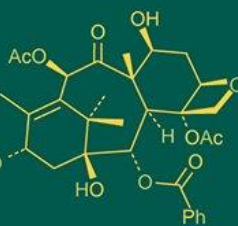
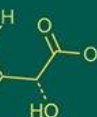
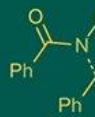


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Formulation, physicochemical characterization, and *in vitro* antioxidant and antimicrobial evaluation of a gum Arabic-stabilized oil-in-water emulsion enriched with *Bacopa monnieri* (Brahmi) leaf extract

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Abstract

Bacopa monnieri (Brahmi), an important Ayurvedic herb, has potential for functional food and nutraceutical applications due to its neuroprotective and antimicrobial properties. In this study, olive oil-in-water nanoemulsions containing aqueous Brahmi leaf extract were prepared using gum Arabic as a natural emulsifier. Four formulations (1, 2, 4, and 6% olive oil) were evaluated, and the 1% formulation (NBE1) showed the highest visual stability and was selected for detailed characterization alongside the 4% formulation (NBE4) and an extract-free control. NBE1 exhibited significantly smaller droplet size (226.1 ± 4.3 nm) than NBE4 (297.9 ± 3.1 nm; $p < 0.001$), while creaming stability and UV-Vis absorbance did not differ significantly from the control. FTIR confirmed the presence of phenolic compounds, and rheological analysis demonstrated shear-thinning behavior. Both formulations displayed concentration-dependent DPPH radical scavenging activity, although the control showed slightly higher antioxidant activity. The Brahmi emulsion exhibited reproducible antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, but its efficacy remained significantly lower than amoxicillin. Overall, gum Arabic effectively stabilized the Brahmi nanoemulsion, producing a formulation with favorable physicochemical and functional properties. Statistical re-analysis using Welch's *t*-test, paired *t*-test, and one-way ANOVA strengthened the reliability and transparency of the findings.

Keywords: *Bacopa monnieri*, Brahmi, gum Arabic, oil in water emulsion, nanoemulsion stability, DPPH antioxidant activity and antimicrobial activity

1. Introduction

Emulsions are multiphasic systems of two immiscible liquids, and are commonly used throughout the food, pharmaceutical, and cosmetic industries to convey lipophilic and hydrophilic bioactive components (McClements, 2016; McClements, 2015) [10, 11]. Emulsion systems are thermodynamically unstable and rely on the presence of emulsifiers and/or stabilizers that reduce the interfacial tension and create steric or electrostatic barrier to droplet coalescence, flocculation and Ostwald ripening (Gupta et al., 2016; McClements, 2015) [6, 10]. Gum Arabic, which is the exuded gum of *Acacia senegal*, is a very attractive food-grade biopolymer emulsifier due to its highly branched polyanionic arabinogalactan-protein structure that results in strong interfacial activity, long-term colloidal stability, and relatively low viscosity even at high concentrations of gum (Jayme et al., 1999; Matsumura et al., 2000; Wang et al., 2011) [8, 9, 18].

The parallel and continued interest in using plant bioactives (Kumar et al., 2021) [19] as additives in emulsion formulations can be explained by the aim of protecting the bioactive from degradation by the environment as well as providing functional property to the emulsion (most often antioxidant and antimicrobial activity) (Donsì et al., 2011; Ha et al., 2015; Sugumar et al., 2013; Sugumar et al., 2015) [4, 7, 15, 16]. Emulsified essential oils and polyphenol extracts have been proven time and time again to inhibit food-related pathogens including *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Salmonella* spp. (Donsì et al., 2011; Nostro et al., 2016) [4, 12].

Brahmi (*Bacopa monnieri*) is an ayurvedic medicinal herb that's been traditionally used in India to aid cognitive function, and for the management of stress or inflammatory conditions. The aqueous and ethanolic extracts of the plant is rich in bacoside type triterpenoid saponins and phenolic components, and ethanolic extract of Brahmi has been reported to inhibit foodborne bacteria such as *Salmonella typhimurium*, *Proteus vulgaris*, *S. aureus*, *E. coli*, and *Bacillus subtilis* independently (Dhanze & Mane, 2012) [3]. To the best of our knowledge, Brahmi extract was not, at the time of this writing, incorporated into a food grade emulsion system and tested for both antimicrobial and antioxidant activity while also maintaining physicochemical stability. The formulation of the olive-oil-in-water emulsion stabilized by gum Arabic enriched with aqueous solution of Brahmi leaf extract was reported at four different concentrations of oil, the emulsion was characterized in terms of droplet size, creaming stability, UV-Vis behaviour, electrolyte tolerance, FTIR fingerprint, flow behaviour, DPPH radical-scavenging capacity and antibacterial activity against *E. coli* and *S. aureus*. The central purpose of this paper differs from the thesis, for which this paper was written, in that every comparison of the Brahmi emulsion with its extract-free control is made here using an explicit, inferential statistical test (Welch's t-test, paired t-test, or one-way ANOVA with Bonferroni-corrected post-hoc comparisons) for which means and standard deviations are reported; therefore, similarity or difference claims are not made just by visual inspection of tabulated values, but can be evaluated against a stated significance level ($\alpha = 0.05$). If this re-analysis confirms the original story, it is reported; if it does not it is reported as well. To develop an oil-in-water emulsion with a stable concentration of Brahmi extract in the oil phase and determine the most stable concentration for the oil phase. 2. To stabilize the selected formulation using gum Arabic and to describe the physicochemical properties of the stabilized formulation. 3. To compare the antioxidant and antimicrobial activity of the stabilized emulsion with extract-free emulsion in a statistical manner.

