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BIOCHEMICAL EVALUATION AND FERMENTATION-BASED STUDY OF *TINOSPORA CORDIFOLIA* (GILOY)

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Tinospora cordifolia (Giloy) is an important Ayurvedic medicinal plant known for its immunomodulatory, antidiabetic, hepatoprotective and antioxidant properties. This study assesses the biochemical composition of Giloy before and after microbial fermentation. It also investigates how microbial fermentation adds to its nutritional and therapeutic ability.
Unfermented Giloy had 68,993 mg/g carbohydrates, 4.25 mg/g phenolics, 448.21 mg/g protein, 39.4 mg/g niacin and 12.5 mg/g vitamin C. After fermentation, significant increase was observed in various parameters. Protein increased to 687.709 mg/g, phenolics to 5.0 mg/g, vitamin C to 20 mg/g, and niacin to 65.4 mg/g. Carbohydrates reduced to 43,540 mg/g due to microbial utilization. Both Gram-positive and Gram-negative bacteria along with spore-forming microorganisms were observed.
These results indicates that fermentation improves bioavailability of active compounds and boosts nutritional potency. This study indicates that the fermented Giloy can be more effective for developing nutraceuticals, fermented herbal tonics, and functional foods.

Keywords: Antioxidant, Fermentation, Proteins, *Tinospora cordifolia* (Giloy) and Vitamins.

Introduction

Tinospora cordifolia (Giloy) is a perennial climbing shrub found throughout tropical India. It is traditionally revered as "Amrita" (root of immortality) due to its various medicinal uses in Ayurvedic and Siddha systems of medicine. Giloy contains a wide range of bioactive compounds such as alkaloids, diterpenoid lactones, glycosides, steroids, flavonoids, and polysaccharides, which contribute to its medicinal activities[1].

Different parts of the plant, including the stem, leaves, roots, flowers, and fruits, possess specific medicinal benefits. The stem is rich in immunomodulatory compounds and is commonly used in formulations for fever, diabetes, liver disorders, and immune enhancement. The leaves exhibit antioxidant and anti-inflammatory effects, while the roots support digestive health and possess anthelmintic properties[2].

Recent scientific studies have validated the antimicrobial, anti-inflammatory, adaptogenic, and antioxidant potential of Giloy. Fermentation, a biological process carried out by microorganisms, is naturally known to enhance nutritional value, increase vitamin content, release phenolics, and improve digestibility. However, limited research exists on the fermentation-driven enhancement of Giloy[3].

Therefore, this study evaluates the biochemical changes occurring in Giloy due to fermentation and compares raw, unfermented, and fermented samples to

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understand the impact of fermentation on its nutritional and medicinal properties.

Material and Methods

Raw Material Collection:

Tinospora Cordifolia (Giloy) stem was collected from the Campus of Maharaja Ganga Singh University, Bikaner, Rajasthan, India. The Giloy stem sample weighing 770 grams was washed thoroughly with distilled water and dried at room temperature for 4 to 5 days. The weight of the dried Giloy sample was found to be 280 grams. The dried sample was finely ground to prepare its powder, which was subsequently stored in a glass bottle at room temperature.

Fermentation Process

A mixture of Giloy powder (2g), Jaggery (10g), NaCl (2g) was kept in a sterile airtight vessel and allowed to ferment at room temperature for 7 days, during which sour aroma developed due to lactic acid fermentation.

Biochemical Tests

Determination of carbohydrates in Giloy samples:

Determination of carbohydrates in raw Giloy powder, unfermented and fermented Giloy samples was performed by anthrone method[4].

Sample Preparation:

(i) Hydrolysis of complex carbohydrates present in Giloy stem powder sample:

Raw Giloy stem powder (100 mg) was weighed into a centrifuge tube, and 5 ml of 2.5N HCl was added. The tubes were then placed in a boiling water bath for 3 hours. After cooling to room temperature, the hydrolyzed sample was neutralized by adding solid sodium carbonate (until effervescence ceased). The neutralized sample was transferred to a volumetric flask, and distilled water (DW) was added to make the volume up to 100 ml. The diluted sample was centrifuged at 10000 rpm for 10 minutes, and the clear supernatant was collected in separate tubes. 1 ml samples were taken for glucose estimation.

(ii) Estimation of total carbohydrates in the unfermented and fermented samples: -

One ml each of unfermented and fermented samples were centrifuged separately at 10,000 rpm for 10 minutes to obtain clear supernatants. To 0.1 ml of each supernatant (from unfermented and fermented sample tubes), 1.9 ml of distilled water was added to make the total volume to 2 ml in each tube. Then 4 ml of freshly prepared anthrone reagent was added to each tube. The blank was prepared by mixing 1 ml distilled water with 1 ml anthrone reagent in a tube. The contents in all tubes were mixed thoroughly and heated in a boiling water bath for 8 minutes until green colour developed. The tubes were rapidly cooled in ice water to stabilize the colour. Optical density (OD) of each tube was measured at 630 nm against the blank.

