The Effect of a Herbal Product Containing Vernonia Amygdalina L. (Asteraceae) and Hibiscus Sabdariffa L. (Malvaceae) on Glycemic Index in Sub-Acute Dexamethasone Treated Mice.

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ABSTRACT

This study focused on evaluating the potentials of two variants of water soluble polyherbal nutraceutical products containing *Vernonia amygdalina* (Bitter leaf) and *Hibiscus sabdariffa* (Zobo calyces) in mitigating the toxicity due to dexamethasone a widely used synthetic corticosteroid associated with adverse effects, including hyperglycemia, and oxidative stress among others. The two polyherbal products variants: POWSG-D-(10% w/w bitter leaf decoction and 10% w/w Zobo decoction extract), and POWSG-C-10% w/w bitter leaf cold maceration extract and 10% w/w Zobo decoction extract) were used in this study. Phytochemical analysis and physico-chemical parameters (residue on drying, total ash and loss on drying) were done following standard methods as recommended by the World Health Organization (WHO) for herbal product.

In vitro antioxidant assay was done using the DPPH (2,2-diphenyl-1-picrylhydrazyl) spectrophotometric method. Effect on glycemic index (fasting blood sugar) was done *in vivo* in sub-acute dexamethasone treated mice. phytochemical analyses confirmed the presence of phenolics with the UV-Visible spectra showing corroborating characteristic absorption peaks at 198-202 nm and 273–275 nm, evidence for phenolics. Quality assessments demonstrated good granule flow properties, with cold maceration granules exhibiting superior compressibility. Antioxidant activity was moderate, with IC_{50} values of 500 µg/mL and 800 µg/mL for POWSG-C and POWSG-D respectively, compared to Vitamin C (IC_{50}: 9 µg/mL). From the result of the bioassay, the high dose (800 mg/kg body weight) decoction successfully mitigated hyperglycemia on the sub-acute dexamethasone induced mice with 1.75mMol/L and 1.80mMol/L on day 18 and 25 respectively compared to the other group of animals. These findings highlight the potential of this polyherbal formulation as a cost-effective, accessible nutraceutical for the treatment of hyperglycemia especially for immuno-compromised subjects undergoing corticosteroid regimen, and for general health and wellbeing with further optimization recommended to enhance efficacy and antioxidant activity.

Key words: Standardization, Hibiscus sabdariffa, Vernonia amygdalina, Polyherbal granules dosage form**,** Corticosteroid hyperglycemia.

BACKGROUND

Dexamethasone is a synthetic corticosteroid widely recognized for its potent anti-inflammatory and immunosuppressive effects, making it valuable in the management of inflammatory and autoimmune disorders. As a glucocorticoid analog, it mimics endogenous hormones such as cortisol but with enhanced potency and prolonged biological activity, mediated through genomic and non-genomic mechanisms[1]. Its long half-life (36–54 hours) supports sustained therapeutic action, enabling its use in conditions including asthma, severe allergic reactions, rheumatoid arthritis, systemic lupus erythematosus (SLE), chronic obstructive pulmonary disease (COPD), and cancer-related complications such as edema and chemotherapy-induced nausea[1-2].

Despite its clinical utility, prolonged dexamethasone use is associated with significant adverse effects, notably steroid-induced hyperglycemia. This arises from impaired insulin secretion and increased hepatic glucose production, elevating fasting and postprandial glucose levels, particularly in individuals with pre-existing metabolic disorders[3-4]. Other complications include musculoskeletal disorders (e.g., osteoporosis and myopathy), neuropsychiatric disturbances, and cardiovascular effects, which are often dose- and duration-dependent[5-6]. Additionally, dexamethasone can induce oxidative stress by disrupting the balance between reactive oxygen species and antioxidant defenses, contributing to cellular damage[7].

These limitations have stimulated interest in nutraceuticals, particularly plant-based products, as complementary strategies for disease management and health promotion. However, ensuring their safety, efficacy, and consistency necessitates robust standardization and quality control. *Hibiscus sabdariffa* (Roselle) is a medicinal plant rich in antioxidants and widely used in managing hypertension, obesity, and diabetes, although standardized incorporation into nutraceutical formulations remains limited[8-9]. Similarly, *Vernonia amygdalina* (bitter leaf), commonly used in tropical Africa, possesses diverse bioactive compounds such as vernodalin and luteolin derivatives, supporting its traditional use in managing diabetes, malaria, and gastrointestinal disorders[9-10].

Water-soluble granules offer a pharmaceutically robust dosage form for phytopharmaceuticals, enhancing dissolution, bioavailability, and dose uniformity while improving stability relative to liquid preparations. By reducing hydrolytic degradation and microbial contamination, and enabling rapid reconstitution, they provide an optimal balance between performance and patient compliance[11-14].

