

Investigating the effectiveness of ayurvedic plants on lowering uric acid levels to combat hyperuricemia

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SUMMARY

Millions of people around the world who suffer from hyperuricemia, a condition of excess uric acid that can become other life-threatening diseases, do not have access to treatment. This study aimed to find a natural, cost-effective solution by investigating the potential of six different ayurvedic plants (turmeric, ginger, moringa, bitter gourd, coriander, and neem) to reduce uric acid levels when used alone or in combination with other therapeutics. We hypothesized moringa to be the most effective, as it was previously shown in many studies to reduce uric acid significantly. To investigate this, we created plant powders from each plant and mixed them with water to create extracts. Then, we added uric acid solution and measured the resulting uric acid levels using colorimetric test strips. Ginger and turmeric were found to be the most effective because they lowered the uric acid levels the most out of all six plants. Ratios of turmeric and ginger were also found to be effective synergistically. Overall, our hypothesis was not supported because moringa's effectiveness had only been previously studied individually, not in relation to other plants, and it did not perform as well comparatively. We conducted a literature search which revealed that the amounts of a uric acid-reducing substance called quercetin matched up with the performance of each of the plants, suggesting that it may be involved in the uric acid reduction. However, more detailed investigations must be conducted in the future directly comparing quercetin levels in different plant samples to validate this pattern.

INTRODUCTION

Uric acid is a bodily waste product that is the byproduct of purine (compound commonly found in food and drinks) metabolism and is excreted through the urine (1). Hyperuricemia is a condition that occurs when the body has elevated uric acid levels in the blood over 400 mg/L (2). In the United States, hyperuricemia is very common, as every 1 in 5 people is affected by it (3). Ninety percent of cases are caused by renal urate underexcretion, which is where impaired kidney function prevents uric acid from being removed properly from the body. The other 10% of cases are due to overproduction of uric acid, where the body creates an excess amount of waste product when breaking down purines (4). Another common cause of hyperuricemia is the excessive intake of purine-rich foods, including meat, seafood, and legumes (5).

If left untreated, hyperuricemia leads to several health issues which are both painful and reduce quality of life significantly. Excess uric acid clumps up into sharp crystals, which can be deposited in various parts of the body (6). When these crystals are stuck in the kidney, they are known as uric acid nephrolithiasis (kidney stones) and can cause extreme pain and blockage of urine flow (7). Another condition hyperuricemia can lead to is gout, which occurs when the uric acid crystals are deposited in joints and can cause a painful form of arthritis (6,8). Gout usually begins in the joint of the big toe and can flare up very painfully (8). It has four critical stages including: asymptomatic hyperuricemia, acute gouty arthritis, intercritical gout, and chronic tophaceous gout, which needs surgical intervention (9). If left untreated, hyperuricemia can quickly progress into this advanced form of gout and become deadly for patients (10). In the United States alone, 9.2 million individuals suffer from gout, and approximately 14.6% of the total population suffers from hyperuricemia in general (11,12). Gout is found more frequently in men and ethnic minorities, specifically Asian and Black Americans (12). Worldwide, the burden is estimated to be over 41 million individuals (13).

Due to health disparities across the world, early screening and care for hyperuricemia is highly limited in many areas, which is why prevention and reduction are imperative (14). However, even when patients are diagnosed, hyperuricemia and gout management are poor, as many areas also do not have access to the medications needed to treat these conditions. In fact, only 69.3% of patients had access to medications that lowered their uric acid levels, known as urate lowering therapies (ULTs) (15). On the other hand, 30.7% of gout patients were left without any treatment (16). In individual patients, this can potentially lead to increased gout flare-ups, advanced stage gout, irreversible hyperuricemia, and progress into other conditions, including type 2 diabetes (15,16). Many people living with uncontrolled and potentially lethal uric acid levels have no way to intervene and improve their condition due to these disparities in access to proper medications, highlighting why finding alternative and more accessible options are necessary (17). The goal of this study, therefore, is to explore the effectiveness of natural solutions, which could be more accessible to patients globally who do not have access to conventional treatments.

