

Standardization and bioactivity evaluation of a distillate from a traditional herbal formula

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This study aimed to standardize and evaluate the bioactivity of a distillate prepared from a traditional herbal formula using *Phyllanthus emblica* Linn, *Tinospora cordifolia*, and *Aerva lanata* var. *rotundifolia*. Standardization parameters assessed included colour, odour, pH, total solid content, ethanol content, viscosity, and specific gravity. The distillate was colourless with an aromatic odour, had a pH of 4.64 ± 0.06 , and showed no detectable solid content, indicating its predominantly volatile composition. The alcohol content of the distillate at 25 °C (v/v%) was $0.49 \pm 0.14\%$; determined by specific gravity. Also, no detectable viscosity was observed. Microbial contamination tests revealed aerobic plate count (APC) values of 5666.7 CFU and yeast and mold count (YMC) of 3.6 CFU, both within acceptable limits, ensuring microbiological safety. Pathogenic bacteria, including *Escherichia coli*, *Salmonella spp.*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, were not detected. No heavy metals or sugars were detected. The phytochemical analysis of ethyl acetate extract of the distillate (EAED), obtained through solvent partitioning, revealed a total phenolic content of 1.55 ± 0.06 mg/g GAE, total flavonoid content of 2.36 ± 0.09 mg/g, and total condensed tannin content of 0.76 ± 0.24 mg/g. EAED demonstrated significant antioxidant

activity, with DPPH assay showing higher radical scavenging activity (182.47 ± 6.23 µg/mL) compared to the BHT standard (52.04 ± 4.68 µg/mL, $p < 0.05$). The FRAP assay confirmed antioxidant potential with mean absorbance of 0.251 ± 0.002 compared to BHT: 0.514 ± 0.009 . Human Red Blood Cells membrane stabilization assay showed anti-inflammatory activity: EAED (IC₅₀: 807.16 ± 22.39 µg/mL), aspirin (IC₅₀: 88.85 ± 2.14 µg/mL). Acute toxicity testing using *Moina macrocopa* indicated a safe LC₅₀ value of 22.26 ppm, suggesting a favourable safety profile. HPLC analysis of the ethyl acetate extract identified 9 major compounds. In conclusion, the distillate from this traditional herbal formulation exhibits strong antioxidant activity, microbiological safety, and potential as a bioactive product.

Keywords:

Traditional herbal formula; antioxidant activity; phytochemical analysis; microbial contamination; acute toxicity

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