Present (+); Prevalent (++)

The outcome of the quantitative phytochemical screening of the Jobelyn® sample is shown in Table 3. This reveals various concentrations of secondary metabolites present in Jobelyn®. Quantitatively, the composition of the phytocompounds in Jobelyn® (Aqueous ethanol extract, 70 %v/v) is in the following order: Saponins > Tannins > Terpenoids > Phenols > Phlobatanins > Cardiac glycosides > Flavonoids > Reducing sugar > Alkaloids.

Table 3: Average weight of Phytochemicals (mg/100g) found in the extracts

Phytochemical	99% v/v Ethanol Mean±S.D	Water Mean±S.D	70% v/v Aqueous Ethanol Mean ± S.D
Alkaloids	24.05±0.50	23.33±0.17	24.54±0.22
Saponins	42.59±0.51	26.80±0.17	48.81±0.18
Tannins	33.61±0.36	14.26±0.04	45.34±0.02
Phenols	25.35±0.17	25.35±0.17	32.33±0.34
Phlobatannins	28.19±0.84	17.57±0.22	32.08±0.51
Flavonoids	28.85±0.44	26.59±0.14	28.51±0.44
Cardiac glycosides	27.99±0.47	21.82±0.40	30.45±0.10
Reducing sugar	18.36±0.29	13.58±0.49	25.11±0.39
Terpenoid	30.43±0.05	16.50±0.84	40.58±0.06

According to the findings of this research, both qualitative and quantitative phytochemical analysis of Jobelyn® revealed the existence of alkaloids, cardiac glycosides, flavonoids, phenols, saponins, steroids, tannins, and terpenoids. Thus, Jobelyn® has the potential to serve as a source of valuable pharmaceuticals due to the presence of these secondary metabolites.

Quantitative phytochemical screening showed that the aqueous ethanol (70 % v/v) extract contains the highest concentration of the phytochemicals.

Cardiac glycosides, found to be a phytoconstituent of Jobelyn®, elicit their therapeutic effect via the inhibition of the Na^+/K^+ pump. This promotes an increase in the myocytes' sodium ions and a resultant increase in Ca^{2+} level. This elevated Ca^{2+} ion level stimulates the contraction of the heart muscle, which mitigates cardiac output and eases the tension/pressure of the heart, and so is utilized in the management and treatment of cardiac arrhythmia and congestive heart failure.[18,19] Anti-cancer and immunologic potentials of cardiac glycosides have also been highlighted. Cardiac glycosides are promising agents for cancer target treatment based on their ability to act as a ligand for Na/K-ATPase.[18,19]

Flavonoids, which are found in predominant amounts in the outer layer of the sorghum plant, have been found in abundance in Jobelyn® as well. They are known to offer a variety of medicinal effects, including anti-inflammatory, antioxidant, antiviral, anti-cancer, neuroprotective, and cardioprotective activities.[18,20] However, these pharmacological effects depend largely on the types of flavonoids present.[18,20] Flavones, dihydroflavonols, flavan-3-ols, flavan-4-ols, 3-deoxyanthocyanidins, flavonols, and flavanones are the classes of flavonoids established in sorghum,[2] the unique source of Jobelyn® dietary supplement. Anti-edematogenic, anti-ulcer, antihistamine, and antinociceptive activities of the flavonoids have also been reported.[18,21] The health impact of flavonoids on diabetes and obesity has been systematically explored. Flavonoids can thus be a lead compound for the development of anti-diabetic and anti-obesity medications that can be safer and affordable.[22,23]

Saponins have vast applications, both health-wise and otherwise. The health benefits of saponins include their potential as an anti-cancer, antioxidant, anti-fungal, anti-glycemic, anti-inflammatory, antihypertensive, anti-asthmatic, antimicrobial, Cholesterol reduction, and management of hypercalciuria in man.[24,25] A diet rich in saponins can ameliorate and inhibit platelet aggregation and dental caries. It is also used as an antidote for lead poisoning. [24,25]

Established pharmacological impact of tannins (a major phytoconstituent of Jobelyn®) includes Antihypertensive, lipid-lowering, modulates immune response, reduction of serum lipid levels, production of liver necrosis, promoting blood clotting, inhibiting HIV replication, mutagenic, hepatotoxic, anti-helminthic, antimicrobial, anti-inflammatory, anti-cancer, and antioxidant.[26] The antidiarrheal effect of tannins has also been scientifically demonstrated using animal models.[27] The most significant pharmacological effects of tannins are cardioprotective, anti-diabetic, antioxidant, antibacterial and antiviral, and anti-inflammatory.[26] Sorghum's tannins include flavan-3-ols and/or flavan-3, 4-diols - catechin oligomers or polymers.[2] The concentration of tannin previously detected in Sorghum was in the range of 0.2 to 48.0 mg/g,[2] which is comparable to the concentration of tannin in Jobelyn® in this study.