Ayurveda is a practice derived from traditional Indian medicine and emphasizes holistic approaches to health, especially through natural solutions and combinations of natural substances (18). The six plants –turmeric, ginger, moringa, bitter gourd, coriander, and neem– were chosen to be tested due to their importance in ayurvedic medicine and proven benefits for many health conditions (19).

In this study, we hypothesized moringa to exhibit higher

efficacy in reducing uric acid levels, due to its notable phenolic activity against hyperuricemia, which had been studied more extensively compared to the other ayurvedic plants in the group being tested (20). The moringa plant has been shown numerous times to inhibit xanthine oxidase (XO), which is an enzyme that contributes to producing a high number of reactive oxygen species (ROS). These ROS are usually accompanied by increased levels of uric acid and can damage cells and tissues (21). It has been shown that the molecules 3-p-coumaroylquinic acid, 5-p-coumaroylquinic acid, and catechin in moringa inhibit XO. Additionally, moringa was shown to have a significant effect on reducing serum uric acid by regulating renal expression of ABCG2, URAT1, and GLUT9, which are uric acid transporters that play a role in reabsorption and excretion of uric acid (20).

The question of interest in this study was: among six ayurvedic plants (turmeric, ginger, moringa, bitter gourd, coriander, and neem), which plant demonstrates the highest efficacy in lowering uric acid levels? We hypothesized that moringa would perform the highest in terms of reducing the uric acid levels, because of the previous, extensive research on its mechanisms (20-22). The results of the study did not support this hypothesis, as ginger and turmeric turned out to be the most effective. Combined ratios of these turmeric and ginger powders also had a high efficacy and were tested, as the synergistic effects of plants are often utilized in Ayurveda (22). These results show that ayurvedic plants could possibly be effectively incorporated into diets to aid in uric acid reduction as a preventative measure in early stages when conventional treatments are unavailable.

RESULTS

In order to investigate which ayurvedic plant would be the most effective at reducing uric acid levels, we assessed each plant alone and in combinations and their impact on uric acid levels. We placed extracts of each plant in vials and then added uric acid solution. The levels were then measured with uric acid test strips, which displayed the uric acid levels on a colorimetric scale (**Figure 1a**). The original value of the uric

acid solution alone was greater than or equal to 1500 mg/L of uric for every plant test, which was the highest the test strip could measure. We analyzed the modes of each plant's dataset rather than the medians because the data consisted of discrete points on the colorimetric scale. In this case, the most frequent value, or mode, provided the most accurate representation of how much each extract reduced uric acid levels, as it showed the most probable performance of each plant extract.

The modes for each plant showed how much each was able to reduce the uric acid levels (**Figure 1b**). Ginger was the plant that brought the uric acid levels down the most (to less than or equal to 100 mg/L) and decreased the levels by at least 93.3%, making it the most effective. Turmeric was the second most effective plant as it reduced the uric acid levels by at least 80% overall. Both plants effectively reduced the uric acid to levels below 400 mg/L. Moringa, bitter gourd, and coriander all most frequently reduced the uric acid by at least 26.6%, while neem was the least effective plant (reduced by only 13.3%). Even though neem had lower values for some of the concentrations, its overall mode was still greater than or equal to 1500 mg/L. Additionally, the control group (no plant powder) remained unchanged at greater than or equal to 1500 mg/L across all trials. Moringa was the most varied in its data (largest error bar), while ginger was the least, and the control had no variance (**Figure 1b**).

We measured three different concentrations for each extract (100 mg, 600 mg, and 1000 mg) and calculated their modes (**Figures 2a-c**, respectively). The extract ratio of 600 mg of plant powder to 5mL water, which was the middle concentration of the three tested, consistently showed the greatest reduction of the uric acid levels, making it the most effective ratio. Additionally, we conducted experiments using multiple turmeric-ginger extract ratios, as these were the two most effective plants based on the previous tests (**Figure 3**). All trials of these ratios reduced the uric acid levels to the lowest possible measured on the strip, less than or equal to 100 mg/L every time. We increased only the amount of ginger in these ratios, since it had shown to be the most effective