2. Materials and Methods

2.1 Plant material and chemicals

Commercial Brahmi (*Bacopa monnieri*) leaf powder was obtained from Alka Ayurvedic Pharmacy, Ahmedabad, India. The olive oil and gum Arabic was collected in the local market (Solan, India). Sodium chloride, nutrient agar, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), L-ascorbic acid and amoxicillin (positive-control disc) were used as such. The reference strains of *Staphylococcus aureus* (MTCC 96) and *Escherichia coli* (MTCC 443) were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India.

2.2 Preparation of brahmi extract

To get the Brahmi powder 10 g was dispersed in 150 mL of distilled water and boiled for 40 min. The mixture was allowed to cool down to room temperature, filtered with Whatman No. 40 filter paper and centrifuged at 5000 rpm for 10 min. Supernatant was removed and sediment fraction was used as working extract for emulsion formulation.

2.3 Formulation of oil-in-water emulsions

Oil-in-Water emulsions are prepared by mixing oil and water. Oil-in-Water emulsions are made by combining oil and water.

To prepare four O/W emulsions, mechanical (magnetic-stirrer) emulsification was performed with different proportions of olive-oil (1%, 2%, 3%, and 4% w/w) and a constant proportion of 1% each (w/w) of Brahmi extract and gum Arabic. The 250 mg formulation of Brahmi extract contained 250 mg of Brahmi extract, 250 mg olive oil, 250 mg gum Arabic and a small volume of Tween 80 (with water as the diluent) mixed for 30 min at 1000 rpm whereas NBE2, NBE4 and NBE6 formulations contained 250, 500 and 750 mg of olive oil, respectively, and a matched 1% extract-free control was similarly prepared but without Brahmi extract. All 4 formulations were kept at room temperature (30 °C) for a period of 6 h and evaluated for the creaming stability by visual examination of the creamed layer, where the more stable formulation was retained for further characterization, along with NBE4 to compare the size.

2.4 Droplet size analysis

Dynamic light scattering (Zetasizer Nano ZS90, Malvern Instruments, UK) was used to measure the mean droplet size of the samples at 25 °C scattering angle of 90° diluted 1:10 (v/v) in deionized water ($n = 1.59$, $a = 0.01$). The mean of three scans is reported for each value.

2.5 Creaming index

Aliquots (5 mL) of emulsion were then placed in graduated glass tubes and allowed to stand at room temperature, and the height of the serum and the cream layer were measured using a digital Vernier caliper. The separated (cream) layer height was measured and the ratio of this to the total emulsion height was calculated to produce a value known as cream index (CI, %).

2.6 UV-visible spectrophotometry

The turbidity/absorbance of emulsion and control were measured at 600nm in triplicate using a distilled-water blank.

2.7 Ionic-strength stability

Freshly prepared and 30-day stored (27 °C) emulsion and control samples were used and NaCl was added to the emulsion samples to produce final concentrations of 0.5 M, 1.0 M, and 2.0 M; and droplet-size related spectrophotometric readings were taken at each concentration on days 0 and 30.

2.8 FTIR spectroscopy

The selected emulsion (NBE1) and the control were analysed in triplicate by FTIR spectrometer with ATR (Agilent Technologies, India) with a resolution of 4 cm⁻¹ and the spectra were corrected for water-vapour and CO₂ (4000-600 cm⁻¹).

2.9 Rheological measurements

A controlled-stress rheometer (cone-plate geometry, 25 °C) was used to determine the apparent viscosity of each product at various shear rates and at various temperatures (5-50 °C).

2.10 Antimicrobial assay

The antimicrobial activity was determined by agar well diffusion method against *S. aureus* (MTCC 96) and *E. coli* (MTCC 443). Overnight bacterial suspension was tested by

swabbing onto nutrient agar, 6 mm wells were punched and 100 µL of emulsion, extract-free control or amoxicillin (positive control) was placed. The plates were incubated for 24 hours at 37 °C and the zones of inhibition were recorded in mm (Kumar et al., 2025; Tripathi et al., 2025) [20, 21].

2.11 DPPH radical-scavenging assay

The extract in methanolic stock solution (25mg/mL) was serially diluted 25-fold, 50-fold, 75-fold and 100-fold. 100 µL of each dilutions was added to 2.9 mL of 0.1 mM DPPH in ethanol and incubated in the dark for 30 min before absorbance reading at 517 nm. The reference standards used were ascorbic acid and Trolox. The percentage inhibition (%I) was determined by the equation: $%I = [(A_0 - A_1)/A_0] \times 100$, A_0 is the absorbance of the blank and A_1 is the absorbance of the sample. Note on terminology: the four dilution levels are given here as "dilution level" (25×-100×) but this is not the same as a "time series" (as mentioned in the narrative of the source study, but not in the assay design that was actually used in the study, and corrected here for consistency).