Terpenes, another major phytochemical present in Jobelyn®, have established therapeutic impact on a wide range of ailments, ranging from anti-malaria or antiplasmodial (artemisinin and its derivatives). They exist in simple (essential oil) and complex forms, such as triterpenes and tetraterpenes. Terpenes in Jobelyn® should encourage additional research for enhanced drug efficacy and novel indications. Studies have shown that terpenes, known as limonene, reduce cholesterol and triglyceride levels in the body. Linalool, another terpene, acts on the brain as an anti-stress agent. Other therapeutic impacts of terpenes systematically highlighted include the mitigation of chronic obstructive pulmonary disease, bronchitis, ear oedema, osteoarthritis, and Skin inflammation.[28] Anti-cancer, antioxidant, anti-diuretic, and anti-inflammatory potentials of terpenes have been extensively reported.[29]

Alkaloids were found in the Jobelyn® sample screened. The presence of alkaloids in Jobelyn® could have contributed largely to its enormous benefits, both known and unknown. Several studies have highlighted the health impact of alkaloids in both *in vivo* and *in vitro* studies. These

include antibacterial, antiproliferative, antiviral, antimetastatic impact of different types of cancers, cardioprotective, anaesthetics, anti-inflammatory, anti-fungal agent,[30,31] hepatoprotective, antihypertensive, and anti-psychotic.[30]

Intake of reducing sugars has been shown to lower the chances of gaining excess body weight, and in turn drastically decrease the risk of developing diabetes, cardiovascular diseases, and dental caries. [32,33]

Phenols, an essential component of Jobelyn®, have demonstrated a range of pharmacological impacts including relief of cancer, Kidney disease, Alzheimer's disease, scavenging of free radicals, enhancement of anti-inflammatory ability of human beings, immunoregulatory, cardioprotective effect, Antilipidemic, anti-depressant, improvement of vision, and prevention of diabetes.[34,35] Polyphenols can attack the pathogenic cell and exert their antimicrobial activity [35]

Phlobatannins, an essential phytoconstituent in Jobelyn®, have been documented for their analgesic, anti-inflammatory, and wound-healing properties.[2]

The potential health impacts of the phytochemicals identified in Jobelyn® in this study have been extensively highlighted. Thus, Jobelyn® may offer numerous health benefits due to its robust phytochemical composition. Moreover, the safety, efficacy, and therapeutic potential of Jobelyn are directly linked to its phytoconstituent.

Proximate analysis

The result obtained from the proximate analysis, as shown in Table 4, indicates that Jobelyn® is rich in carbohydrate with a percentage content of 81.32% and it does not contain crude fibre. It also showed that Jobelyn® contains little amount of crude protein, ash, nitrogen, moisture and fatty acid/oil.

Table 4: Summary of proximate analysis of Jobelyn® Powder

S/N	Parameter	Sample Quantity (gram)	% Composition
1	Moisture	0.2	10.20
2	Ash	0.2	0.003
3	Fatty Acid/Oil	1	3.00
4	Crude Fibre	0.2	0.00
5	Nitrogen	0.2	0.76
6	Crude Protein	0.2	4.73
7	Carbohydrate	0.2	81.32

The proximate screening of Jobelyn® showed that it contains 0.003% ash value, 81.32% carbohydrate content, 4.73% crude protein value, 3.0% fatty acid content, 10.20% moisture content, 0.76% nitrogen content, but crude fibre was not detected.