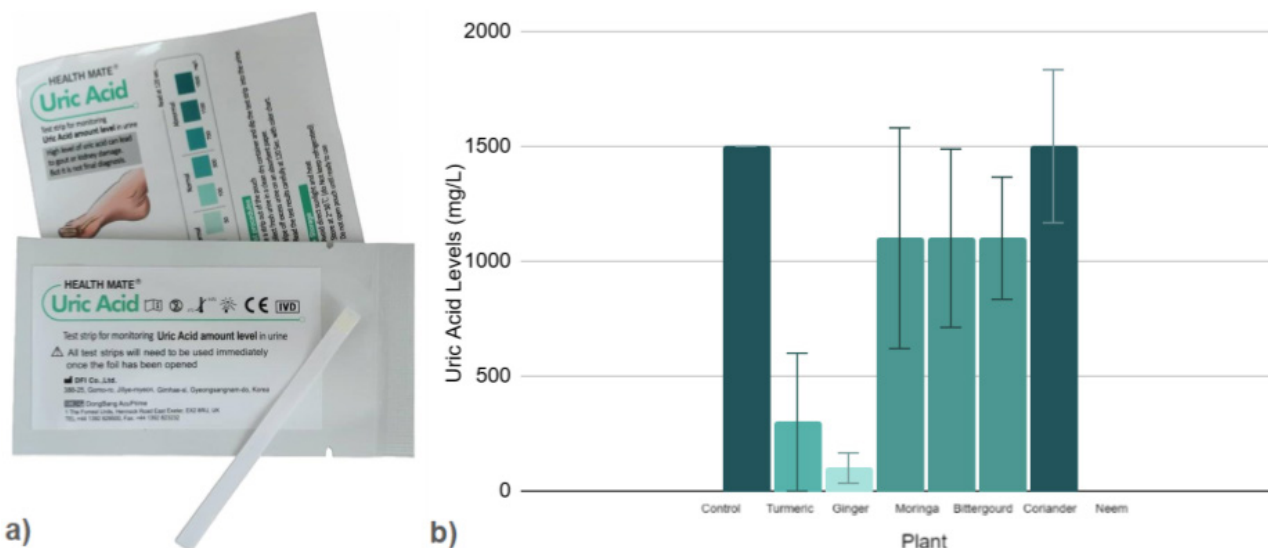


Figure 1: Test strips and modes of the effects of various ayurvedic plant extracts on uric acid levels. (A) Test strips used for uric acid measurement showing colorimetric bars. **(B)** Bar graph showing error bars and the average uric acid levels each plant resulted in (n=3). Extracts were combined with uric acid solution and read after two minutes to measure uric acid-reducing effects of each plant.