This study therefore evaluates the application of an oral water-soluble polyherbal granule formulation containing *Vernonia amygdalina* and *Hibiscus sabdariffa*. Two variants—PHOWSG-D (decoction method) and PHOWSG-C (cold maceration method), each containing 10% extract, were used in this study.

MATERIALS AND METHODS

Materials

Reagents, Solvents, and Drugs: normal saline, dexamethasone, metformin (Diabetmin®), potable water, hydrochloric acid, concentrated sulphuric acid, acetic anhydride, glacial acetic acid, copper sulphate, sodium hydroxide, potassium sodium tartrate, 5% ferric chloride solution, chloroform, dinitrophenylhydrazine, ethanol, mercuric chloride, potassium iodide, picric acid (2,4,6-trinitrophenol), bismuth subnitrate, and ammonia solution.

Instruments and Glassware: analytical balance, desiccator, fume cupboard, Accu-Answer® multi-monitoring system device, refrigerator, water bath, beakers, Bunsen burner, blender, food dehydrator, UV–visible spectrophotometer, tripod stand with wire gauze, retort stand with clamp, burette, sieve, spatula, conical flask, ceramic crucible, ceramic mortar and pestle, filter paper, funnel, glass rods, measuring cylinder, muslin cloth, 1 mL syringe, muffle furnace, furnace tongs, test tubes, oven, and oral gavage needle.

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Samples evaluated: Two variants of the water-soluble granules—PHOWSG-D (prepared by the decoction method) and PHOWSG-C (prepared by the cold maceration method)—each containing 10% (w/w) herbal extract, were provided by the Institute of Natural Medicine, University of Port Harcourt, Nigeria. For validation purposes using reference plant materials, fresh leaves of *Vernonia amygdalina* (bitter leaf) were collected from the Botanical Garden, University of Port Harcourt. The plant material was authenticated by Dr. Suleiman, assigned voucher specimen number UPHM0652, and deposited in the Herbarium of the Department of Pharmacognosy and Phytotherapy, University of Port Harcourt. Dried calyces of *Hibiscus sabdariffa* (zobo) were obtained from a local market; these were similarly authenticated, assigned voucher specimen number UPHM0653, and deposited in the same herbarium.

Phytochemical Analysis: This process involves screening the formulated dosage forms(POWSG-C and POWSG-C granules) and the reference bitter leaf and Zobo calyces to confirm the presence or absence of secondary plant metabolites, such as alkaloids(Dragendorf, Mayer, and Hager test), phenolic compounds(Ferric chloride test), cardiac glycosides(Kedde and Keller-Killiani test), saponins(Frothing test), Triterpenoids(Libermann test), anthraquinones (Borntrager test), Carbohydrates(Molisch and Fehlings tests) using standard methods[15-16]. The determination of the spectral fingerprint of phytochemical characteristics was conducted using a scanning UV-Visible spectrophotometer[15]. Each sample was scanned over a specified wavelength range (190–900 nm) to identify characteristic absorption peaks indicative of specific phytochemicals. This method enabled precise identification and validation of the characteristic absorption bands for key phytochemicals within the samples, serving as a reliable measure of batch-to-batch consistency and formulation stability.

Quality Assessment of the Water-Soluble Polyherbal Granules

Determination of Moisture Content Using Loss on Drying Method[17]

The weight of the empty crucible was determined with the aid of an analytical balance which was noted as Wd. A 2g of the formulated nutraceutical was weighed on an analytical balance into a previously tarred crucible and the weight of the crucible and the weighed crude drug was noted as W1. The glass dish containing the crude drug was placed in the oven at 100-105^{o}C for an hour and was cooled in a desiccator. The crucible containing the crude drug was placed in the oven at the same temperature for another 1 hour and was cooled and reweighed. The same procedure stated above was done until there was no constant weight (i.e. no further loss in weight) and was noted as final weight W2. The moisture content of the sample was calculated. Using this formula

Moisture content= 100 (W1-W2) / W1-Wd.

Determination of Total Ash[17]

A 2.0g of the powered formulated herbal sample was accurately weighed in a tarred silica or platinum crucible and the weight was noted as W1 which contained the sample and the crucible Using the furnace tong, it was transferred inside a muffle furnace and set to 550^{o}C, and was allowed to incinerate until the sample turned to ash. With the aid of the furnace tong removal the remnant of the ash content in the crucible was transferred to a desiccator and allowed to cool. After cooling the crucible was weighed and noted as W2 containing the ash

Total ash content= 100 (W1-W2) / W1-Wd

Angle of Repose Determination Using the Fixed Funnel Method[18]

The angle of repose was determined using the fixed funnel method to assess the flow properties of the powder. A piece of paper was placed on a flat, horizontal surface, and a funnel with a wide outlet was positioned vertically above it. With the funnel closed, the powder was loaded into the funnel. Upon opening the funnel, the powder was allowed to flow freely, and the funnel was gradually raised to maintain a constant height between the tip of the funnel and the developing powder cone. This approach minimized particle impact on the measurement. Pouring was stopped once the powder cone reached a predetermined height. The angle of repose (Θ) was then calculated as the angle between the slope of the powder cone and the horizontal plane. The height