2.12 Statistical analysis

All values are given as mean±SD (triplicate or otherwise indicated) determinations. The comparisons between the Brahmi emulsion and its extract-free control were done on the reported summary statistics based on a Welch's two-sample t-test (independent sample of unequal variance) or paired t-test (where the same set of conditions salt concentration or dilution level were measured in both groups). Pairwise t-tests were used following a one-way ANOVA to compare the diameters of the inhibition zones between *E. coli*, *S. aureus*, and the amoxicillin positive control. $p < 0.05$ was considered significant. All calculations were done in Python 3 with SciPy and the degrees of freedom for unpaired comparisons were approximated by the Welch-satterthwaite approximation.

3. Results and discussion

3.1 Screening of oil-phase concentration and selection of the stable formulation

After 6 h at room temperature, all four formulations (NBE1, NBE2, NBE4 and NBE6; 1, 2, 4 and 6% olive oil) exhibited a visible cream layer, except for NBE1, which did not show any visible layer (Table 1). Only the 1% oil-phase formulation was judged kinetically stable, and was selected for complete characterization, along with NBE4 for size comparison, and was used for all subsequent antioxidant and antimicrobial testing against a matched extract-free 1% control.

Table 1: The creaming stability and composition of emulsions prepared from *Bacopa monnieri*

Formulation	Olive oil (%)	Extract (%)	Gum Arabic (%)	Cream layer at 6 h	Outcome
NBE1	1	1	1	None observed	Stable selected
NBE2	2	1	1	High	Unstable
NBE4	4	1	1	High	Unstable (used for size comparison)
NBE6	6	1	1	Highest	Unstable

3.2 Droplet Size

NBE1 exhibited a significantly smaller mean droplet diameter (226.1 ± 4.3 nm) than NBE4 (297.9 ± 3.1 nm; Welch's $t = -23.5$, $df = 3.65$, $p < 0.0001$; Table 2). Both values are in the size regime typically considered as nanoemulsions (droplets $< \sim 500$ nm) (Gupta et al., 2016; Solans et al., 2005) [6, 14]; and as the dispersed oil-phase fraction increased, the droplet sizes increased, as is to be expected as the increasing amount of dispersed oil-phase will not be able to be fully covered by a fixed amount of gum Arabic, leaving larger and less completely covered droplets (McClements, 2015; Ozturk & McClements, 2016) [10, 11, 13].

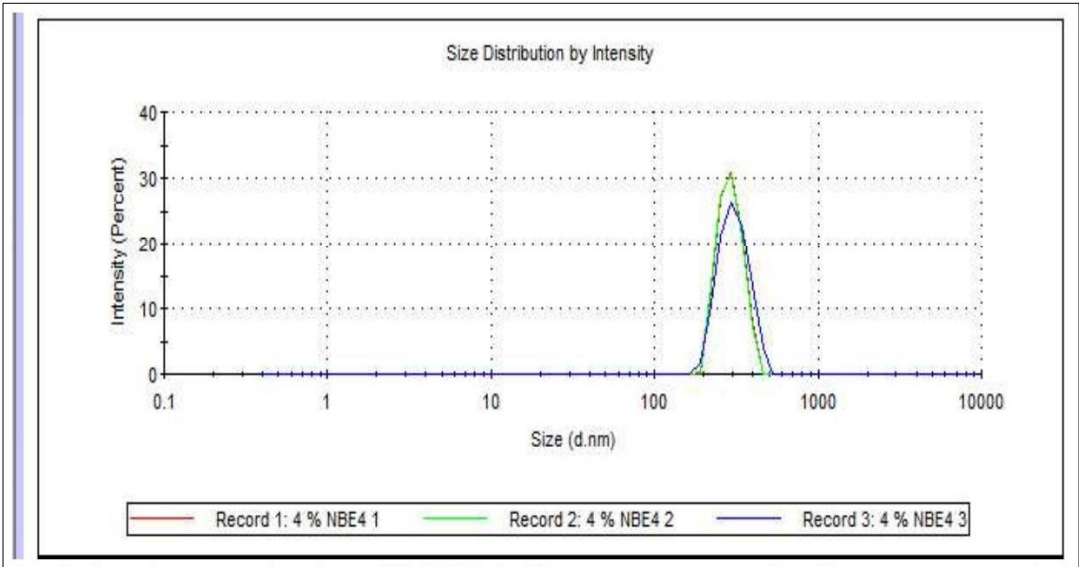


Fig 3 (A): Droplet size distribution (dynamic light scattering) of NBE4 (4% olive oil)