Moisture content in the pharmaceutical industry is an essential quality explored in the screening of finished pharmaceutical products.[36,37] For finished capsules and tablets, moisture content is a significant quality/feature that affects the physical form, solubility, chemical degradation and the product's overall shelf life. High moisture content of any pharmaceutical formulation or raw material promotes the growth of microorganisms and short shelf-life. When the moisture content of a granule is not well regulated or handled, powder cohesion in downstream capsule filling or particle friability is bound to occur.[36] Thus, Jobelyn® could have a moderate shelf life and reduced chances of microbial growth due to its moisture content of 10.20%. Jobelyn® tends to contain minute moisture-sensitive active pharmaceutical ingredient (API) that must have formed a hydrate or undergone hydrolysis either during the manufacturing or distribution process. Poor storage conditions, which can promote water uptake from the ambient atmosphere, could also be responsible for the moisture content of Jobelyn® as seen in this study. Howbeit, measures must be taken to ensure that Jobelyn® is properly stored, and thorough studies are conducted on the hygroscopicity of each batch produced for prolonged shelf-life.

Ash value has been highlighted as one of the essential parameters for assessing the quality of dietary and herbal supplements.[37,38] Determination of ash content forms the basis for assessing the uniqueness and purity of a drug and provides valuable information regarding adulteration with inorganic compounds. Ash value indicates the total mineral composition of a pharmaceutical or food product. It is useful in the determination of the purity and quality of the pharmaceutical product, especially those in powder form.[38] The result of the total ash value in Jobelyn®, which is low, indicates that the inorganic component of Jobelyn® is low. This implies that the drug has a high organic constituent with zero to minimal adulteration.

Carbohydrates are vital components of all ingestible including drugs. Quite a few dietary supplements are explored daily for their carbohydrate content. Drugs containing carbohydrates are being approved globally on a daily basis.[39] From 2000 to 2021, drugs rich in or containing carbohydrate moieties as their main structural entity have been approved as diagnostic agents or therapeutics.[40,41] It is therefore important to explore the properties of carbohydrates to develop drugs that are rich in carbohydrates. Their irreplaceable health benefits have been well highlighted. These include anti-diabetic drugs, cardioprotective drugs, antiviral, anti-cancer, antibacterial, and neuroprotective. [39] The high carbohydrate content in Jobelyn® positions it as a drug that can exhibit most of these pharmacological impacts, if not all, with little or no structural modifications, if thoroughly investigated. Carbohydrates have also been confirmed in Sorghum, from which Jobelyn® is derived. According to the author, the major carbohydrate found in sorghum is starch (Amylopectin and Amylose), which is present in the endosperm as granules.[2]

Protein and amino acids are crucial for a range of biological functions in living organisms. The presence of these proteins fundamentally enhances intestinal morphology, growth performance, and immunological status of man. For instance, amino acids such as arginine, glutamate, and glutamine enhance intestinal health by reducing inflammation and improving the functional integrity of the intestinal epithelial mucosa.[42] Glutamine, which is the major energy source for enterocytes, helps to retain the functional and structural integrity of the intestinal mucosal.[43] It has also been established to elevate the cell proliferation and differentiation rate and decrease intestinal cells' oxidative stress through enhanced synthesis of glutathione.[43] The deficiency of protein results in anaemia, weakness, delayed wound healing, infection-prone, and kwashiorkor. Research proposes that the consumption of dietary protein offers many benefits throughout the stages of life. Consumption of protein above the recommended daily intake (0.66g $kg^{-1}day^{-1}$ and 0.8g kg^{-1} day^{-1}) helps in the improvement of bone health, cardiovascular health, wound healing support, maintains/enhances energy balance, and prevents sarcopenia.[44] Jobelyn® contains crude protein value and thus can be a good source of protein and amino acids with health impacts, including anti-diabetic, antiparkinson, and anti-cancer.[42]

Another essential component of the human body is nitrogen, which is present in a range of dietary drugs or supplements. It enhances the healthiness of bones, tissues, organs, muscles, and overall body functions. It helps in the reduction of inflammation and the treatment of a range of chronic diseases. Nitrogen is a vital building block of DNA and RNA. Their pharmacological impacts include anti-diabetic, anti-HIV, anti-cancer, antimicrobial, anti-tubercular, and anti-malaria effects.[45] The high nitrogen content of Jobelyn® indicates that Jobelyn® may elicit all the pharmacological activities already mentioned. Therefore, research must be carried out systematically to evaluate the potential of Jobelyn® in mitigating these diseases.

