doi.org/10.36721/PJPS.2023.36.1.SP.277-280.1

Investigation of medicinal properties and chemical and biochemical characterization of apple cider vinegar for anti-hyperuricemia in female human subjects in controlled randomized trial

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Abstract: Serum uric acid levels are an important parameter in health care. If uric acid levels are not in the normal range, uric acid becomes crystallized and leads to the development of joint pains, gout, and renal health issues. There are natural ways to reduce elevated uric acid levels. In this regard, apple cider vinegar can inhibit uric acid accumulation. In this study, 60 females with elevated uric acid levels were enrolled and were divided into three groups; control group (G_o) and treatment groups G_1 and G_2. Treatment groups were given different doses of apple cider vinegar ACV. Serum uric acid levels were measured before and after the trial. Chemical characterization and phytochemical analysis were also performed. ACV has 89.47% moisture, 2.27% ash and 4.56% NFE in addition to 0.64% crude protein, 0.29% crude fat, and 3.05% crude fiber. Moreover, total phenolic content (TPC is 42.87±0.02ug GAE/ml and total flavonoid content (TFC) is 34.87±0.11ug CE/ml in ACV. Two sample t-test showed a significant reduction in uric acid levels when ACV was given to subjects.

Keywords: Uricemia, apple cider vinegar, uric acid levels, medicinal properties, chemical characterization, mineral analysis, phytochemical, total soluble solids, proximate values.

INTRODUCTION

Uricemia has elevated serum urate or uric acid levels and it is associated with other metabolic syndromes and arterial hypertension (López-Jiménez *et al*., 2012). Uric acid produces a product of purines and has no role in the body's physiological functions. Scientific studies and recent studies have explained the physiological contribution of urate ion as a part of enzymes that play role in preventing the inactivation of endothelial enzymes thus preventing endothelium from oxidative stress (Ousaaid *et al*., 2022). It also has a role in preventing oxidative stress-induced complications. Uric acid within normal ranges plays an important role in the body. However, when it exceeds safe limit, it begins to accumulate in the body in crystallized form and leads to the development of gout, joint pains, permanent bone damage, heart diseases, and renal diseases (Tripathi & Mazumder, 2022). Studies have also reported an association between elevated uric acid levels and the development of hypertension, high blood pressure, and impaired glucose metabolism (Rock *et al*., 2013). All these factors; make continuous monitoring of uric acid levels important and necessary (Lin *et al*., 2000). Elevation in uric acid level is observed due to over functionality of the xanthine oxidase enzyme. Elevated uric acid gets crystallized and starts accumulating in the knee and ankle joints, which leads to swelling and pain (Dawson & Walters, 2006). Different medicines and natural products are used to reduce elevated uric acid levels. People around the globe are paying attention to the use of plants and natural products due to less cost and side effects (Bnouham *et al*., 2002). Apple cider vinegar (ACV) is among the most popular fermented products that have tremendous health benefits. ACV contains phytochemicals including flavan-3-ol, phenolic acid and dihydrochalcones. The presence of all these compounds plays a role in inhibiting the over-activity of the xanthine oxidase. These phytochemicals also have a role in reducing oxidative stress by preventing the escape of the hydroxyl group from phenols. ACV can create hydrophobic pockets of the enzyme xanthine oxidase. These polyphenols also bind with the catalytic site of xanthine oxidase and prevent its activation. In this way xanthine oxidase is unable to perform its activity and xanthine is not converted into uric acid. This mechanism is also helpful in reducing the inflammation of joints in hyperuricemia (Ramesh, 2021). This study is aimed at

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determining the chemical composition, phytochemical analysis, mineral analysis, and therapeutic properties of ACV against hyperuricemia.

MATERIALS AND METHODS

Collection of materials
Bragg ACV was purchased from the local store with net weight packaging of 473 ml in bulk.

Investigation # 1
Characterization of apple cider vinegar
ACV was subjected to various assays described as follows:

***Proximate profile of apple* cider vinegar**
ACV was analyzed for the following proximate profile including moisture content, ash, carbohydrates, crude protein, crude fiber and nitrogen-free extract (NFE) according to the AOAC method (Garcia-Amezquita *et al*., 2018).

***Phytochemical analysis of apple* cider vinegar**
Phytochemical analysis of ACV was investigated for the determination of total phenolic content (TPC) and total flavonoid content (TFC). The amount of TPC and TFC was measured in ug of gallic acid equivalent per ml of sample and ug of catechin equivalent per ml of sample respectively (M'hiri *et al*., 2015).