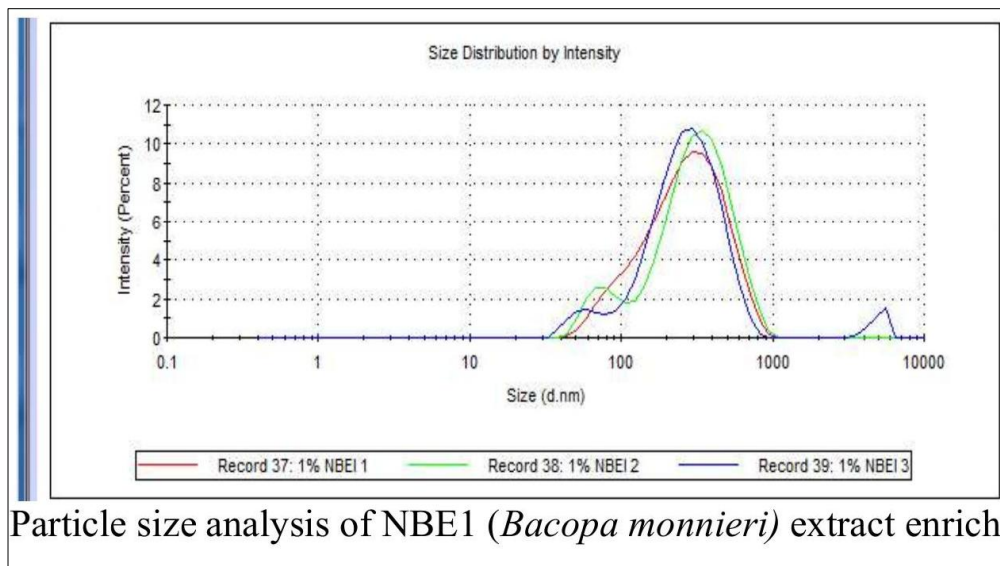


Fig 3 (B): NBE1 (1% olive oil) was chosen for full characterization, and its droplet size distribution (dynamic light scattering) is shown

3.3 Creaming stability

However, no significant difference was observed in the creaming index of NBE1 with the control without extraction ($1.56 \pm 0.63\%$) (welch's $t = -0.39$, $df = 3.54$, $p = 0.72$; Table 2), suggesting that the incorporation of NBE1 did not

significantly affect the short term creaming stability provided by gum Arabic. This is in line with the report that gum Arabic-based system is able to suppress creaming in plant oil emulsion (Wang et al., 2011)^[18].



Fig 1: The cream-layer formation in the emulsion samples enriched with *Bacopa monnieri* extract after 6 h at room temperature increased with the increase in the olive-oil content (NBE1 (1%), NBE2 (2%), NBE4 (4%), NBE6 (6%)). NBE1 was the only one that was not visually unstable



Fig 2: Visual comparison of the selected 1% Brahmi-extract emulsion (NBE1, right) and extract-free 1% control (left)

3.4 UV-vis absorbance

Absorbance at 600 nm was statistically similar between the Brahmi emulsion (3.12 ± 0.04) and control (3.13 ± 0.09 ; Table 2) indicating that in both the formulations, emulsification

was comparable, as the result was an O/W turbid dispersion irrespective of the addition of extract, and thus serves as a simple confirmatory test for the completeness of the emulsification.

Table 2: A summary of the particle size, creaming index and UV-Vis absorbance

Parameter	Brahmi emulsion	Comparator	Test	df	p-value	Significant ($\alpha=0.05$)
Particle size (nm)	226.13 ± 4.28 (NBE1)	297.9 ± 3.1 (NBE4)	Welch t = -23.52	3.65	<0.0001	Yes
Creaming index (%)	1.31 ± 0.92	1.56 ± 0.63 (control)	Welch t = -0.39	3.54	0.72	No
UV-Vis abs. (600 nm)	3.12 ± 0.04	3.13 ± 0.09 (control)	Welch t = -0.18	2.76	0.87	No

3.5 Ionic-strength tolerance

The Brahmi emulsion and the control showed an upward trend in apparent particle-size-related readings with increasing concentrations of NaCl at day 0 and the two were not significantly different as a paired series at the three concentrations of NaCl (paired t = -1.24, df = 2, p = 0.34). At day 30 the same comparison was very close to the conventional level of significance (paired t = -2.87, df = 2, p = 0.10), the control showing a slight trend to higher values than the emulsion. When viewed longitudinally, there was a significant decrease in Brahmi emulsion values from day 0 till day 30 (paired t = 6.93, df = 2, p = 0.02), but not for the control (paired t = -0.67, df = 2, p = 0.57).

This is a pattern to be highlighted explicitly: the data are not consistent with a simple "storage has no effect" story but a stronger conclusion would require more replicates and a direct particle-size (not absorbance) measurement at day 30. The overall trend of higher apparent size with increasing salt concentration is likely due to charge screening at the gum Arabic-stabilized interface; that is, the electrostatic repulsion normally present between droplets of gum Arabic, as a result of its highly anionic arabinogalactan-protein backbone, is progressively reduced as Na^+ concentration increases, allowing droplets to approach each other more closely (Jayme et al., 1999; Wang et al., 2011) [8, 18].