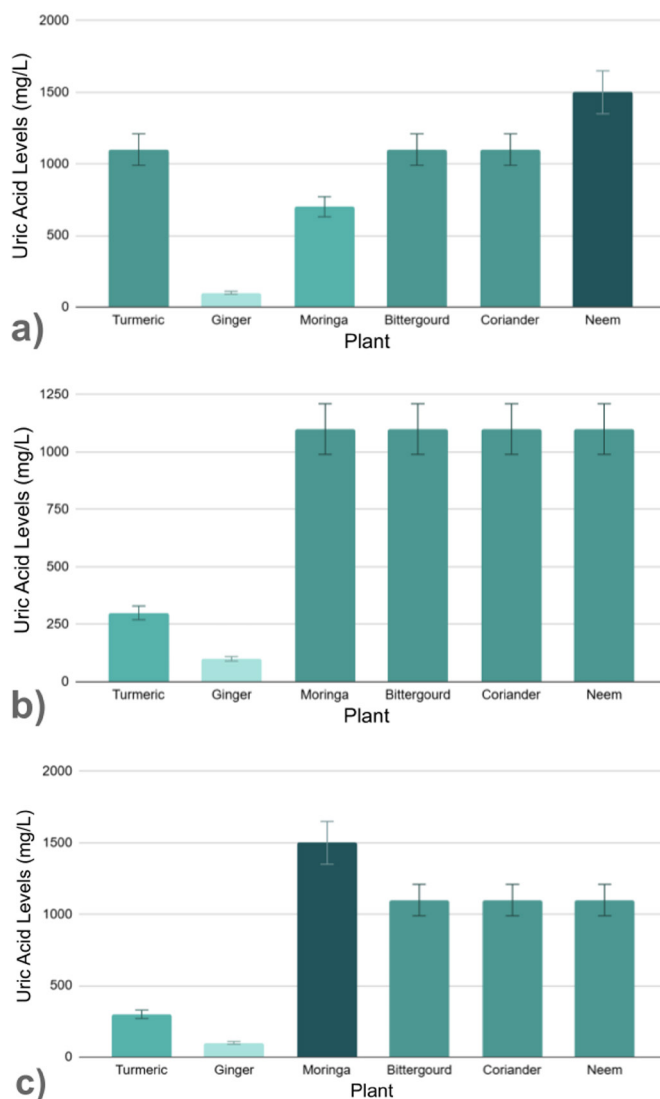


Figure 2: Modes of the effects of various ayurvedic plant extracts on uric acid levels at concentrations of 100 mg, 600 mg, and 1000 mg. Bar graphs showing the most frequently reduced uric acid level for each plant at (A) 100 mg, (B) 600 mg, and (C) 1000 mg (n=3). Extracts were created with 5 mL water and varying amounts of plant powder, and each extract was combined with uric acid solution and read after two minutes to measure uric acid-reducing effects.

in the previous experiment. The ratio of 1 part turmeric to 4 parts ginger was the best at reducing the uric acid level. This combination reduced uric acid levels down to less than or equal to 100 mg/L. The ratio of 1 part turmeric to 2 parts ginger was an outlier compared to the rest of the data because it was less effective than the first ratio of 1:1 turmeric to ginger (Figure 3). We performed a Kruskal-Wallis Rank Sum Test to examine the relationship between plant type and uric acid levels. This test was chosen because the data collected was ordinal qualitative, meaning the values were hierarchical and not continuous (23). This test also compares the ranks instead of averages, which are not valid with this kind of data. This test determines if the modes of 2+ groups are the same and does so by ranking the data points in every group and comparing the sums and means of those ranks (24). The values of rank sum and mean rank for the data from

each plant extract were also calculated (H statistic = 39.6223, critical value ≈ 12.5916 with 6 degrees of freedom, p -value < 0.05). Based on these values, the p -value was less than 0.05, equaling about 5.40×10^{-7} (0.00000054), meaning that the results are statistically significant.

After further researching the results, a significant relationship between the performance of the plants and the levels of a substance called quercetin (that was present in all the plants at a level that approximately matched their effectiveness) was found. Quercetin is a type of polyphenol called a flavonoid, and a type of flavonoid called a flavanol (flavonoids with a ketone group). It is also a plant-specialized metabolite, a substance found in a plant that contributes to its survival that has also been used in natural medicine by humans due to its specific biological activities (25). Quercetin has been proven to reduce uric acid levels through many mechanisms, one of which includes reducing XO (25). Additionally, since one of the main causes of increased uric acid levels is renal urate underexcretion (less elimination of uric acid from the kidneys), studies have shown that quercetin could increase urate excretion through regulating renal transporters and promoting the activity of excretion transporters (26).

Per gram, ginger has the highest level of quercetin: ~ 40.25 mg (27). Turmeric, the second-most effective at reducing uric acid levels, has 0.6 mg of quercetin per gram (28). All of the other plants have similar averages, which mostly match up with how they performed: moringa has 0.207 mg/g (29), bitter gourd has 0.00017038 mg/g (30), coriander has 0.0241 mg/g (31), and neem has 0.06-0.48 mg/g (32).

These quercetin levels matched up with the results of the experiment, revealing a trend: the highest amount of quercetin resulted in the lowest uric acid level (Figure 4). To show the significance of this trend, we computed Pearson's correlation coefficient. The correlation coefficient was calculated to be -0.883765, showing that the quercetin levels and uric acid results for each plant were strongly negatively correlated. It also appeared that, at the lower amounts of quercetin, there was no significant trend regarding the uric acid levels, as the amounts might have been too trivial to have any effects.