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Fatty acids/oils are another major compound detected in Jobelyn®. Studies have shown that fatty acids such as omega-3 (eicosapentaenoic acid, alpha-linolenic acid, and docosahexaenoic acid) have the potential to alleviate depression.[46,47] Other therapeutic impacts of fatty acids/oils include reduced risk of macular degeneration,[48] enhanced cognitive development and performance,[49] Improved cardiovascular health[50] through the reduction of triglycerides, prevention of blood clot formation, increased HDL, anti-inflammatory action,[50] and many more. It should, however, be noted that saturated fatty acids (oleic, linoleic and α-linoleiec) may be harmful to human health. The specific oil/fatty acid present in Jobelyn® should therefore be systematically evaluated for proper elucidation and utilization of its health-related benefits.

Light and Heavy metals

The results in Tables 5 and 6 revealed that the Jobelyn® sample contains a high concentration of potassium (9.14 ppm) compared to other metals with low concentration, such as mercury (0.001 ppm).

Table 5: Beneficial metals present in Jobelyn® Powder

Metals	Actual Concentration (ppm)	WHO Maximum permissible limit (ppm)
Calcium (Ca)	4.96	100
Potassium (K)	9.14	12
Magnesium (Mg)	2.50	150
Sodium (Na)	23.30	180

Table 6: Heavy metals found in Jobelyn® Powder

Heavy Metals	Actual Concentration (ppm)	WHO Maximum permissible limit (ppm)
Arsenic (As)	0.02	0.05
Chromium (Cr)	0.05	0.05
Copper (Cu)	0.03	1.5
Iron (Fe)	1.07	0.3
Mercury (Hg)	0.001	0.001
Lead (Pb)	0.05	0.05
Zinc (Zn)	0.05	5.0

Heavy metal analysis of Jobelyn® conducted using ICP-OES (Inductively coupled plasma - optical emission spectrometry) also revealed that the dietary supplement contains beneficial metals such as calcium, potassium, iron, magnesium, sodium, and zinc, which plays pivotal role in biological functions of the body such as immune response, enzyme activation, hormone regulation and kidney function.[51] The analytical instrument, ICP-OES, has enormous advantages over atomic absorption spectrometry (AAS), because it can screen metals and non-metals, thus offering all-inclusive analysis of samples containing a wide range of elements. In addition, AAS is slower in the analysis of many elements than ICP-OES, based on the capacity of the latter to analyse multiple samples simultaneously through a single run.[52] Toxic heavy metals present in Jobelyn® are lead, aluminium, arsenic, mercury, copper, and chromium, which are deleterious and harmful to human health. These toxic heavy metals (which are within the WHO permissible limit) are suspected to have come into contact with Jobelyn® during the growing stages of the *Sorghum bicolor* plant from which Jobelyn® is derived, such as fertilizer application, as well as heavy toxic metals in the air and water.

HPLC Chemical Profiling

High-performance liquid chromatography was used to ascertain the presence of significant bioactive compounds in Jobelyn®, where each peak corresponds to different components such as quercetin, gallic acid, formononetin, caffeine, and luteolin in the sample. The result obtained from the HPLC analysis is presented in Table 7. According to this finding, it was discovered that Jobelyn® contains a high concentration of quercetin, followed by formononetin, luteolin, caffeine, and gallic acid.

Table 7: Concentrations of bioactive compounds present in Jobelyn® powder

Bioactive compounds	Concentration (µg/mg)
Quercetin	16.95
Formononetin	3.23
Luteolin	2.83
Caffeine	0.31
Gallic acid	0.13

Figure 1-5 below presents the chromatogram obtained from the HPLC profiling of quercetin, formononetin, luteolin, caffeine, and gallic acid standards and Jobelyn® powder. Major peaks were observed at 1.4, 1.5, 1.6, 3.1, and 5.7 min, respectively, for gallic acid, caffeine, quercetin, luteolin, and formononetin. The peak resolution indicates adequate separation, allowing for accurate quantification.