Determination of minerals
For the determination of minerals, a 20ml sample of ACV was taken in a 100ml volumetric flask. In this flask, 10 ml of hydrochloric acid (HCL) was added and 100ml volume was prepared by using distilled water. To remove the impurities, the sample was filtered. The mineral standard was prepared with HCL and lanthanum concentrations. The sample was analyzed using atomic absorption spectrophotometer (Hernández *et al*., 2005).

Total soluble solids
Total soluble solids (Brix) were measured through a digital refractometer (Bielmann *et al*., 2010).

Investigation # 2
Investigation of therapeutic potential of apple cider vinegar *in hyperuricemia patients*
Selection of Subjects 60 female subjects with elevated serum uric acid levels were randomly selected from the social security hospital. The age of all subjects was between 30-60 years.

Exclusion criteria
Patients with any other chronic health condition and patients of obese solves category were selected. Male patients and patients with obesity i.e., BMI> 30 were not selected. Pregnant and lactating women were not included in the study. Subjects taking medicines to reduce uric acid levels were not included in the study.

Inclusion criteria
All female subjects who went blood biochemical analysis by checking their serum uric acid at the start of the study included.

Study duration
The study was conducted for eight weeks.

Treatment groups and treatment plan
60 female subjects were divided into three groups of 20 subjects each, with the following conditions. The control group was given no treatment. Treatment group-I and treatment group II were given the following doses (table 1).

Table 1: Treatment groups and treatment plan

Groups	Title	Treatment
G_0	Control group	No treatment
G_1	Treated group 1	15 ml ACV was given in 100 ml water two hours after breakfast to the participants.
G_2	Treated group 2	20 ml ACV was given in 100 ml water two hours after breakfast to the participants.

Collection of blood samples
At the beginning and end of the trial, blood samples of participants from the antemedial cubital vein were drawn in sample collecting tubes.

STATISTICAL ANALYSIS

Descriptive statistical analysis using the Two sample t-test was performed under a Completely Randomized Design (CRD) was carried out to investigate the level of significance ($p<0.05$). All statistical analyses are done with IBM SPSS Statistics 20 version (Larson, 2008).

Ethical approval
Ethical approval has been taken from the Biosafety Committee of the institute.

RESULTS

***Proximate analysis of apple* cider vinegar**
The quality of raw material used is significantly influenced by the measurement of resolved proximate values. To evaluate the quality characteristics of ACV, proximate determination including moisture, ash, crude fat, crude protein, fiber and the nitrogen-free extract was evaluated. The presence of 89.47±1.24% moisture in ACV makes the liquid state of vinegar. The total solid content was measured by subtracting the moisture from

100 which comes out to be 10.81±0.57%. 2.27±0.13% ash is present in ACV while 4.56±0.09% of carbohydrates are present in ACV. Moreover, 0.64±0.08, 0.29±0.57 and 3.05±0.02 of crude protein, crude fat, and crude fiber are present in ACV. Values of proximate analysis for ACV are shown in table 2.

Table 2: Mean±S.D for proximate values of ACV.

Proximate analysis	Composition (%)
Moisture	89.47±1.24
Total solid content	10.81±0.57
Ash	2.27±0.13
Nitrogen Free Extract	4.56±0.09
Crude protein	0.64±0.08
Crude Fat	0.29±0.57
Crude fiber	3.05±0.02

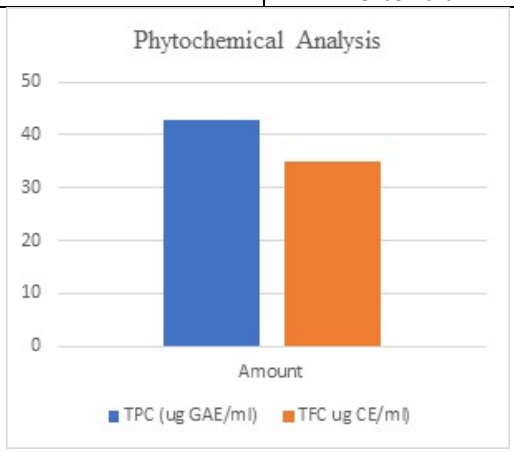

Fig. 1: Phytochemical analysis of ACV

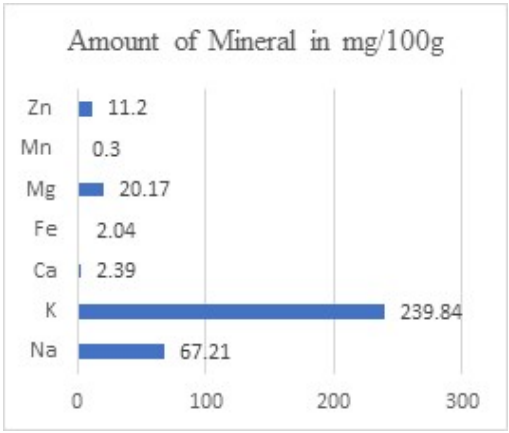

Fig. 2: Mineral Analysis of ACV

Table 3: Mean±S.D for serum uric acid level of male Subjects in mg/dl.

