PhD thesis title**: Extraction and Characterization of Bioactives from Giloy (*Tinospora cordifolia*) and its application in Herbal Yoghurt Formulation**

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Abstract

Giloy (*Tinospora cordifolia*), a medicinal herb, traditionally used in complementary and alternate medicine owing to its various positive impact on human health i.e. antioxidant, anti-inflammatory, anti-allergenic, antidiabetic, and immunomodulatory characteristics. Keeping the therapeutic potential of giloy stem extract (GSE) for the holistic benefit in food, human nutrition and health, a process technology was aimed to develop a herbal yoghurt using GSE as a natural antioxidant. To achieve this, the present research work was comprised of extraction of GSE using microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE), thereafter screeing bioactive compounds using Gas chromatography–mass spectrometry (GC-MS), and High resolution-liquid chromatography mass spectrometry-quadrupole-time-of-flight (HR-LCMS-QToF) techniques. The optimized GSE was further utilized for the formulation of herbal yoghurt along with shelf life under refrigerated storage conditions.

A Box–Behnken design of response surface methodology (RSM) was employed for the optimization of yield, TPC and antioxidant activity (DPPH)) of GSE with solvent-to-solid ratio and MAE variables (extraction time, microwave power) and UAE variables (extraction time and ultrasound amplitude). The MAE was observed to produce a higher GSE yield however, the UAE-assisted GSE was found to have better phenolic and antioxidant values. Based on the numerical optimization, the optimum process conditions under UAE were found to be 30% US amplitude, 10:1 SS ratio and 30 min extraction time. The optimum values of the extract yield: 9.61±0.01%, TPC: 100.04±0.13 mg GAE per 100g extract, and DPPH inhibition: 78.85±0.39%. Screening of optimized extract was done through GC-MS, and HR-LCMS-QTOF and the presence of several polyphenolic compounds like 1,2-oxathiane, 6-dodecyl-, 2,2-dioxide, silanol, trimethyl-, phosphate (3:1), 2-propenoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester, methyl 9,10-octadecadienoate, (-)-catechin quercitrin, malic acid and aurasperone E among others.

Development of GSE-based herbal yoghurt was formulated using a central composite rotatable design of RSM with GSE concentration, incubation temperature (IT), and total soluble solid (TSS) as independent variables and whiteness index, overall acceptability, pH, syneresis, firmness, DPPH scavenging activity, and complex viscosity were measured as response variables. The results revealed that GSE and TSS exhibited maximum effects on all the responses. The optimum formulation condition was found to be 0.08% GSE, 45˚C IT, and 9.1% TSS where whiteness index, overall acceptability, pH, syneresis, firmness, DPPH radical scavenging activity and complex viscosity were predicted to be 84.513%, 8.567, 4.193, 47.268%, 281.190 N, 56.78% and 76.89 cP respectively. Storage qualities of the yoghurt sample were assessed for 21 days under the refrigerated storage condition (4±1°C). Both the control (without the addition of GSE) and herbal yoghurt exhibited a shear-thinning behaviour during the storage. The herbal yoghurt had significantly greater ($p < 0.05$) DPPH activity compared to the control. The herbal yoghurt showed significant α-analyses and α-glucosidase inhibition activity in comparision to control. The estimated cost per 200 mL herbal yoghurt cup was calculated to be 23.50 with a 20% profit margin.