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FORMULATION AND EVALUATION OF HERBAL CHOCOLATE *AS A NUTRACEUTICAL SUPPORT FOR AUTISM SPECTRUM DISORDER (ASD)* IN CHILDREN

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Abstract:

*This study presents the formulation and evaluation of a novel **herbal chocolate as a nutraceutical support for children with Autism Spectrum Disorder (ASD)**. The formulation integrates scientifically studied herbal ingredients such as* Bacopa monnieri, Curcuma longa, Withania somnifera, Camellia sinensis, Ocimum sanctum, *and* Piper nigrum, *incorporated into a dark chocolate matrix to enhance palatability and bioavailability. The lipid-rich nature of chocolate facilitates improved absorption of fat-soluble phytochemicals, while also ensuring better compliance in paediatric populations.*

The research outlines a comprehensive framework covering ingredient selection, formulation composition, preparation methods, and multi-dimensional evaluation parameters including physicochemical, phytochemical, microbiological, and stability studies. In addition, a clinical evaluation model using standardized behavioural assessment tools (such as CARS-2 and ABC-C) is proposed to assess therapeutic outcomes.

The formulation aims to support neurological function by targeting oxidative stress, neuroinflammation, and cognitive deficits associated with ASD. However, it is emphasized that this product is a complementary nutraceutical and not a replacement for conventional therapies. Further randomized clinical trials are necessary to validate efficacy, safety, and long-term benefits.

Keywords:*Autism Spectrum Disorder (ASD) ,Herbal Chocolate ,Nutraceutical ,Paediatric Formulation ,Bacopa monnieri (Brahmi) ,Curcuma longa (Curcumin) ,Withania somnifera (Ashwagandha) Camellia sinensis (Green Tea Extract) ,Ocimum sanctum (Tulsi) ,Piper nigrum (Piperine), Neuroprotection ,Cognitive Enhancement ,Neuroinflammation ,Antioxidant Activity, Phytochemicals ,Bioavailability Enhancement ,Functional Foods ,Chocolate Drug Delivery System ,Complementary Therapy ,Behavioural Assessment (CARS-2, ABC-C)*

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1. INTRODUCTION & RATIONALE:

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental condition characterised by persistent deficits in social communication, restricted and repetitive patterns of behaviour, and atypical sensory processing. Its global prevalence has risen dramatically, now affecting approximately 1 in 36 children (CDC, 2023). While behavioural and educational interventions remain the cornerstone of ASD management, growing interest exists in complementary nutritional and phytotherapeutic strategies that may support neurological function.

Chocolate — particularly dark chocolate — serves as an ideal pharmaceutical and nutraceutical delivery vehicle for herbal actives due to its lipid-rich matrix, which aids the absorption of fat-soluble phytochemicals, high palatability (critical for paediatric compliance), and intrinsic bioactive content including theobromine, phenylethylamine (PEA), and magnesium.

Key herbal candidates studied for ASD include Bacopa monnieri, which may enhance cognitive flexibility via bacosides; Curcuma longa (curcumin), which can reduce oxidative stress and repetitive behaviours; and green tea extract (luteolin), which can modulate neuroinflammation. This document outlines the complete framework for formulating and evaluating a standardised herbal chocolate for ASD support.

Conventional management strategies primarily include behavioural interventions, speech therapy, and educational support. However, increasing attention has been directed towards **nutritional and phytotherapeutic approaches** that may enhance neurological function and improve quality of life in affected children.

In this context, **dark chocolate** serves as an innovative delivery system for herbal actives due to its high palatability, which improves compliance in children, and its lipid-rich composition, which enhances the absorption of bioactive compounds. Additionally, chocolate itself contains beneficial components such as theobromine, phenylethylamine (PEA), and magnesium, which may contribute to mood regulation and cognitive support.

Several herbal ingredients have shown potential benefits in ASD management. For instance, *Bacopa monnieri* is associated with improved memory and cognitive flexibility, *Curcuma longa* exhibits antioxidant and anti-inflammatory properties, and *green tea extract* may help regulate neuroinflammation.

This study aims to develop a **standardized herbal chocolate formulation** that combines these therapeutic agents into a child-friendly dosage form. The work further provides a structured framework for formulation, quality evaluation, and clinical assessment, thereby bridging the gap between traditional herbal medicine and modern nutraceutical development.