DISCUSSION

Overall, in this experiment we found ginger to be the

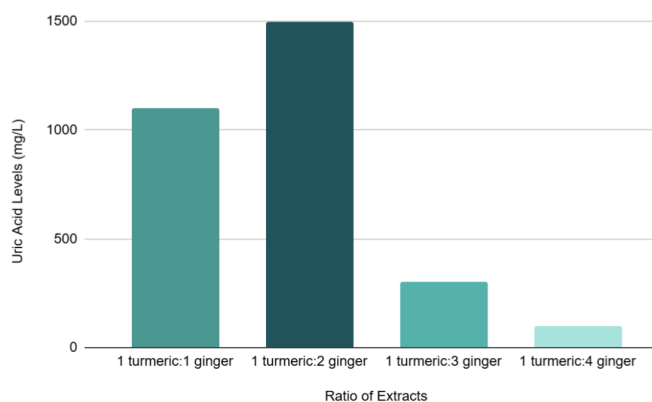


Figure 3: Comparative analysis of different ayurvedic plant ratios on uric acid levels. Bar graph showing the uric acid reduction of each ratio of turmeric to ginger (n=1). Powders were mixed for each ratio, then 5 mL of water and uric acid solution were added. Strips were read after two minutes to check uric acid levels.

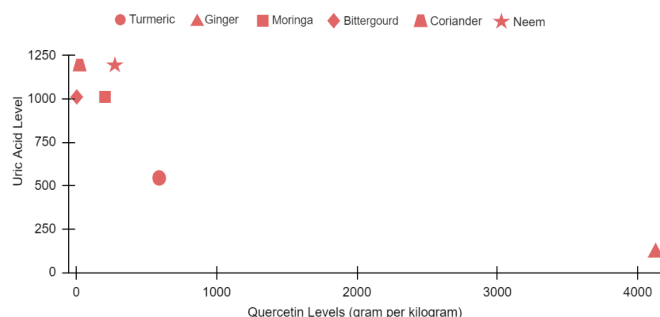


Figure 4: Relationship between the quercetin levels (g/kg) in each ayurvedic plant and their effectiveness in lowering uric acid levels. Graph depicting the relationship between quercetin levels and uric acid level of each plant. Quercetin levels were obtained through literature search and data about uric acid levels came from averaging the results of each plant found in experiment (27-32).

most successful ayurvedic plant in reducing uric acid levels. It reduced the uric acid to a healthy level of 100 mg/L most frequently, from a starting level of greater than or equal to 1500 mg/L (the excess uric acid level), which was a 93.3% reduction ($\pm 8\%$). Additionally, the second most effective plant was turmeric. Turmeric reduced the uric acid most frequently by 80% ($\pm 8\%$) overall. This shows that it also has some properties that make it effective against uric acid. Synergistically, both ginger and turmeric were also quite effective.

Based on this, we found our hypothesis, that moringa leaves would have the greatest potential to reduce uric acid levels, was not supported. Although there were more scientific discoveries regarding moringa's effectiveness against uric acid, there were no available studies comparing it with any of the other plants tested (19-21).

One of the mechanisms that likely caused ginger to be so effective was its more potent ability to target and reduce XO levels in the blood, which effectively reduces uric acid levels because XO is a catalyst in the reaction that turns purines into uric acid (33). Additionally, ginger has been shown to act as an antioxidant that neutralizes harmful free radicals and reduces oxidative stress, which also contributes to protecting against hyperuricemia (34). Ginger likely targets the root of the problem most effectively, which was the reason it turned out to perform the best.

Additionally, the correlation with the levels of quercetin and the performance of ginger revealed a significant trend. However, these values were not found in this study, they were obtained through a literature search. The relationship between the effectiveness of the plants in reducing uric acid and the amounts of quercetin in the extracts is speculative and was not directly tested. However, research has shown that quercetin's properties of reducing XO and regulating renal transporters make it effective against hyperuricemia (25, 26).

Overall, the data answered the question but did not support the hypothesis. After thorough statistical analysis, it was concluded that ginger was the highest-performing plant in reducing uric acid levels. The standard deviation was found to be only about 200 mg/L (one level on the test strip), which is relatively low in terms of the scale used to measure the uric acid levels (scale of 200 mg/L). Additionally,

the statistical significance of results was determined through the Kruskal-Wallis Rank Sum test, which showed that the groups were statistically very different. A post hoc Dunn's test with Bonferroni correction revealed that the ginger group differed significantly from all other treatment groups ($p < 0.0024$), while no other pairing comparisons were statistically significant. This indicates that ginger had the most significant effect compared to the other plant extracts.