The carbohydrate content of crude Giloy powder, unfermented and fermented samples were determined using the standard glucose curve and the results were expressed as mg carbohydrate per g or per ml of sample[5].

Protein estimation in the crude, unfermented and fermented Giloy samples:

The protein content of the crude Giloy powder, unfermented & fermented Giloy samples were determined using the Lowry's Method.

Sample preparation:

(i) *Extraction of proteins from crude Giloy powder:-*

500mg of the crude Giloy powder was ground in 10 ml of phosphate buffer (0.1M, pH 7.0) and kept at 40 °C for 1 h for extraction of the proteins in buffer. Subsequently the protein extracted buffer was centrifuged for 10 min at 10000 rpm. The protein estimation was done using clear supernatant.

(ii) Extraction of proteins from unfermented and fermented Giloy samples:-

One ml of both unfermented and fermented samples was taken in separate tubes. The tubes were centrifuged at 10,000 rpm for 10 minutes and then 5 ml of potassium phosphate buffer (pH 7.0, 0.1mM) was added to each tube. Collect 1 ml of the clear supernatant extract for Protein estimation.

Determination of total phenolics:

Determination of total phenolics in crude Giloy powder, unfermented & fermented Giloy samples was performed as per the method given by[5].

Sample preparation**:-**

(i) Giloy powder extract, unfermented and fermented Giloy extracts:-

To 0.2 grams of dried Giloy powder, 10 ml of distilled water was added, and the mixture was vortexed for 10 minutes. From this extract, 1 ml was further diluted using 10 x saturated sodium carbonate to maintain alkaline conditions. To 1 ml of the diluted extract 7.5 ml of distilled water was added. Subsequently, 0.5 ml of diluted Folin-Ciocalteu reagent was added to each tube and the contents were mixed thoroughly. After 3 minutes 1 ml of saturated sodium carbonate solution was added to each tube. Using distilled water, the total volume was made up to 10 ml. The reaction mixture was left to stand for 1 hour at room temperature for full colour development. The total phenolics in the samples were then determined by measuring the OD_{725nm} of samples and converting those values into concentration as per Joy et al. (2015). In case of unfermented and fermented Giloy samples, 1 ml of supernatants were directly used for estimation of total phenolics.

Determination of Vitamin C by DCPIP method:-

This method is based on reduction of blue-coloured dye 2,6-dichlorophenolindophenol (DCPIP) with ascorbic acid, which results in the formation of a colourless leuco compound. The endpoint of the titration is observed when a light pink colour appears and persists for a few seconds. The amount of dye used is directly proportional to the amount of ascorbic acid present in the sample[5].

Reagents:

(i) Oxalic acid solution (4% w/v): used as the extraction and titration medium.

(ii) Dye solution: This was prepared by mixing 42 mg of sodium bicarbonate and 52 mg of 2,6-dichlorophenolindophenol (DCPIP) in 200 ml distilled water.

(iii)Stock standard solution: This was prepared by mixing 100 mg of ascorbic acid in 100 ml of 4% oxalic acid.

(iv) Working standard solution: 10 ml of the stock solution was diluted with 4% oxalic acid (100 µg/ml) and the final volume was made up to 100 ml.

Procedure:-

(i) Preparation of Standard: Five ml of the working standard was pipetted into a conical flask. To this 10 ml of 4% oxalic acid was added and the mixture was titrated against DCPIP dye solution. The volume of dye used (V_1) was recorded.

(ii) Sample Extraction: Five ml each of fermented and unfermented samples was taken in 100 ml volumetric flasks. To each flask, 4% oxalic acid was added to make the final volume to 100 ml. Ten ml aliquots from the unfermented and fermented samples were centrifuged at 10000 rpm for 10 minute and supernatant from each tube were collected.

(iii) Sample titration: Five ml supernatant from unfermented and fermented samples were taken separately in 150 ml conical flasks. To each flask, 10 ml of 4% oxalic acid was added and the mixtures were titrated against DCPIP dye. The volume of the dye consumed was recorded and represented as V_2[5].

Estimation of Niacin in Unfermented and Fermented samples:-

This method is based on the reaction of niacin with cyanogen bromide, which forms a pyridinium compound that undergoes rearrangement to produce derivatives. These derivatives react with aromatic amines to form a yellow pigment. The intensity of the resulting yellow color is proportional to the amount of niacin present and is measured spectrophotometrically at 420 nm.