(h) of the cone was measured along with the diameter (d) of the cone's base, and the angle was determined using the formula:

$\Theta = \tan^{-1} (2h/d)$

Bulk and Tapped Density Determination, Carrs Compressibility Index, and Hausner Ratio[18]

To determine bulk and tapped densities, a known mass of powder was passed through a screen into a calibrated volume-measuring device under free fall conditions. The bulk density was calculated by dividing the mass by the initial measured volume of the powder:

Bulk density = mass/bulk volume

For tapped density, the same mass of powder was placed into a graduated measuring cylinder, which was then tapped mechanically by raising it and allowing it to drop a specified distance. This tapping continued until there was minimal change in the volume readings. The tapped density was calculated by dividing the mass by the final tapped volume:

Tapped density = mass/tapped volume

Based on the bulk and tapped density values, the flow properties of the powder were further analyzed by calculating Carr's Compressibility Index and the Hausner Ratio, both of which provide insight into the powder's compressibility and flowability. Carr's Compressibility Index was determined using the formula:

Carrs Index = (Tapped density - Bulk density) 100/Tapped density

The Hausner Ratio was calculated as follows:

Hausner Ratio = Tapped density / Bulk density

Biological quality assessment of the water -soluble polyherbal granules

Determination of Antioxidant Activity of the Nutraceutical Product

To determine the antioxidant activity of a nutraceutical product using Vitamin C as a reference antioxidant standard for comparison. The procedure for the antioxidant activity was by using the DPPH free radical scavenging assay as described by[19] with slight modifications as reported by[20]. A 0.1 mM solution of DPPH was freshly prepared in methanol with the optical density adjusted to fall within the range 0f 1.0-1.5 absorbance unit). Serial concentrations of each two products variants aqueous stock solution of 10 mg/ml respectively prepared were appropriately diluted separately in methanol to obtain test solutions in the concentration range:1000-12.5 µg/mL). Two milliliters of each concentration was added to 2 mL of the DPPH solution, mixed thoroughly, and incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a UV–Visible spectrophotometer. Methanol served as the blank, and ascorbic acid (vitamin C) at the same concentration range was used as the positive reference standard. All assays were performed in triplicate. The percentage of DPPH radical scavenging activity was calculated as:

% Radical Scavenging Activity = $[(A_0 – A_1) / A_0] \times 100$

where A_0 = absorbance of the control (DPPH only) and A_1 = absorbance of the test sample or standard. The IC_{50} value—the concentration required to scavenge 50% of DPPH radicals—was extrapolated from the

linear regression plot of percentage inhibition against extract concentration.

In Vivo Biological Assay of the Nutraceutical Product

Experimental Animals

Healthy albino mice with an average body weight of approximately 25 g were used for this study. The animals were obtained from the Animal House of the Department of Animal and Environmental Biology (AEB), Faculty of Science, University of Port Harcourt. They were maintained on standard laboratory feed (Premier Feed Mills, Rivers State) and provided with water ad libitum. The mice were housed in clean, well-labeled, and uniform cages under standard environmental conditions and allowed to acclimatize for seven days prior to the commencement of the experiment. All procedures were conducted in accordance with established ethical guidelines for the care and use of laboratory animals.

Animal Study

Determination of the Effect of Nutraceutical Products on Glycemic Index in Dexamethasone-Induced Sub-Acute Mice[21]

A total of 28 mice were initially weighed to obtain baseline body weights prior to treatment. Each mouse was administered dexamethasone at a pre-standardized dose of 2.5 mg/kg (intraperitoneally) for ten consecutive days. Thereafter, the animals were randomly assigned into seven groups (n = 4 per group). The selected doses were based on previously reported acute toxicity profiles of the constituent edible plants[9].

Group I (dexamethasone control) continued to receive dexamethasone only for an additional 15 days without further intervention.

Group II (metformin group) received metformin at a dose of 20 mg/kg (intraperitoneally), in addition to the daily dexamethasone treatment, for 15 days.

Groups III and IV were treated with high and low doses of POWSG-C at 800 mg/kg and 400 mg/kg, respectively, administered orally.

Groups V and VI received high and low doses of POWSG-D at 800 mg/kg and 400 mg/kg, respectively, also administered orally.

All treatment groups (II–VI) continued to receive dexamethasone concurrently with their respective interventions for 15 days.

Group VII served as the normal control and did not receive dexamethasone, metformin, or any test formulation. This group was maintained under the same experimental conditions to provide baseline comparative data.