Table 3: Ionic strength (spectrophotometric particle-size index) at day 0 and day 30

NaCl (M)	Emulsion, Day 0	Control, Day 0	Emulsion, Day 30	Control, Day 30
0.5	2.72 ± 0.06	2.70 ± 0.08	2.44 ± 0.12	2.65 ± 0.03
1.0	2.52 ± 0.09	2.58 ± 0.02	2.17 ± 0.08	2.90 ± 0.84
2.0	2.63 ± 0.70	2.83 ± 0.57	2.42 ± 0.04	2.80 ± 0.07

3.6 FTIR spectroscopy

The IR spectra of both the Brahmi emulsion and the control all exhibit a broad band at $\sim 3330 \text{ cm}^{-1}$ corresponding to the O-H/N-H stretch, a band at $\sim 1640\text{-}1745 \text{ cm}^{-1}$ corresponding to carbonyl/amide stretches of residual protein in the gum Arabic emulsifier and a band at $\sim 2700\text{-}3000 \text{ cm}^{-1}$ corresponding to the C-H stretch. The broad shoulder in the

$3100\text{-}3400 \text{ cm}^{-1}$ region, observed in the Brahmi emulsion, corresponds to the O-H stretching mode of the phenolic components which is suggested by the addition of the extract, and is reported here as qualitative fingerprint evidence for the presence of the extract in the emulsion as opposed to a calculated peak-area difference because the peak-area data was not provided in the source dataset.

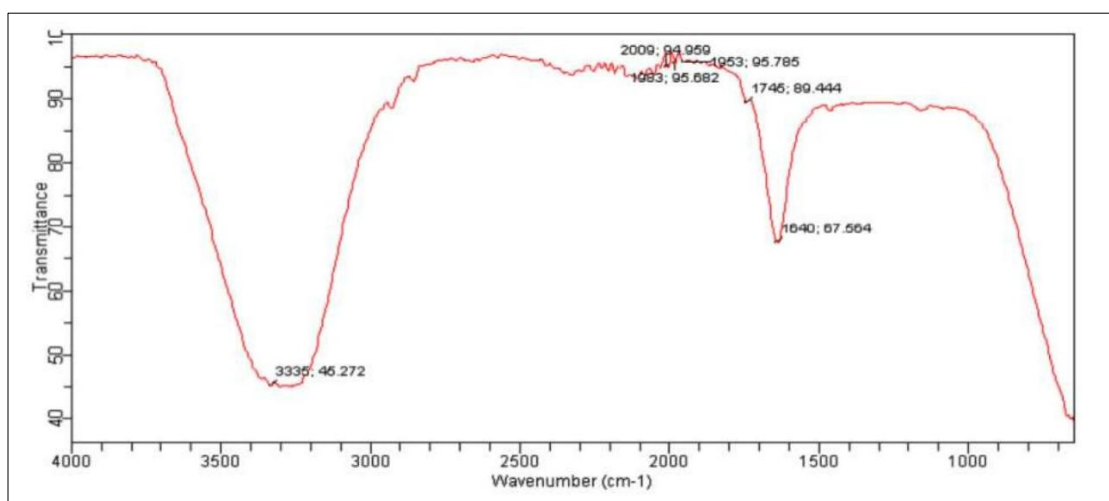


Fig 7 (A): FTIR spectrum of the emulsion containing the brachmi extract stabilised by gum Arabic (NBE1)

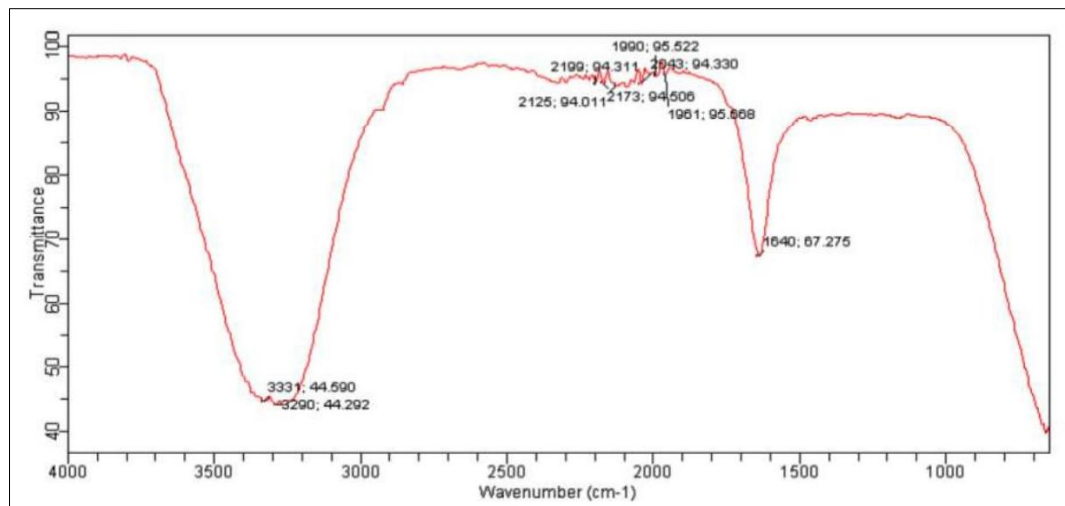


Fig 7 (B): FTIR spectrum of the gum Arabic-stabilized, extract free control emulsion