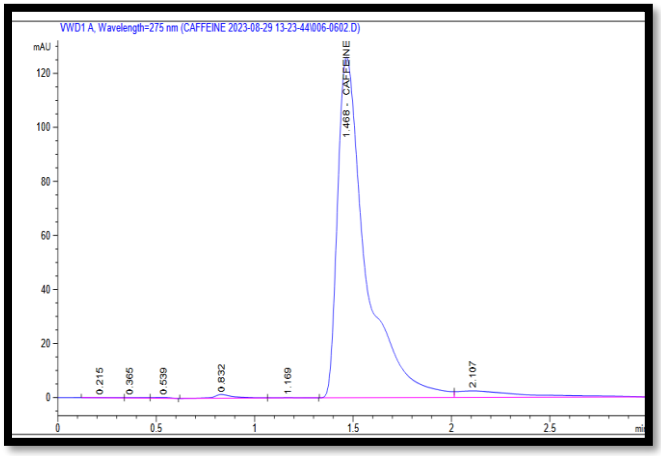

Figure 1: Chromatogram showing Caffeine standard at 100 µg/mL

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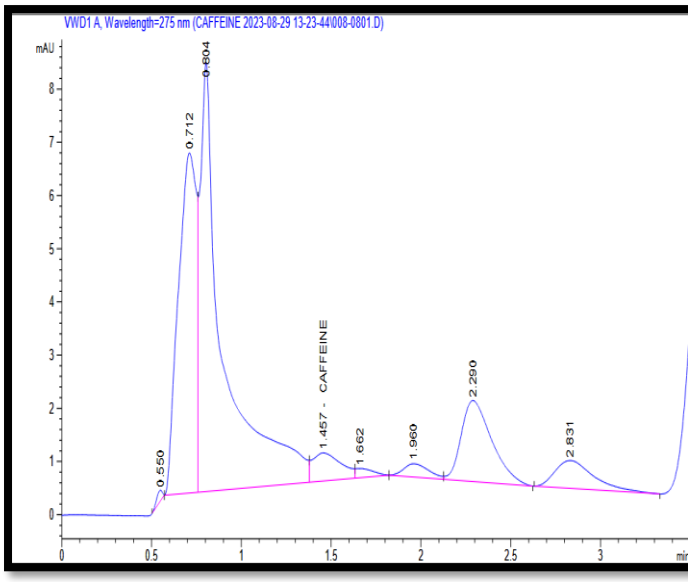

Figure 2: Chromatogram showing the concentration of Caffeine in Jobelyn® powder (1000 µg/mL) sample

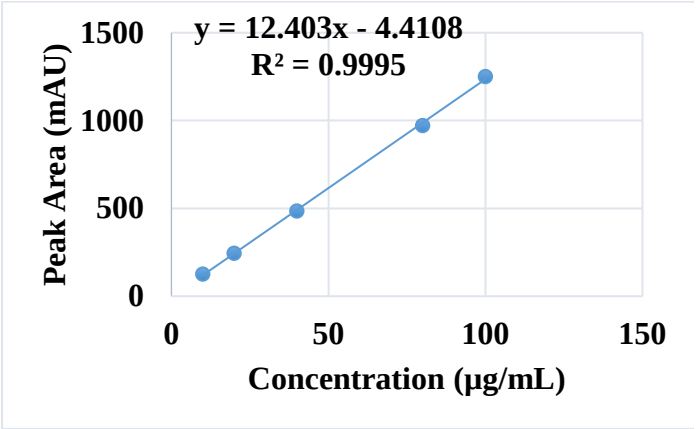

Figure 3: Calibration curve of Caffeine standard in the concentration range of 10 – 100 µg/mL

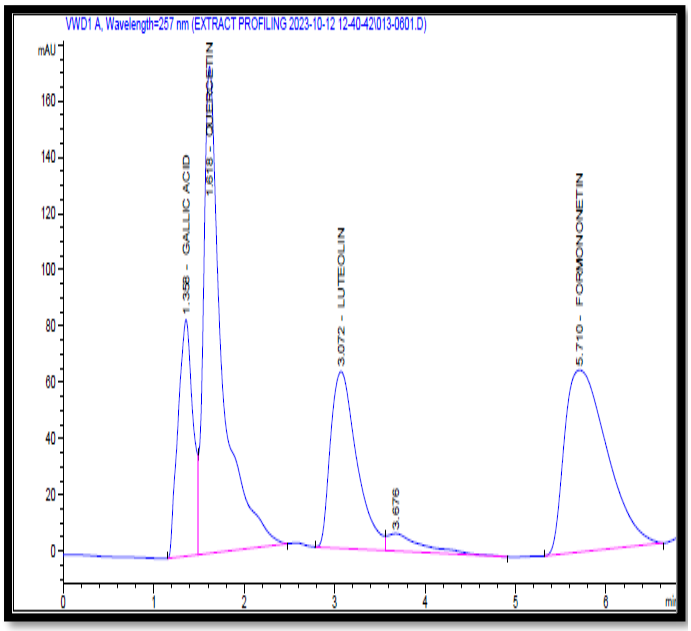

Figure 4: Chromatogram of Gallic, Quercetin, Luteolin, and Formononetin standards (Mixed standards @ 100 µg/ml).