Glycemic indices were assessed on days 18 and 25 post-treatment by measuring fasting blood glucose levels using an Accu-Answer® multi-monitoring system. Blood samples were obtained from the tail vein of each animal and applied to the test strip, where a fixed volume was automatically drawn by capillary action. The device determines blood glucose concentration using an electrochemical biosensor, with results displayed digitally.

Data and Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical significance was evaluated using analysis of variance (ANOVA) followed by Student's t-test, with $p < 0.05$ considered statistically significant.

Ethical Approval

This was obtained from the University of Port Harcourt Research Ethics Review Committee (UPREC) with reference number: UPH/R&D/REC/EXEC/192

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RESULTS

Table 1: Phytochemical Screening Test Results for the evaluated polyherbal products variants compared to the reference medicinal plants

TEST	*Vernonia amygdalina* Aqueous Extract	*Hibiscus sabdariffa* Aqueous Extract	POWSG-D	POWSG-C
Saponins: Frothing test	Present	Present	Absent	Absent
Alkaloid				
Mayer's test	Present	Absent	Absent	Absent
Dragendorff test	Present	Absent	Absent	Absent
Hagers test	Present	Absent	Absent	Absent
Anthraquinone: Borntrager test				
Free anthraquinone	Absent	Present	Absent	Absent
Combined anthraquinone	Absent	Present	Absent	Absent
Phenolics				
Ferric Chloride test	Present	Present	Present	Present
Carbohydrate				
Molisch's test	Absent	Absent	Present	Present
Reducing sugar				
Fehling's test	Absent	Absent	Absent	Absent
Triterpenoid				
Lieberman Burchard's test	Present	Present	Absent	Absent
Salkowski's test	Present	Present	Absent	Absent
Cardiac glycoside				
Kedde's test for lactone ring	Absent	Absent	Absent	Absent
Keller -Killiani's test	Absent	Absent	Absent	Absent

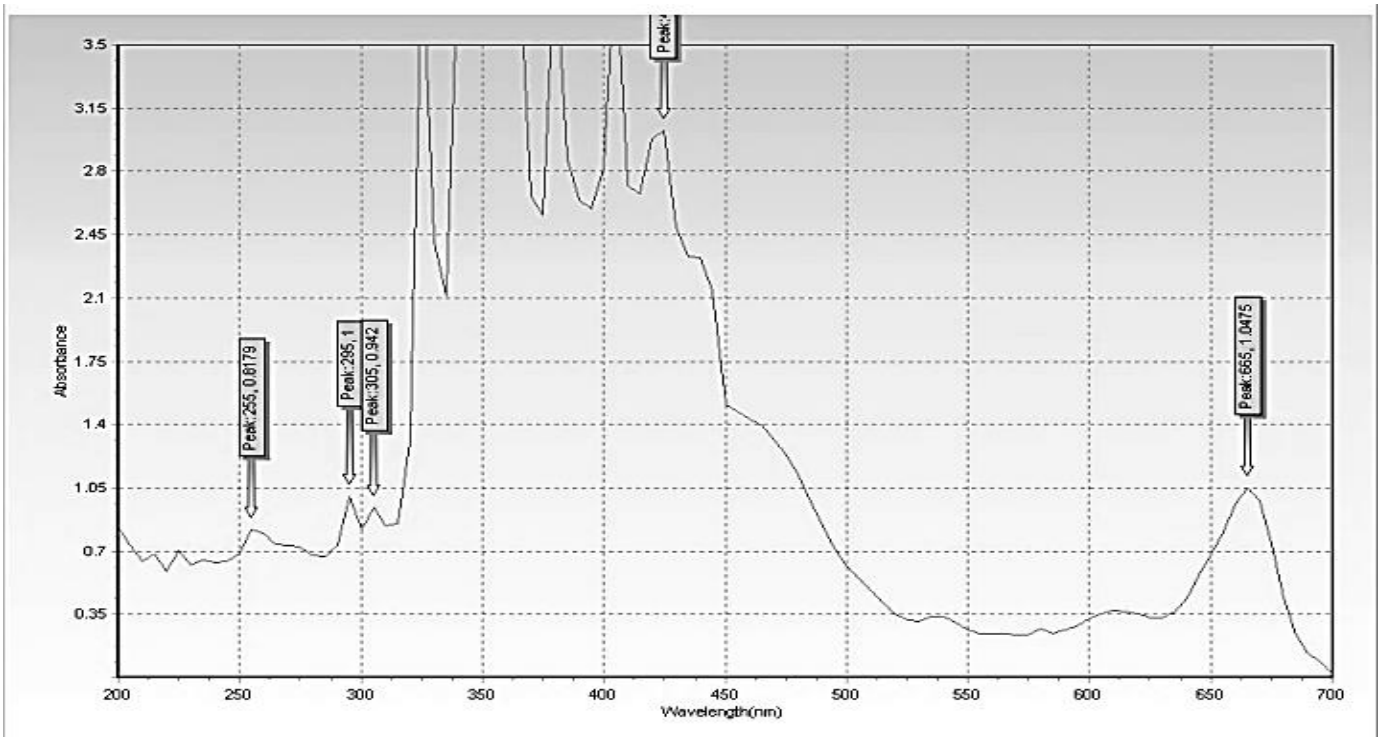

Fig 1. UV-Visible spectrum showing peaks for *Vernonia amygdalina* aqueous extract