Duration	G_0	G_1	G_2	*p*-value
0 week	6.23±0.87	6.78±1.01	6.57±1.24	p<0.05
8thweek	6.88±1.84	6.18±11	5.18±2.2	p<0.05

G_0 = No treatment, G_1= 15ml apple cider vinegar in 100 ml water G_2= 20 ml apple cider vinegar in 100ml water.

***Phytochemical characters of apple* cider vinegar**

ACV carries a significant amount of TPC and TFC as depicted in fig. 1. Total phenolic content (TPC) was 42.87±0.02ug GAE/ml and total flavonoid content (TFC) was 34.87±0.11ug CE/ml.

Mineral analysis

Mineral analysis was performed *via* atomic absorption spectrophotometer for Ca, Zn, Fe, Mg, Mn, K, and Na. Mean±S.D for sodium (67.21±9.32), potassium (239.84±15.74), zinc (11.20±1.69), magnesium (20.17±1.52), calcium (2.39±0.09), manganese (0.30±0.05) and iron (2.04±0.87) were determined.

Total soluble solids

Total soluble solids were measured with a refractometer and its Mean±S.D. is 3.58±0.017.

Investigation of therapeutic potential of apple* cider vinegar *for uricemia in female subjects

The objective of this study was to investigate the medicinal effect of ACV on uricemia. Participants of the control group (G_0) were given no treatment while participants of treatment groups G_1 and G_2 were given an oral dose of ACV in water in doses of 15 ml and 20 ml in 100 ml water, two hours after meal once a day respectively for 8 weeks. Before the commencement and after the standstill of the trial, blood samples were gathered from each subject for analysis of uric acid level.

Changes in uric acid level of female subjects

Results of serum uric acid levels at 0 week and in the 8th week are shown in table 4.

The investigation has shown a significant ($p<0.05$) reduction in uric levels in response to uric acid powder capsules in both treatment groups. Treatment group G_1 showed a reduction in the serum uric acid level from 6.78±1.01mg/dl to 6.18±1.71mg/dl while treatment group G_2 showed a reduction in serum uric acid level from 6.57±1.24mg/dl to 5.18±2.12mg/dl. Treatment group G_2 with a 20 ml ACV dose showed a better reduction in uric acid levels than treatment group G_1. However, the control group showed a slight increase in uric acid level levels.

DISCUSSION

ACV contains a negligible amount of crude fat and crude protein. Moisture is a major part of the composition of fiber and nitrogen-free extracts are present in ACV comparatively in more amounts than fat and protein. Proximate analysis helps researchers in chemical characterization and quality determination of raw materials. The proximate values of this study, for ACV, are consistent with the proximate values of previous studies in literature. Minor differences are present in values which can be due to differences in environmental conditions, the efficacy of chemicals used, or due to

location (Judith *et al*., 2021). Mineral analysis showed the presence of minerals in the mentioned amounts in fig. 2. These findings were consistent with the values of previously existing literature. Sodium and potassium are majorly found minerals in ACV. Potassium and sodium play an important role in the body including nerve impulse conduction and the value for Brix for ACV was also consistent with the literature. Phytochemical analysis showed the presence of phenolic and flavonoid content. These phytochemicals are useful in reducing oxidative stress in the body and reducing uric acid levels (Techakanon & Sirimuangmoon, 2020). An oral dose of 15ml and 20ml in 100ml water showed a reduction in elevated uric acid levels. An oral dose of 20ml in 100ml water once a day showed better results of reduction in uric acid levels. These findings were consistent with a previous study that tested a different level of ACV for elevated uric acid levels (Singh *et al*., 2019).

CONCLUSION

ACV is a fermented product, and it has many health benefits. It is widely used for uricemia, diabetes, weight loss, and hyperlipidemia as well. ACV contains a significant amount of minerals Na, K, Zn and Mg, which have important physiological roles in the body. ACV is useful in lowering uric acid levels by preventing the conversion of xanthine into uric acid. In this way, it is also useful in uric acid-linked complications like gout, joint pain, and swellings and it also possesses anti-inflammatory properties. All these therapeutic properties make ACV a useful product with tremendous medicinal properties.

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