Reagents:

(i) *Niacin stock solution*: 0.1 g in 10 ml citrate buffer; *Cyanogen bromide solution*: 10 g in 50 ml ice-cold ethanol; *Aniline solution*: 2 ml aniline in 48 ml absolute alcohol; *Ascorbic acid solution*: 0.5g in 100ml distilled water; *Lead acetate solution* [2% (w/v)]: 2 g in 100 ml distilled water. *TCA solution* [15% (w/v)]: 15 g in 100 mL with distilled water; *Citrate buffer* (pH 6.0, 50 mM): prepared by dissolving 4.85 g of sodium citrate dihydrate and 0.6716 g of citric acid in double distilled water to a final volume of 200 ml.

(ii) *Preparation of Niacin Standards*: For the preparation of niacin standards, 0.1, 0.25, 0.5, 0.75 and 1.0 ml of the niacin stock solution were taken in test tubes. The final volume in test tube was made up to 1.0 ml with citrate buffer.

(iii) *Niacin extraction from unfermented and fermented samples*: To 5 ml of the unfermented and fermented samples, 20 µl ascorbic acid solution was added. This was followed by centrifuging the samples at 10,000 rpm for 10 min. Then 1 ml of 2% lead acetate was added to each sample, the components were mixed properly and then centrifuged for 10 minutes at 10000 rpm. The supernatants were collected and 5 ml of 15% TCA was added to each tube. The tubes were then centrifuged at 10000 rpm for 10 minutes and the clear supernatants (sample extracts) were collected. The extracts were used to prepare assay tubes with 0.10 ml, 0.25 ml, and 0.50 ml aliquots.

(iv) *Estimation by Spectrophotometer*: To 5 ml of both unfermented and fermented sample-extracts 10 ml of citrate buffer (pH 6, 0.1mM) was added. After mixing, 3.0 ml of cold cyanogen bromide was added to each tube, and the mixture was kept for 10 min at 4 ℃. Finally, 0.5 ml of aniline solution (8%) was added, and the mixture was incubated at room temperature for 5 min. Absorbance of the yellow colour developed was measured at 420 nm against reagent blank.

Determination of Proximate Composition:-

Determination of dry matter (Dm%), total ash (mineral matter), acid - insoluble ash, crude fibre, neutral detergent fibre (NDF/NDS) and acid detergent fibre (ADF) were performed as per the methods given in Analytical Techniques in Animal Nutrition [6].

Microbial Characterization: -

Gram staining and endospore staining were performed to characterize microbial populations present in the fermented sample.

Results and Discussion

The results presented in Table1 and Figure 1 support that fermentation of the Giloy sample has resulted in breakdown of complex carbohydrates and enriched the fermented product with released nutrients and those produced by the natural microbes which responsible for the fermentation process. The biochemical profiling of raw *Tinospora cordifolia* (Giloy) powder revealed that carbohydrates constitute the major nutritional component, with an exceptionally high concentration of 689,930 mg/g, whereas the fermented sample showed a drastically lower value of 43,540 mg/g. This sharp decline is consistent with microbial fermentation, during which carbohydrates are used as the

Table 1. Biochemical Composition of Crude Giloy sample.

Parameter	Unfermented	Fermented
Carbohydrates (mg/g)	68,993±108	43,540±45
Protein (mg/g)	448.21±91	687.709±9.23
Phenolic Content (mg/g)	4.25±0.32	5.0±0.21
Vitamin C (mg/g)	12.5±0.12	20.0±0.43
Niacin (mg/g)	39.4±3.24	65.4±3.8