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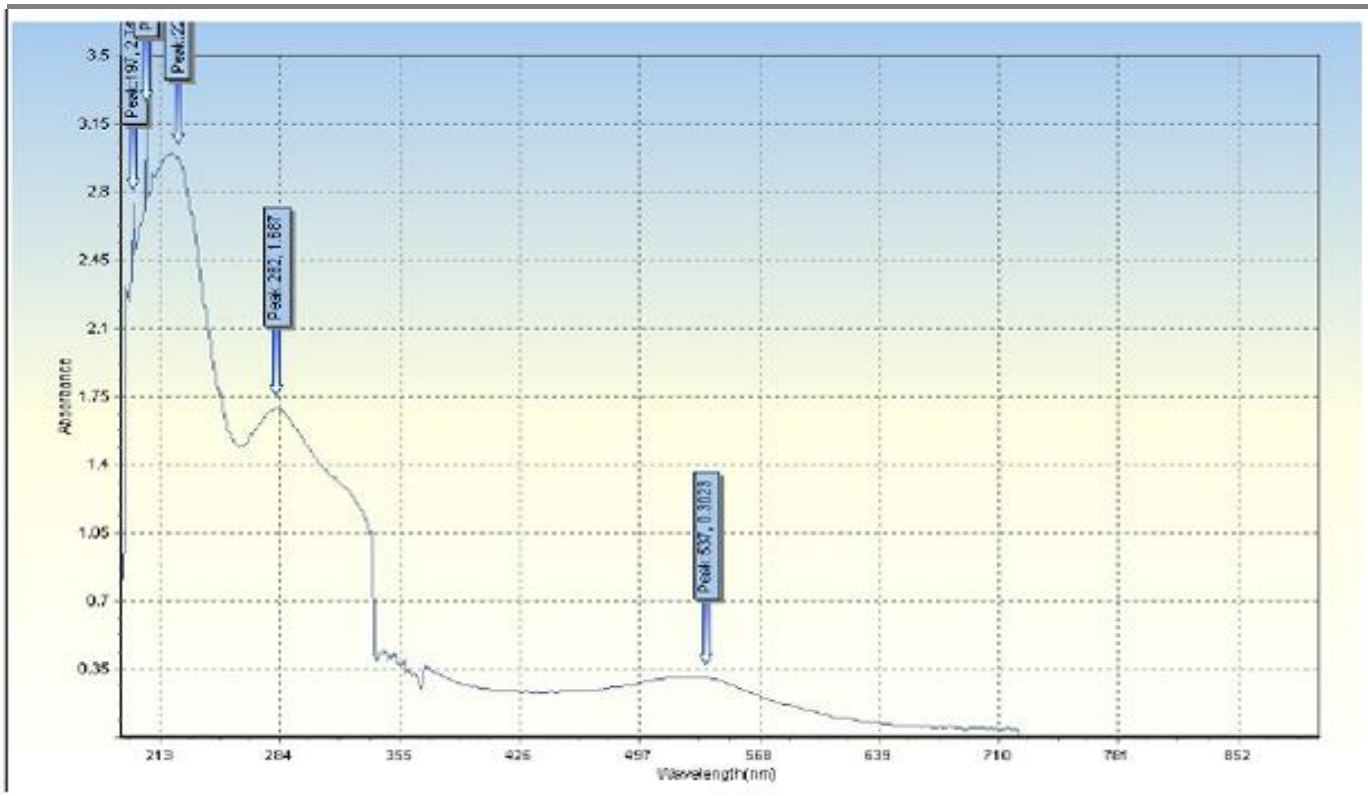

Fig 2. UV-Visible spectrum showing peaks for *Hibiscus sabdariffa* aqueous extract