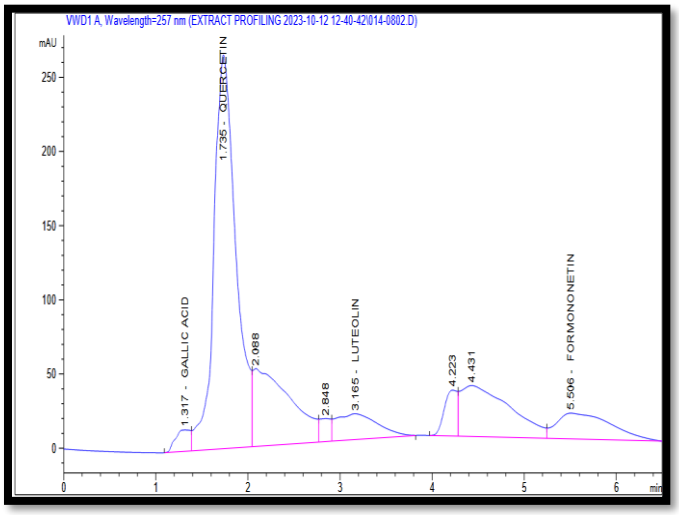

Figure 5: Chromatogram of Gallic, Quercetin, Luteolin, and Formononetin in Jobelyn® sample.

HPLC profiling of Jobelyn® buttresses the quantification of bioactive compounds that are present in Jobelyn®, and the result obtained from the HPLC analysis showed that Jobelyn® contains quercetin, formononetin, luteolin, caffeine, and gallic acid. The result of caffeine analysis conducted using HPLC reveals that the dietary supplement contains an infinitesimal amount of caffeine in the range of 313 µg/g. This implies that in every 1 g of Jobelyn® taken, there is 313 µg of caffeine present.

Quercetin, a common flavonoid found in abundance in Jobelyn®, has a track record of enormous medicinal properties such as anti-cancer, anti-diabetes, anti-arthritis, bladder infection alleviation, amelioration of blood and heart vessel diseases, reduced blood pressure,[53] and as a complementary agent for COVID-19 management.[54] Quercetin has also been shown to elicit some synergistic effects when combined with catechin, resveratrol, and genistein flavonoids to inhibit platelet function via antagonizing the intracellular production of hydrogen peroxide[55] and also with an antiviral drug in COVID-19 treatment.[56] However, studies have shown that taking above 1 g of quercetin daily could cause stomach aches, tingling sensations, and headaches.[54] Quercetin present in Jobelyn® is therefore within the acceptable limit (16.95 µg/mg) that may not stimulate any side effects. Interaction of quercetin in Jobelyn® with other drugs is possible,[57] so there is a need to consult one's physician should Jobelyn® be taken with other drugs.

Formononetin, an isoflavone reported for first time in Jobelyn® have been found with a lots of health benefits; ranging from suppression of inflammation, enhancement of cardiovascular health, alleviation of cancer, improvement of bone health, management of anxiety, mood enhancement, amelioration of diabetes, reduced blood pressure, management of digestive health and obesity to enhancement of cognitive function.[58,59] Additional pharmacological benefits include treatment of menopausal indications such as vaginal dryness, hot flashes, and night sweats. [58,59,60] Formononetin, however, has some important adverse effects that must be taken into consideration. These include stroke, an increased incident of blood clots, and a heart attack. Moreover, interaction with other medications could result in liver damage, nausea, diarrhoea, and vomiting.[61] Thus, caution must be taken when ingesting any compound having formononetin in abundance. It is wise to consult the doctor when taking Jobelyn® with other medications to prevent unwanted drug interactions or events.