A limitation that may have influenced the results of this project was the number of replicates that were able to be produced, due to cost and availability of materials. The dried plants only created a certain amount of powder, and the amount of uric acid powder was limited, which determined the number of trials that were conducted. Additionally, advanced lab equipment to test the uric acid levels more accurately was inaccessible. This may have affected the accuracy of the data, since the strips could only test up to 1500 mg/L. Future testing of larger sample sizes could improve consistency in the results and improve scientific understanding of this correlation. Additionally, we were unable to directly measure quercetin levels, instead inferring a relationship between quercetin levels and uric acid reduction based on previously published data obtained through a literature search (27-32).

Further research of the effectiveness of these plants in other organisms and humans can lead to an understanding of how these plants can be used as targeted treatments. More work in this field will greatly benefit the millions of people around the world suffering with asymptomatic hyperuricemia, which can progress into other diseases if not treated in a timely manner. This study found a correlation between uric acid reduction and multiple ayurvedic plants, which can possibly be applied as a preventative solution. Ayurvedic plants, which are extremely accessible and cost effective, can be used to target hyperuricemia early on, especially in places where traditional medications are inaccessible. Combining this plant into diets or using it as a treatment when needed may have the potential to prevent the worsening of hyperuricemia in patients across the world.

MATERIALS AND METHODS

Preparation of Powders

Various amounts of all six ayurvedic plants were obtained fresh from a local market and were washed thoroughly. Turmeric (*Curcuma longa*), ginger (*Zingiber officinale*), and bitter gourd (*Momordica charantia* L.) were cut into thin pieces, and the leaves were removed from the moringa (*Moringa oleifera*), neem (*Azadirachta indica*), and coriander (*Coriandrum sativum*). All slices/leaves were spread evenly on trays lined with foil. The trays were then covered with plastic wrap to prevent contamination and were placed in direct sunlight until all plants dried fully. The dried plants were placed in a blender (one plant at a time, sterilizing all supplies in between) and blended until they each became powders. Each plant powder was then sifted four times to ensure it was as fine as possible. All powders were placed inside sterile, labeled plastic cups with lids for safe storage. This method of deriving dry matter from plants is the most basic step in most plant extraction procedures (35).

Uric Acid Reduction Experiment

Uric acid solution was prepared by mixing 3,000 mg of uric acid powder (Sigma Aldrich, U2625-25G) with 200 mL of

water. This concentration would read as a high uric acid level (greater than or equal to 1500 mg/L) on the test strip, meaning that any observed decrease would be attributed to the effect of the plant extracts. Appropriate amounts of plant powder (100 mg, 600 mg, 1000 mg) were added to the vials for each plant and trial, followed by the addition of 5 mL of water using a syringe to ensure proper dilutions. Then, 3 mL of the prepared uric acid solution was added to each vial. Vials were shaken for 5 seconds to let the contents mix, and a uric acid test strip (Amazon, ASIN B01NAV5BL7) was immediately dipped into each vial for 2 seconds. Strips were then placed on labeled paper towels and left for 2 minutes to develop color as per the instructions provided with the test strips. After 2 minutes, the color on each strip was compared to the given chart to determine the uric acid level: <100 mg/L, 300 mg/L, 700 mg/L, 1100 mg/L, or >1500 mg/L. This procedure was repeated for each plant extract tested.

Ratio Experiment

Following analysis of the initial results, the two highest-performing plant extracts were selected based on their ability to reduce uric acid levels: turmeric and ginger. Using the same testing procedure, combinations of these top two extracts were prepared in ratios of turmeric to ginger of 1:1, 1:2, 1:3, and 1:4, with the base unit being 100 mg. Each ratio was tested by combining the appropriate amounts of the two powders in labeled vials, followed by the addition of 5 mL of water and 3 mL of the prepared uric acid solution using syringes. Vials were shaken for 5 seconds, and uric acid test strips were dipped for 2 seconds, then placed on labeled paper towels for 2 minutes of color development. After 2 minutes, test strips were read and recorded.

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