3.7 Rheological behaviour

The shear-thinning (pseudoplastic, non-Newtonian) behavior was observed in the Brahmi emulsion, as apparent viscosity decreased with shear rate; with low shear, weak interactions between the droplets and the biopolymer make it difficult for the emulsion to flow, and as the shear rate increases, these interactions are broken. The viscosity also decreased as temperature increased (5-50 °C), which is in

line with the expectations that the viscosity of the continuous phase decreases with increasing temperature and the interaction between the droplets is lower at higher thermal energy. The strong shear-thinning of the NBE1 indicates that it could flow well for practical applications like tube-feeding or drop-wise dosing when it is sheared, but not flow when stored under the near-quiescent conditions required for phase separation.

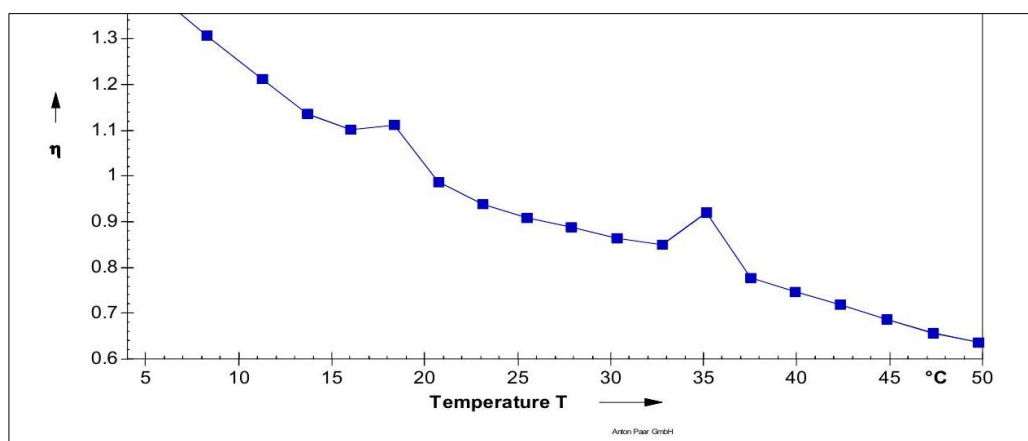


Fig 8 (A): Apparent viscosity of the Brahmi extract-enriched emulsion as a function of temperature (5-50 °C)

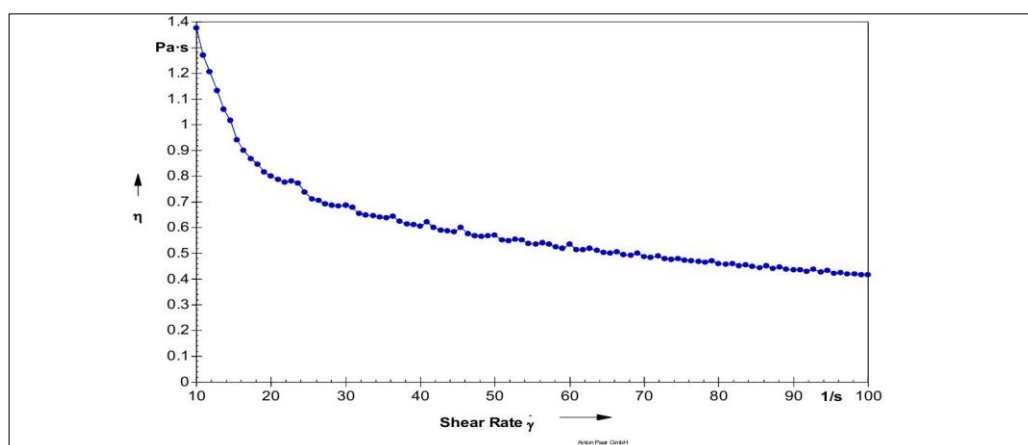


Fig 8 (B): Apparent viscosity of the Brahmi extract-enriched emulsion as a function of shear rate, showing shear-thinning behaviour

3.8 Antioxidant (DPPH) activity

DPPH inhibition declined modestly and near-linearly with increasing dilution (i.e., decreasing extract concentration)

for both the Brahmi emulsion (79.56% at 25× to 78.39% at 100×; linear regression $r = -0.97$, $p = 0.03$) and the control (81.33% to 80.09%; $r = -0.99$, $p = 0.01$) (Table 4). The

result was contrary to what was expected where the antioxidant activity with the inclusion of antioxidant rich plant extract was shown to be higher than the extract without the inclusion of the antioxidant extract; the antioxidant activity of the extract with no antioxidant extract (control) was found to be marginally but consistently higher than the antioxidant activity of the antioxidant rich plant extract (Brahmi emulsion) (mean difference 1.78 percentage points; paired $t = -19.3$, $df = 3$, $p < 0.001$) when compared at all dilutions.