2. Selection of Herbal Ingredients

The following herbs are selected based on current evidence, traditional use, and safety profile in paediatric populations:

Herb (Common Name)	Active Constituent(s)	Therapeutic Relevance in ASD	Standardisation
Bacopa monnieri (Brahmi)	Bacosides A & B	Cognitive flexibility, memory enhancement, learning	Bacosides ≥ 20%
Curcuma longa (Turmeric)	Curcuminoids (curcumin, bisdemethoxycurcumin)	Antioxidant, reduces neuroinflammation & repetitive behaviour	Curcuminoids ≥ 95%
Withania somnifera (Ashwagandha)	Withanolides	Anxiolytic, adaptogenic, stress & cortisol reduction	Withanolides ≥ 5%
Camellia sinensis (Green Tea)	Luteolin, EGCG	Neuroinflammation modulation, GABA-A modulation	EGCG ≥ 45%
Piper nigrum (Black Pepper)	Piperine	Bioavailability enhancer (especially for curcumin)	Piperine ≥ 95%
Ocimum sanctum (Tulsi)	Eugenol, ursolic acid	Neuroprotective, antianxiety, antioxidant	Eugenol ≥ 1.5%

Note: All extracts must be dry, standardised, and food-grade certified. Avoid solvent residues exceeding ICH Q3C limits.

3. Proposed Formulation Composition
Target dose unit: 10 g chocolate bar (child-appropriate serving). Suggested daily intake: 10–20 g/day for children aged 4–12 years.

Ingredient	Quantity per 10 g bar	Category / Purpose
Dark cocoa mass (70% cacao)	7.00 g	Base vehicle; antioxidants, PEA, theobromine, magnesium
Organic cane sugar / stevia blend	1.50 g	Sweetener; palatability
Cocoa butter	0.80 g	Lipid matrix; texture, fat-soluble phytochemical absorption
Brahmi extract (standardised)	50 mg	Herbal active — cognitive support
Curcumin extract (standardised)	50 mg	Herbal active — anti-inflammatory, antioxidant
Ashwagandha extract (standardised)	50 mg	Herbal active — adaptogen, anxiolytic
Green Tea extract (luteolin/EGCG)	25 mg	Herbal active — neuroinflammation modulation
Tulsi extract (standardised)	25 mg	Herbal active — neuroprotective
Piperine (black pepper extract)	5 mg	Bioavailability enhancer (≥ 20× for curcumin)
Soy lecithin (emulsifier)	50 mg	Stability; homogeneous herbal distribution
Natural vanilla flavour	q.s.	Palatability; masking of herbal bitterness

q.s. = quantum sufficit (as much as sufficient). Adjust sugar level based on clinical tolerability.

4. Preparation Method
The manufacturing process involves five sequential stages designed to maintain herbal active integrity and ensure uniform distribution throughout the chocolate matrix.

4.1 Stage 1 — Herbal Extract Preparation
- Procure or prepare standardised, spray-dried or freeze-dried herbal extracts from validated suppliers.
- Confirm active constituent content by HPLC prior to incorporation.
- Store extracts in sealed, airtight containers away from light and moisture until use.

4.2 Stage 2 — Cocoa Mass Melting
- Melt dark cocoa mass and cocoa butter together using a bain-marie / double boiler at 45– 50°C.
- Maintain temperature strictly below 50°C throughout to prevent degradation of thermolabile phytochemicals (bacosides, EGCG).
- Stir gently and continuously to ensure a homogeneous melt.

4.3 Stage 3 — Herbal Blend Incorporation

- Pre-blend all dry herbal extracts with lecithin and piperine — dry mixing ensures uniform distribution.
- Gradually sift the herbal blend into the molten cocoa mass with continuous mechanical stirring at low speed (30–50 rpm).
- Add sweetener and natural vanilla flavour; continue stirring for 20–30 minutes until fully homogeneous.
- Sample for visual uniformity (no visible particulate agglomerates).

4.4 Stage 4 — Conching and Tempering

- Conch the mixture at 45°C for 2–4 hours to develop smooth texture and uniform flavour.
- Temper the chocolate to develop stable Form V cocoa butter crystals:

○ Heat to 50°C → cool to 27°C → reheat to 31–32°C (dark chocolate tempering protocol).