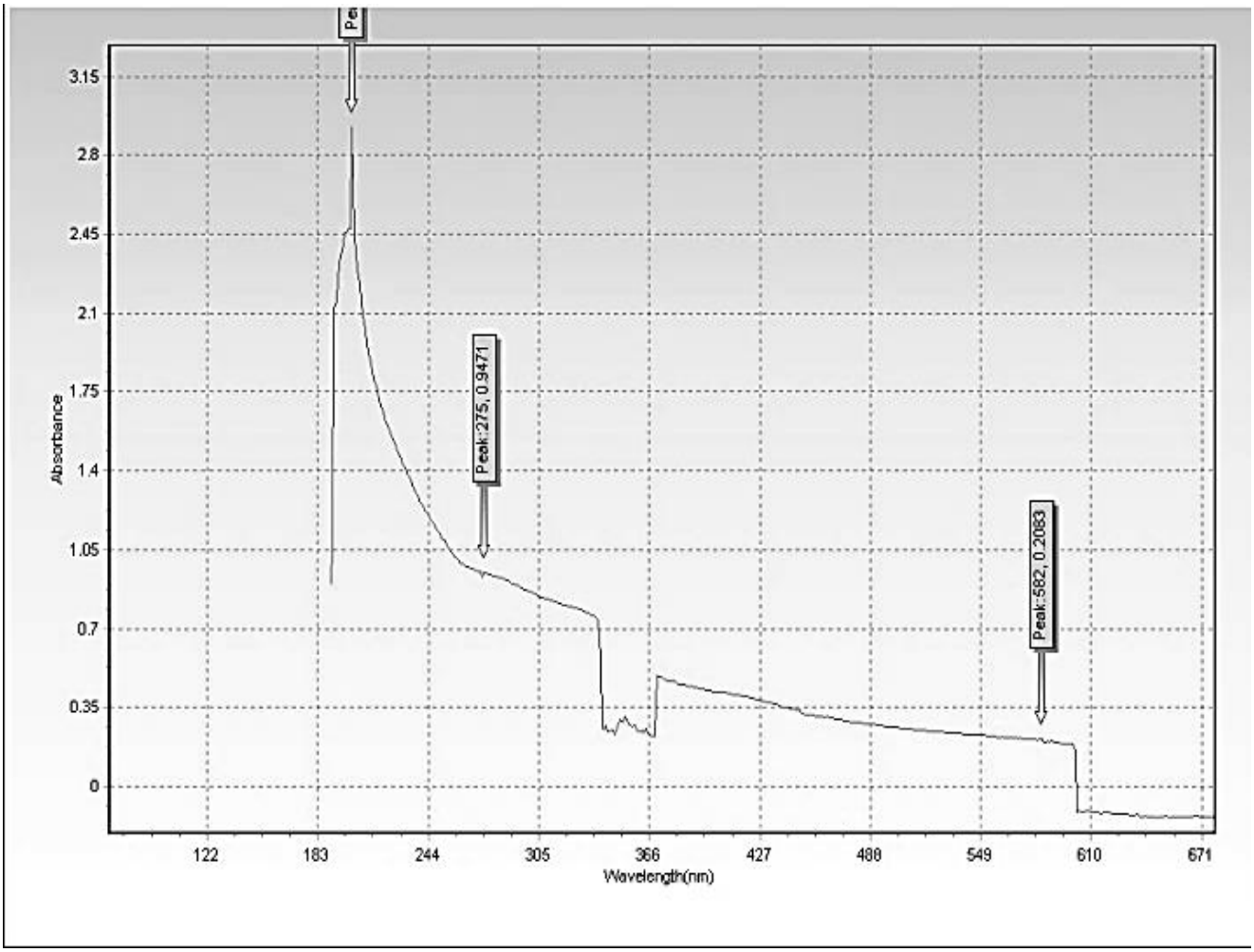

Fig 3. UV-Visible spectrum showing peaks for decoction variant of nutraceutical product

