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Evaluation of Phytochemicals, *In-vitro* Antioxidant, and Anti-inflammatory Properties of an Arishta Formula under Three Aging Conditions

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Traditional literature suggests that Arishta can be stored at room temperature for up to two years while maintaining its potency. Arishta has been widely used as a fermented Ayurvedic formulation to treat various illnesses. However, the impact of different aging conditions on its phytochemical composition, antioxidant, and anti-inflammatory properties remains underexplored, highlighting a key research gap. This study aimed to investigate the bioactivity of three samples of one Arishta formula given for tumors: freshly prepared (A), prepared >1 year ago and kept at room temperature (B), and prepared >1 year ago and kept in the refrigerator at 4^0C (C). Comparing these three samples, we aimed to understand the influence of aging and storage conditions on anti-oxidant and anti-inflammatory activities. Oxidative stress and inflammation are linked to tumour formation. Total phenolic content (TPC) was assessed by the Folin Ciocalteu method, and it was highest in A (34.77±2.82mg GAE/g), followed by B (20.92±0.91mg GAE/g), and lowest in C (17.05±0.41mg GAE/g). Total flavonoid content (TFC) was measured by the aluminium chloride method where the values were 3.23±0.40mg QE/g in A, 3.17±0.48mg QE/g in B, and 3.46±0.18mg QE/g in C. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay showed similar radical scavenging activity (RSA) ($p>0.05$) for all samples: 25.63±0.31% for A, 22.52±0.53% for B, and 23.20±0.72% for C. The 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay ($p>0.05$) showed RSA percentages of 48.11±1.02% for A, 45.52±0.23% for B, and 21.84±0.82% for C. The anti-inflammatory percentages of human red blood cell membrane stabilization assays ($p>0.05$) for the samples are 44.93±0.75% for A, 50.98±0.96% for B, and 40.72±1.23% for C. The protein denaturation percentages are 75.51±0.23% for A, 73.60±1.6% for B, and 72.437±0.67% for C. Room temperature storage appears to preserve Arishta's therapeutic benefits, though proper preparation and storage are essential for efficacy. Further research may identify optimal conditions for herbal medicine storage.

Keywords: *arishta formula, in-vitro, storage temperature, antioxidant, anti-inflammatory*