○ Proper tempering ensures glossy appearance, clean snap, and extended shelf life.

4.5 Stage 5 — Moulding, Setting & Packaging

- Pour tempered mixture into child-appropriate moulds (small squares or rectangular bars, 10 g each).
- Tap moulds gently to remove air bubbles.
- Allow to set at 10–15°C for 30–60 minutes.
- Demould and inspect for surface defects (bloom, cracks, uneven colour).
- Package in light-resistant, moisture-proof, food-grade aluminium foil with child-safe secondary packaging.
- Label to include: ingredient list, allergens, herbal content per dose, storage instructions, and cautionary statement.

5. Evaluation Parameters

Comprehensive evaluation is conducted across six domains to ensure quality, safety, and efficacy of the formulation.

5.1 Physical / Organoleptic Evaluation

Parameter	Method / Tool	Acceptance Criteria
Appearance	Visual inspection	Uniform glossy surface; no fat or sugar bloom
Colour	Colorimetry (CIE L*a*b*)	Dark brown; consistent batch-to-batch
Parameter	**Method / Tool**	**Acceptance Criteria**
Odour	Sensory panel	Characteristic chocolate aroma; no offodour
Taste / Palatability	Child sensory panel (5-point hedonic scale)	Score ≥ 3.5 / 5.0
Texture / Hardness	Texture Profile Analyser (TPA)	Firm snap; hardness 15–30 N
Weight Uniformity	Analytical balance (n=20)	±5% of label claim

5.2 Physicochemical Tests

Test	Method	Acceptance Criteria
Moisture content	Loss on Drying (LOD); 105°C, 2 h	< 3.0%
pH (aqueous dispersion)	pH meter	6.0 – 7.0
Melting point	Differential Scanning Calorimetry (DSC)	30 – 34°C
Viscosity	Brookfield viscometer (45°C)	300 – 600 mPa·s
Fat content	Soxhlet extraction	30 – 40% w/w
Total solids	Gravimetry	≥ 97%

5.3 Phytochemical & Chemical Analysis

Analysis	Method	Target / Acceptance
Curcuminoid quantification	RP-HPLC (λ = 425 nm)	45–55 mg/10 g bar
Bacosides quantification	RP-HPLC (λ = 278 nm)	9–11 mg/10 g bar
Withanolide quantification	HPLC-MS	2.3–2.7 mg/10 g bar
Piperine quantification	RP-HPLC (λ = 343 nm)	4.5–5.5 mg/10 g bar
HPTLC fingerprinting	TLC densitometry	Matches reference standard
Total polyphenol content (TPC)	Folin-Ciocalteu method	Report value (mg GAE/g)
Total flavonoid content (TFC)	AlCl3 colorimetric method	Report value (mg QE/g)
Antioxidant activity (DPPH)	IC50 determination	Report IC50 value
Antioxidant activity (ABTS)	Radical scavenging %	Report % inhibition at test conc.
Heavy metals (Pb, Cd, As, Hg)	ICP-MS	< WHO/ICH limits for paediatric products
Pesticide residues	GC-MS/LC-MS	Below MRL (EU regulation EC 396/2005)

5.4 Microbiological Tests

Conducted as per Food Safety and Standards Authority (FSSAI) / WHO guidelines for food products:

Test	Method	Limit
Total aerobic plate count (TPC)	Pour plate; 37°C, 48 h	< 10^2 CFU/g
Yeast & mould count	SDA plate; 25°C, 5 days	< 10^1 CFU/g
Coliforms (E. coli)	MPN method / MAC agar	Absent in 1 g
Salmonella spp.	ISO 6579	Absent in 25 g
Staphylococcus aureus	BP agar	Absent in 0.1 g

5.5 Stability Studies (ICH Q1A Guidelines)

Condition	Temperature / RH	Duration	Parameters Monitored
Accelerated stability	40°C ± 2°C / 75% ± 5% RH	6 months	Appearance, hardness, HPLC actives, microbial load
Intermediate stability	30°C ± 2°C / 65% ± 5% RH	12 months	Appearance, texture, actives, bloom assessment
Long-term / real-time	25°C ± 2°C / 60% ± 5% RH	24 months	All physicochemical + microbiological parameters

- Sampling time points: 0, 1, 2, 3, 6, 9, 12, 18, 24 months.
- Accelerated data used to predict shelf life; confirmed by real-time data.
- Acceptance criterion: HPLC active content ≥ 90% of initial label claim at all time points.