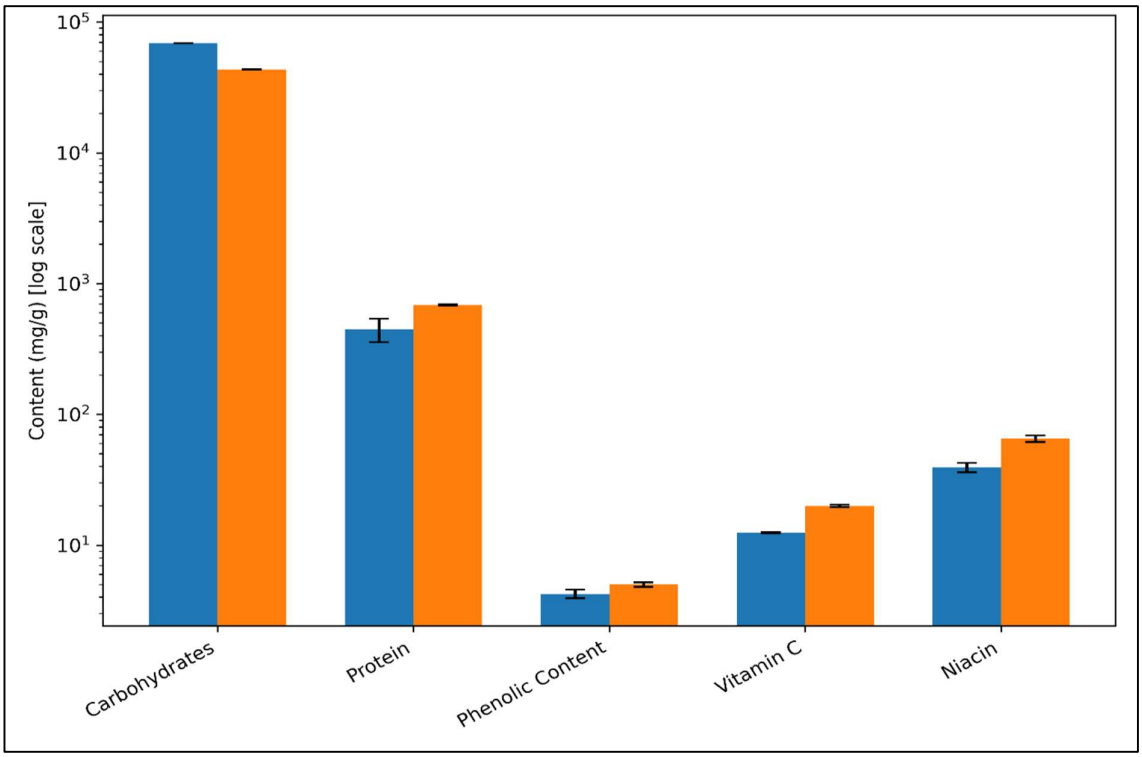

Figure 1: Effect of fermentation on nutritional parameters of the sample. Values are expressed as mean ± SD (n = 3).

primary energy substrates and are converted into organic acids, alcohols, and CO_2 by lactic acid bacteria, yeasts, and other fermenting microbes[7-9]. The moisture (8.72 %) and dry matter (91.28%) content of unfermented Giloy suggest high overall stability during storage. Mineral analysis demonstrated extremely high ash value (91.36%), indicating abundant inorganic content, while acid-insoluble ash (6.46 %) reflected the presence of siliceous materials. Fiber-related parameters (NDF 39.4%, ADF 28.2%) revealed the presence of structural carbohydrates contributing to total dietary fibre in Giloy.

Protein estimation showed a significant increase from 448.21 mg/g in unfermented samples to 687.709 mg/g in fermented samples, suggesting microbial synthesis and release of proteins into the substrate. During fermentation, microorganisms such as *Bacillus*, *Lactobacillus*, *Rhizopus* and yeasts synthesize cellular proteins (single-cell proteins) and also release plant-bound proteins due to enzymatic hydrolysis, thereby enhancing total protein content[10-12].

Total phenolic content showed a slight increase from 4.25 mg/g to 5 mg/g after fermentation, indicating that microbial activity did not significantly degrade these bioactive secondary metabolites.

Fermentation also enhanced micronutrient levels. Ascorbic acid increased from 12.5 mg/g to 20 mg/g, which corresponds with previous studies showing microbial enhancement of vitamin C during vegetable and seed fermentation (Hassan et al., 2021; Wuyts et al., 2018). Similarly, the niacin concentration increased from 39.4 mg/g to 65.4 mg/g, suggesting microbial biosynthesis of B-complex vitamins, which is well documented in fermented cereals, legumes, and soy-based foods[10, 13-14].

Microbiological characterization through Gram staining of the fermented sample showed the presence of both Gram-positive purple rods and Gram-negative pink rods, confirming a mixed microbial population. The detection of green-stained endospores indicated the presence of spore-forming bacteria. Based on morphological and staining characteristics, the bacteria most likely belong to species such as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, or *Bacillus pumilus*. These species are well-known for their ability to produce proteases, cellulases, keratinases, and antimicrobial compounds such as surfactin and iturin, as well as their roles in plant fermentation, nutrient release, and probiotic activity[14-15].

Overall, the results clearly indicate that fermentation significantly enhanced the nutritional profile of *T. cordifolia* by increasing protein, niacin, and ascorbic acid levels, while decreasing carbohydrate concentration through microbial metabolism. The retention of phenolic compounds and the presence of beneficial *Bacillus* species further support the functional and therapeutic value of fermented Giloy preparations.

Conclusion

This study demonstrates that fermentation is an effective biological process that enhances the biochemical composition of *Tinospora cordifolia*. Raw Giloy is already nutritionally rich, but fermentation significantly improves its protein, vitamin C, niacin, and phenolic content while reducing carbohydrate levels. These changes indicate greater antioxidant activity, higher medicinal value, and improved digestibility.

Microbial analysis confirmed that fermentation involved diverse microbial groups, which contributed to biochemical transformations. These findings validate traditional Ayurvedic practices, where fermentation is often used to increase the potency of herbal formulations.

Overall, fermented Giloy is a more efficient nutraceutical option with improved therapeutic potential. Future research should focus on identifying specific microbial strains, optimizing fermentation conditions, and studying clinical effects on human health.

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