This does not appear to be an actual antagonistic effect of Brahmi extract on the radical scavenging. The other two likely explanations are: First, the extract itself may have a measurable reducing/radical-scavenging effect on both formulations, and any contribution of the extract may not be detectable when compared to the background by the emulsifier; Second, background absorbance or light

scattering due to oil droplets in the emulsion prepared from an extract may include a small systematic absorbance bias at 517 nm compared to the control. The numerical difference between the emulsion and control results (~2 percentage points on an ~80% baseline) is not very large even though it is statistically significant, so we interpret the result as evidence of a reproducible, low-noise assay (small SDs, hence a large t -statistic), rather than of a practically significant loss of antioxidant capacity in the emulsion when compared to the control. The present re-analysis of the original thesis narrative reports the within-group concentration trend but does not directly compare emulsion to control; in fact, the original thesis is counter-intuitive in that regard, which we suggest be reexamined in future emulsion/exposure studies using a properly matched extract-free blank at each dilution, and, ideally, a Trolox-equivalent calibration curve.

Table 4: The DPPH radical-scavenging inhibition (%) with respect to dilution level

Dilution	Brahmi emulsion (%)	Control (%)
25×	79.56±0.08	81.33±0.23
50×	79.07±0.49	81.10±0.14
75×	78.97±0.10	80.57±0.15
100×	78.39±0.08	80.09±0.08

Statistics Paired t -test, emulsion vs. control (all 4 dilutions): $t = -19.32$, $df = 3$, $p < 0.001$ (control significantly higher at every level). Linear trend across dilution: emulsion $r = -0.97$, $p = 0.03$; control $r = -0.99$, $p = 0.01$ (both significant declining trends with increasing dilution).

All 4 dilutions: emulsion vs. control Paired t -test $t = -19.32$, $df = 3$, $p < 0.001$ (control significantly higher at all dilutions). Both emulsion and control had significant negative trends with increasing dilution (emulsion, $r = -0.97$, $p = 0.03$; control, $r = -0.99$, $p = 0.01$).

3.9 Antimicrobial activity

For both *E. coli* and *S. aureus*, the ZOI were 12.0±2.0 mm and 11.5±0.71 mm respectively, compared to 18.5±0.71 mm for the amoxicillin positive control (Table 5) and no ZOI was observed with the negative control. A one-way ANOVA showed that there was a significant difference between the three groups overall [$F(2, 6) = 27.41$, $p < 0.001$]. No significant differences were observed with *E. coli* vs. *S. aureus* (corrected $p = 1.00$), and *E. coli* survived correction to < 0.001 compared to the amoxicillin control, but the gap was not significant after correction (corrected $p = 0.06$) when compared to the amoxicillin control.

The antibacterial activity of the Brahmi emulsion is therefore considered real and reproducible, as it exhibited a distinct and reproducible inhibition zone against both

organisms, with the extent of inhibition being similar in both species in comparison to that of a clinical strength antibiotic which was smaller. However, the antibacterial activity of Brahmi emulsion is considered real and reproducible as it showed distinct and reproducible inhibition zone against both the organisms and the extent of inhibition was also found to be same in both organisms in comparison to the clinical strength antibiotic which had a smaller inhibition zone. This is the more conservative and accepted statement than "comparable antibacterial activity", and is presented here as being the more defensible interpretation of the data considering the attained sample size ($n = 3$ in each group) and the resulting wide confidence intervals. The general ability of Brahmi extract to exhibit antibacterial activity against *E. coli*, *S. aureus*, and related organisms is comparable with previous reports on ethanolic extract of Brahmi (Dhanze & Mane, 2012) [3] and suggests that the delivery system of Brahmi-emulsion has potential as a natural antimicrobial delivery vehicle, but potency optimization such as increasing loading of Brahmi in the extract, exploring alternative extraction solvents, or combining with a synergistic compound would likely be required before the system could be marketed as an antibiotic adjunct or replacement instead of as a mild, stand-alone antimicrobial.

Table 5: Antimicrobial zone of inhibition (mm) and statistical comparison

Group	Mean±SD (n = 3)	vs. Positive control (Bonferroni-corrected p)
<i>E. coli</i>	12.0±2.0	0.06 (ns; uncorrected $p = 0.02$)
<i>S. aureus</i>	11.5±0.71	<0.001 (significant)
Positive control (amoxicillin)	18.5±0.71	-
Negative control	No inhibition	-

One-way ANOVA: $F(2,6) = 27.41$, $p < 0.001$. *E. coli* vs. *S. aureus* (Bonferroni-corrected): $p = 1.00$ (ns).