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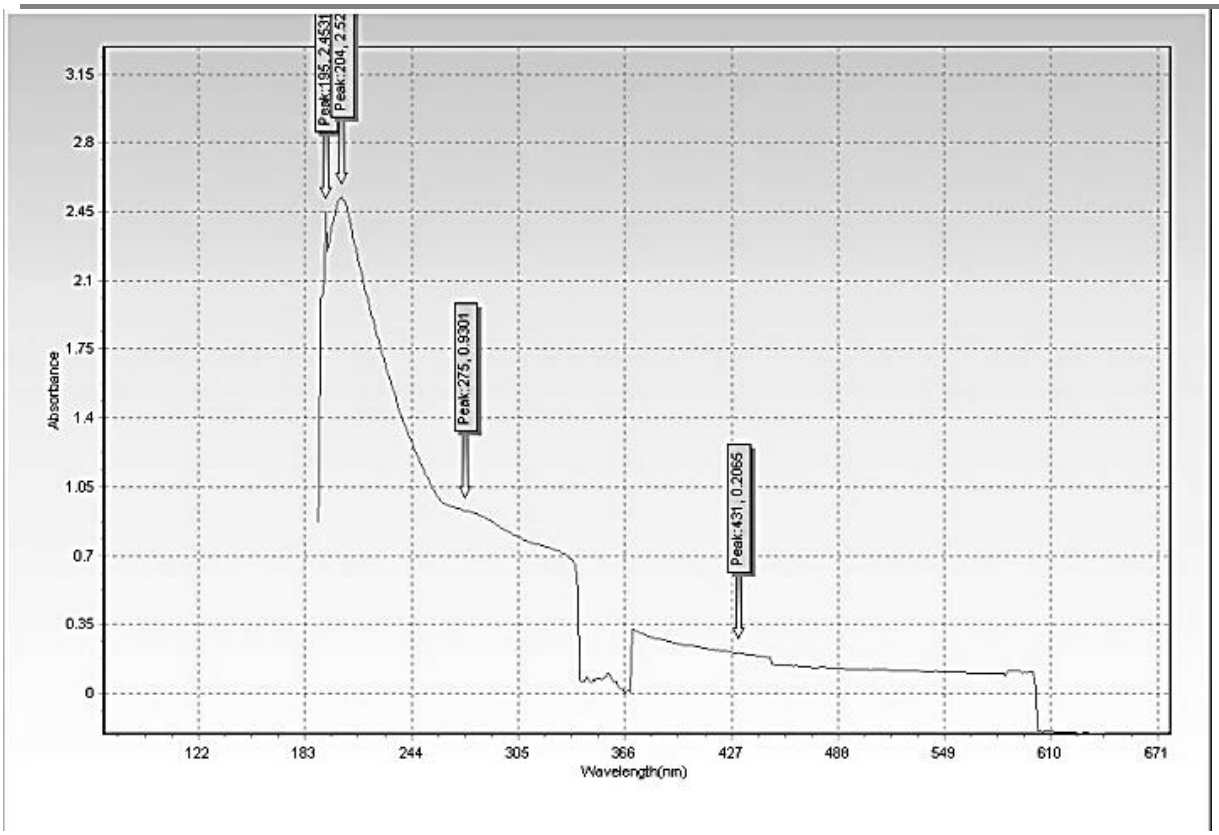

Fig 4. UV-Visible spectrum showing peaks for cold maceration variant of nutraceutical product

Table 2. Determination of physico-chemical (Proximate) properties of the evaluated polyherbal product variants

Parameter	POWSG-D	POWSG-C
Total ash	0	0
Loss on drying (%)	1.90	3.60

Table 3. Granule flow properties of the evaluated products

Parameter	POWSG-D	POWSG-D
Angle of repose (º)	29.7	29.7
Bulk density (g/ml)	0.400	0.455
Tapped density (g/ml)	0.625	0.588
Carr's compressibility index (%)	0.360	0.226
Hausner ratio	1.560	1.290

Table 4. Sieve analysis of the evaluated products

Sieve Size (mm)	POWSG-D (%w/w)	POWSG-C (%w/w)
0.063	3.520	0.650
0.125	15.620	3.460
0.250	10.050	24.560
0.500	12.390	29.670
1.000	48.820	35.650
2.000	1.540	1.790

Table 5. Antioxidant activity of nutraceutical product

Sample	IC_{50} (µg/ml)
Decoction variant of nutraceutical product (POWSG-D)	800
Cold maceration variant of nutraceutical product (POWSG-C)	500
Vitamin C (positive control)	9

Table 6. Glycemic control effect of the two Polyherbal granules dosage variants POWSG-D and POWSG-C) and Metformin on the blood glucose level of dexamethasone-induced mice

Groups	Pre-Treatment			Post-treatment	
	Day 0	Day 7	Day 10	Day 18	Day 25
High-dose cold maceration	2.60±0.20	4.0±0.10	4.4±0.20	2.00±0.25(54%)	2.35±0.05(46%)
Low-dose cold maceration	3.10±0.10	4.1±0.20	4.8±0.80	1.95±0.15(59%)	3.50 ±0.60(27%)
High-dose decoction	1.90±0.80	3.5±0.10	4.5±0.25	1.75±0.45(61%)	1.80±0.20(60%)
Low-dose decoction	2.70±0.10	3.8±0.20	4.8±0.60	1.70±0.50(64.5%)	3.60±0.50(25%)
Metformin	2.40±0.10	3.5±0.30	4.1±0.00	1.20±0.00(70%)	3.40±0.00(17%)
Untreated	2.50±0.20	3.5±0.10	5.2±0.50	3.00±0.30(42.3%)	4.00±0.20(23%)
Normal	2.53±0.16	-	3.56±0.00	-	4.00±0.20

DISCUSSION

Phytochemical Analysis: Phytochemical screening is a simple and cheap quality test use to identify the presence or absence of a diverse array of bioactive compounds, including saponins, alkaloids, triterpenoids, and phenolics. When applied in this study (see Table 1), a diverse array of bioactive compounds, including saponins, alkaloids, triterpenoids, and phenolics were detected in the fresh bitter leaf and Zobo calyces samples used as reference for comparison while for the two dosage forms samples: POWSG-C and POWSG-D), it revealed phenolics as the predominant phytochemical, with other compounds undetected. This shift may be attributed to the formulation process which may have been intended to achieve a phyto polyphenol-rich dosage form. The UV-visible spectrophotometry(see figures 1-4) further corroborated these results whereby the spectra of the reference bitter leaf and Zobo aqueous extracts exhibited multiple peaks indicative of diverse phytochemical profiles, with the spectra of the formulated samples: POWSG-C and POWSG-D showing dominant absorption peaks only in the region 198-202 nm and 273–275 nm, which is potentially indicative of phenolics[15,22]. That at 530-580 nm is due to the anthocyanin, and 650 nm in the bitter leaf spectrum which must have been suppressed in the formulated products due to dilution effect is associated with the chlorophyll pigment[15]. This suggests selective stabilization of phenolics, which are known for their antioxidant properties, critical for modulating oxidative stress.