Luteolin, a flavone, has a track record of several pharmacological impacts, ranging from analgesic, antioxidant, anti-inflammatory, neuroprotective,[62] anti-tumour, and cardioprotective.[63] A study has shown the neuroprotective effect of luteolin in models of spinal cord

injury and intense brain injury and induction of neurite outgrowth in P12 cells.[64]

Gallic acid present in Jobelyn® has been studied and shown to have anti-inflammatory, antioxidant, cardioprotective, neuroprotective, antineoplastic, and anti-diabetic effects.[65] Reports have also been released on its therapeutic impact on neuropsychological, gastrointestinal, and metabolic disorders.[66] In another study, gallic acid exerted potent anti-stroke and traumatic brain injury mitigation.[67,68] Gallic acid promotes the release of insulin and so has been explored for its benefit in the prevention, management and treatment of diabetes and related myocardial infarction.[69,70]

Caffeine, which was detected in minute quantities in Jobelyn®, has also been extensively researched and found to possess a range of pharmacological/therapeutic effects. It has been beneficial in the management of obesity, hypotension, asthma, gallbladder disease, migraines, memory failure, mental lapses, headaches, depression, and Alzheimer's disease (AD).[71] It was discovered in a study that among the tested participants, withdrawal of caffeine triggered migraine in about 2% to 30% of the participants, while all those who were treated with caffeine in combination with an analgesic recovered fully from acute migraine. It is important to note that the daily permissible limit of 200 mg should not be exceeded, according to the author.[72] In an investigation on observational analysis of the link between caffeine and depression, consumption of caffeine was found to reduce the risk of depression.[73] A similar inference was drawn from a logistic regression model using PHQ-9 $\geq$ 10 as a cut-off mark for depression, thus proposing that people can ingest caffeine to alleviate depression.[73]

Jobelyn® also has enormous potential pharmacological activities, such as anti-depressant properties, which are suggested to be related to its antioxidant activity.[74] There are claims that the dietary supplement, Jobelyn®, is beneficial in neurological abnormalities such as psychosis, convulsions, and stroke, as well as boosting the immune system, thus enhancing the body's defensive mechanisms in response to infections, stress, or debilitating maladies.[75] Jobelyn® has also been suggested to exhibit anti-psychotic-like activity with the benefit of lacking extra-pyramidal side effect risks and therefore being postulated to be of possible benefit in the symptomatic relief of psychosis.[75] Another study on Jobelyn® claimed that it has strong anti-oxidative and anti-inflammatory properties that have been utilized in the management and treatment of myriad diseases ranging from diabetes, arthritis, infertility and many other diseases. Jobelyn's anti-aggressive effect and antidepressant-like property are related to its stimulation of serotonergic pathways.[75] The phytochemical (quercetin, formononetin, luteolin, gallic acid, caffeine, and others) content of Jobelyn® observed in this study stems from the phytoconstituent of the major and only component, *Sorghum bicolor* sheath.

The producer/manufacturer and some proponents of Jobelyn® claim that it can aid absolute health and well-being, be an immune system booster, an energy levels enhancer, and have anti-inflammatory properties. These health impacts of Jobelyn® are ascribed to its antioxidant activities, which can help shield cells from damage by free radicals. Although there are currently limited studies on Jobelyn®, which may not provide substantial proof to validate all of its claimed therapeutic impact, the presence of these phytochemicals in Jobelyn® with extensive health benefits suggests that Jobelyn® can offer a wide range of health benefits if maximally explored and systematically investigated. However, some functional group modification may be required to achieve most of these pharmacological impacts. Thus, extensive research is needed to substantiate Jobelyn's effectiveness, safety, and repurposing.

Moreover, based on the claim by NAFDAC on the attached leaflet, Jobelyn® has not been subjected to rigorous regulatory evaluation to ascertain the claim by the manufacturer regarding its indications. It is therefore strongly recommended that a doctor's advice should be sought when taking Jobelyn® alone or in combination with other drugs, to avoid adverse events and unwanted drug interactions.

Conclusion

The findings from this research revealed the phytochemicals and therapeutically significant secondary metabolites, as well as essential and non-essential metals in Jobelyn® dietary supplement. Formononetin, an isoflavone, is reported for the first time in Jobelyn®. These results offer a valuable insight to further explore potential therapeutic benefits (for repositioning) and adverse effect (s) of Jobelyn® in clinical practice aside from the manufacturer's claim, as well as a broader implication for public health, regulatory practices, and the advancement of scientific knowledge.

Conflict of interest

The authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them

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