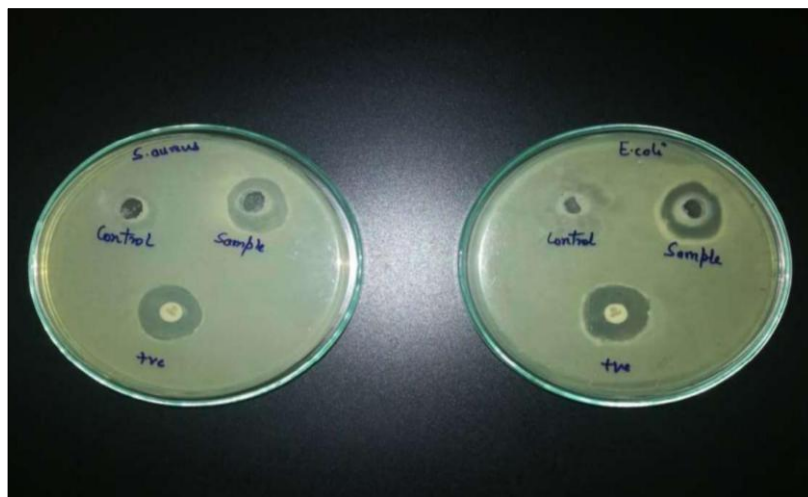


Fig 10: Agar-well diffusion plates showing zones of inhibition of the Brahmi extract-enriched emulsion ("Sample"), extract-free control ("Control"), and amoxicillin positive control ("+ve") against *S. aureus* (left) and *E. coli* (right)

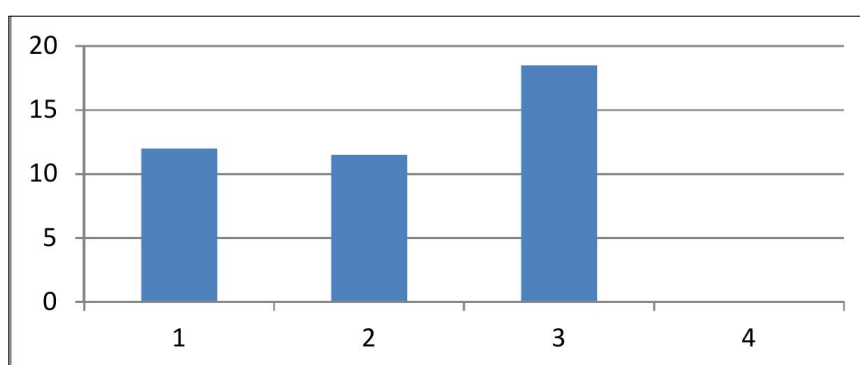


Fig 11: Zone of inhibition (mm) by group: (1) *E. coli*, (2) *S. aureus*, (3) amoxicillin positive control, (4) negative control

4. Conclusion

The formulation of a 1% olive-oil-in-water emulsion that had been enriched with Brahmi extract and stabilized with gum Arabic was successful and was the only one of four different levels of oil loadings that showed no visual instability after 6 h. The selected formulation (NBE1) exhibited a nanoscale mean droplet size (226 nm), was not significantly different from an extract-free control in creaming index and UV-Vis absorbance, had shear-thinning flow behaviour suitable for food delivery and pharmaceutical applications, had significant antibacterial inhibition zones against *E. coli* and *S. aureus*, although smaller than an amoxicillin positive control, and had a significant DPPH radical-scavenging activity, albeit smaller than an extract-free control.

The re-analysis of the reported means and standard deviations, using appropriate statistical tests, confirms most of the qualitative results of the underlying investigation; but also exposes results that require more restrained interpretation than a simple positive narrative: It is worth noting that the extract-free control was slightly, but statistically significantly, superior to the Brahmi emulsion in the DPPH assay, while the apparent day-30 ionic strength effect of the emulsion compared to the control was not statistically significant at the conventional 0.05 level. This preliminary dataset, which was small ($n = 3$) and does not contain any raw replicate values, should be considered a proof of concept and not a full characterization of the ability of gum Arabic-stabilized Brahmi emulsions to successfully deliver natural antimicrobials/antioxidants in food

applications, until confirmed by larger sample sizes ($n > 3$) with full raw replicate values and more readouts.

5. Limitations

All inferential statistics in this paper were reconstructed from summary means $\pm$ SD, not from raw replicate measurements; this restricts the precision of degrees-of-freedom estimates (via the Welch-Satterthwaite approximation) and prevents assessment of normality or outliers. • Sample sizes were small ($n = 3$ per condition in most assays), limiting statistical power most clearly for the day-30 ionic-strength comparison, which fell just short of significance. • Zeta potential and polydispersity index, both standard companions to DLS droplet-size measurement and directly relevant to the electrostatic-stabilization mechanism invoked for gum Arabic, were not measured. • Antimicrobial activity was assessed only by inhibition-zone diameter; no Minimum Inhibitory Concentration (MIC) or Minimum Bactericidal Concentration (MBC) was determined, and only two bacterial species were tested. • The DPPH assay was not benchmarked against a properly matched extract-free absorbance blank at each dilution, which would help resolve whether the emulsion-versus-control gap in Section 3.8 is an assay artefact or a genuine formulation effect. • Long-term storage stability was assessed at only two time points (day 0 and day 30).

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