Physicochemical Evaluation: The physicochemical assessments highlighted good proximate composition quality for the polyherbal granules, with total ash content (see Table 2) measuring 0% for both cold maceration and decoction methods. Moisture content analysis revealed higher loss on drying in cold maceration granules (3.60%) compared to decoction granules (1.90%), suggesting that the cold maceration process retains more residual moisture. While moisture is important for granule stability, excessive moisture could compromise shelf life and promote microbial growth, necessitating additional drying or stabilization measures for cold maceration formulations. Quality control analyses demonstrated good flowability for both granule types, with the cold maceration granules exhibiting superior compressibility (see Table 3) and particle size distribution (see Table 4), suggesting better disintegration properties, uniformity in dosing, and bioavailability compared to decoction granules.

***In-vitro* antioxidant Activity:** Oxidative stress plays a central role in the pathogenesis of dexamethasone-induced toxicity, contributing to both renal dysfunction and hyperuricemia. This stress results from an imbalance between pro-oxidants and antioxidants, leading to tissue damage, including in the kidneys, which regulates uric acid levels. The polyherbal formulations exhibited antioxidant activity (see Table 5), with cold maceration granules showing stronger IC_{50} values (500 µg/mL) compared to decoction granules (800 µg/mL), indicating better preservation of antioxidant compounds in the cold maceration process. However, both

formulations exhibited significantly weaker antioxidant activity compared to Vitamin C (IC_{50}: 9 µg/mL), suggesting room for improvement in enhancing the potency of the herbal formulations. These moderate antioxidant effects could help counteract dexamethasone-induced oxidative stress, thereby mitigating renal dysfunction and potentially regulating uric acid levels.

***In-vivo* glycemic modulation effect in sub-acute dexamethasone treated mice:** *In vivo* bioassays demonstrated the efficacy of the polyherbal formulations in mitigating dexamethasone-induced hyperglycemia (see Table 6) in the dexamethasone sub-acute treated mice. The results in Table 6, demonstrated that dexamethasone significantly elevated fasting blood sugar (FBS) levels, particularly in the untreated group, confirming its hyperglycemic effects. Furthermore, on day 18 post-treatment, a significant reduction ($p < 0.05$) in fasting blood glucose levels was observed across all dose groups. However, the reduction produced by metformin was significantly greater ($p < 0.05$). No significant dose–response relationship was observed ($p > 0.05$) until day 25 post-treatment, when a pronounced and statistically significant dose-dependent effect ($p < 0.05$) became evident. This indicates the polyherbal products' potential to enhance insulin sensitivity and glucose uptake, counteracting the hyperglycemic effects of dexamethasone[23-25].

By day 25 post-treatment, this improvement in glycemic control was significantly greater ($p < 0.05$) than that observed with the reference drug, metformin. Furthermore, on day 25, the effect of the high-dose decoction variant was significantly superior ($p = 0.037$) to that of the cold maceration variant. This observation may be attributed to a higher extraction yield of active constituent(s) resulting from enhanced solubility at elevated temperatures, or possibly to favorable heat-induced chemical modifications, rather than thermal degradation that could otherwise lead to loss of activity. Generally, the decoction variant (POWSG-D) (both high and low doses) showed more effectiveness in lowering glucose levels compared to cold maceration. Both methods are more effective than no treatment, with the decoction treatments resulting in the most significant glucose reductions across the comparisons

CONCLUSION

The two variants of the formulated polyherbal product demonstrated potential to mitigate hyperglycemia. This effect appears to be influenced by the extraction temperature, with the decoction-derived variant showing superior antihyperglycemic activity. A similar dependence on extraction temperature was observed in the antioxidant activity and physicotechnical properties of the granules. Specifically, the cold maceration-derived formulation exhibited advantages in antioxidant activity, granule flowability, and particle size distribution; however, its relatively higher moisture content necessitates additional stabilization measures. These findings further highlight the critical role of phenolic constituents in the therapeutic efficacy of the formulation and suggest that optimization of both extraction and formulation processes can enhance product stability and overall effectiveness. In view of the study scope and the potential for future clinical application, further investigations are required to optimize therapeutic performance, establish long-term stability, and comprehensively evaluate safety and toxicity profiles.

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