5.6 In-Vitro Dissolution / Release Studies

- Media: Simulated Gastric Fluid (SGF, pH 1.2, pepsin) and Simulated Intestinal Fluid (SIF, pH 6.8, pancreatin).
- Apparatus: USP dissolution apparatus II (paddle); 50 rpm; 37°C ± 0.5°C.
- Sampling intervals: 15, 30, 45, 60, 90, 120 minutes.
- Analyse: % release of curcuminoids and bacosides by HPLC at each time point.
- Plot drug release profiles; calculate Q30 and Q60 values.
- Enhanced release expected due to piperine-curcumin synergy and lipid matrix dissolution.

6. Clinical / Behavioural Evaluation Framework

Clinical efficacy is assessed using validated, standardised behavioural assessment tools administered by trained clinicians and caregivers. The recommended study design is a randomised, double-blind, placebo-controlled trial (RDBPCT) with a minimum duration of 12 weeks.

6.1 Recommended Behavioural Assessment Tools

Assessment Tool	Abbreviation	Domain Assessed	Frequency
Childhood Autism Rating Scale (2nd ed.)	CARS-2	Overall ASD severity; social, emotional, cognitive	Baseline, Week 6, Week 12
Aberrant Behaviour Checklist – Community	ABC-C	Irritability, hyperactivity, social withdrawal, stereotypy	Baseline, Week 4, Week 8, Week 12
Autism Spectrum Rating Scale	ASRS	Core ASD symptom domains (DSM-5 aligned)	Baseline, Week 12
Social Responsiveness Scale (2nd ed.)	SRS-2	Social awareness, cognition, communication, motivation	Baseline, Week 12
Vineland Adaptive Behaviour Scale	VABS-3	Social communication, daily living, motor skills	Baseline, Week 12
Parental Stress Index	PSI	Caregiver stress & quality of life	Baseline, Week 12

6.2 Proposed Clinical Study Design

Study Type

- Randomised, double-blind, placebo-controlled trial (RDBPCT)

Population

- Children aged 4–12 years with confirmed ASD diagnosis (DSM-5 criteria)
- Both genders
- Sample size: minimum 60 participants (30 per arm); calculate via power analysis

Intervention

- Group A (Treatment): 10 g herbal chocolate bar twice daily (20 g/day total) for 12 weeks
- Group B (Placebo): Identical chocolate bar without herbal extracts

Exclusion Criteria

- Confirmed epilepsy or active seizure disorder (theobromine caution)
- Known caffeine sensitivity or chocolate allergy
- Concurrent use of psychoactive medications (risperidone, aripiprazole)
- Diabetes mellitus or metabolic disorder
- Severe gastrointestinal conditions

Primary Endpoints

- Change from baseline in ABC-C subscale scores at Week 12

- Change from baseline in CARS-2 total score at Week 12

Secondary Endpoints

- SRS-2, VABS-3, and PSI score changes
- Antioxidant biomarker levels (serum MDA, SOD, GSH) at baseline and Week 12
- Inflammatory markers (IL-6, TNF-α, CRP) before and after intervention

7. Safety Considerations

Paediatric safety is paramount. The following considerations must be addressed before and during clinical use:

7.1 Dosage & Administration

- Recommended dose: 10–20 g/day for children aged 4–12 years, in two divided doses (morning and afternoon).
- Begin with a lower dose (5–10 g/day) during the first week and titrate up to assess tolerance.
- Administer with food to minimise gastrointestinal discomfort.

7.2 Allergen Declaration

- Soy (from lecithin) — must be declared on label.
- Milk / dairy cross-contamination risk from manufacturing facility.
- Tree nuts — assess and declare facility cross-contamination risk.
- Gluten-free status: confirm cocoa mass and extract sources.

7.3 Herb–Drug Interactions

Herb	Potential Interaction	Clinical Action
Curcumin	May potentiate anticoagulants (warfarin); CYP3A4 inhibition	Review concurrent medications before enrolment
Ashwagandha	Additive sedative effect with benzodiazepines, melatonin	Monitor sedation; adjust if needed
Herb	**Potential Interaction**	**Clinical Action**
Brahmi (Bacopa)	May enhance cholinergic activity; caution with anticholinergics	Contraindicated with cholinesterase inhibitors
Piperine	Significantly inhibits CYP3A4/P-gp; increases bioavailability of many drugs	Review all concurrent medications — dose adjustments may be needed
Green Tea (EGCG)	Iron absorption inhibition; caution with iron supplements	Separate administration by ≥ 2 hours

7.4 Monitoring Parameters

- **Liver function tests (LFT)** at baseline and Month 3 (curcumin/ashwagandha).
- Includes: ALT, AST, ALP
- Done at **baseline and after 3 months**
- Important due to herbal components like curcumin and ashwagandha

- **Renal Function Tests (RFT)**
- Includes: Serum creatinine, urea
- Checked at **baseline**
- Ensures no kidney-related toxicity
- Haematology panel at baseline.
- Weight and height (growth monitoring) monthly.
- Parental adverse event diary maintained throughout the study.

8. Limitations & Future Directions

8.1 Current Limitations

- Limited large-scale randomised clinical trial data on herbal chocolate for ASD specifically.
- Bioavailability of herbal actives embedded in a chocolate matrix requires individual study.
- Heterogeneity of ASD phenotypes complicates standardised outcome measurement.
- Long-term effects of the herbal combination in children are unknown.

- Palatability and compliance among children with ASD (who often have food selectivity) may be variable.

8.2 Future Research Directions

- Large-scale, multi-centre RCTs with adequate power and stratified ASD phenotypes.
- Pharmacokinetic studies in children to define optimal dosing intervals.
- Biomarker-guided treatment personalisation (genetic polymorphisms, oxidative stress biomarkers).
- Long-term safety evaluation (12–24 months) with growth and developmental monitoring.
- Exploration of nanoformulation (nano-curcumin, liposomal bacosides) within the chocolate matrix to enhance bioavailability.
- Investigation of microbiome modulation as a mechanistic pathway for herbal effects in ASD.
- Head-to-head comparison with other nutraceutical delivery forms (gummies, capsules) for efficacy and compliance.

9. Regulatory & Ethical Considerations

- The formulation must be classified as a food supplement or nutraceutical under applicable national regulations (e.g., FSSAI in India, FDA GRAS in the USA, EFSA in Europe).
- Clinical trials must obtain Institutional Ethics Committee (IEC) / IRB approval before commencement.
- Written informed consent from parents/legal guardians; assent from children where appropriate.
- CTRI / ClinicalTrials.gov registration is mandatory for clinical studies.
- Compliance with GMP (Good Manufacturing Practice) standards for nutraceutical production.
- All herbal raw materials must carry Certificates of Analysis (CoA) and be free from adulteration.
- Labelling must comply with applicable food safety standards including accurate allergen, ingredient, and cautionary information.

10. CONCLUSION:

The formulation of a standardised herbal chocolate incorporating Brahmi, Curcumin, Ashwagandha, Green Tea extract, Tulsi, and Piperine offers a scientifically grounded, palatable, and accessible nutraceutical approach to supporting neurological function in children with ASD. Dark chocolate as a delivery vehicle synergistically complements the herbal actives through its intrinsic antioxidant, mood-modulating, and magnesium-rich profile.

The comprehensive evaluation framework — spanning physicochemical, phytochemical, microbiological, stability, and clinical assessment domains — ensures that the product meets the highest standards of quality, safety, and efficacy expected for a paediatric nutraceutical. Rigorous randomised clinical trials are essential to confirm therapeutic benefit and establish evidence-based dosing guidelines.

Future research should focus on pharmacokinetic profiling, bioavailability enhancement techniques (such as nanoformulations), and personalized treatment approaches based on individual biomarkers. Overall, this formulation represents a promising bridge between traditional herbal medicine and modern pharmaceutical science, offering a safe, acceptable, and potentially effective supportive therapy for improving the quality of life in children with ASD.

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