



Comparative Analysis of Anti Gout Activities of Ethanolic Extract of *Alternanthera sessilis* and *Moringa oleifera*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i60B34975

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/82131>

Original Research Article

Received 20 October 2021

Accepted 25 December 2021

Published 27 December 2021

ABSTRACT

Introduction: Ethanolic extracts of *Alternanthera sessilis* and *Moringa oleifera* were screened for the presence of different classes of phytochemicals. The phytochemical screening revealed the presence of amino acids, flavonoids, alkaloids, terpenoids, saponins and steroids. Gout is the type of arthritis that causes painful inflammation and develops due to overproduction of urate.

Aim: To analyse the anti gout activities of ethanolic extract of *Alternanthera sessilis* and *moringa oleifera*.

Materials and Methods: Ethanolic extract of *Alternanthera sessilis* and *Moringa oleifera* were prepared as per the standard methods and used for the assessment of preliminary phytochemical screening, antioxidants and anti gout activities. The data were analyzed statistically by a one-way analysis of (ANOVA) followed by Duncan's multiple range test was used to see the statistical significance among the group. The results with the $p < 0.05$ level were considered to be statistically significant.

Results: Antioxidant and Anti gout potential of the ethanolic extract of *Moringa oleifera* was found to be significantly more than the ethanolic extract of *Alternanthera sessilis*.

Conclusion: Both the ethanolic extracts exhibited anti gout activity, further studies are needed to validate the herbal extracts as a drug formulation.

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Keywords: Extract; phytochemicals; antioxidants; xanthine oxidase; innovative technology; novel method.

1. INTRODUCTION

Plants possess a wide variety of secondary metabolites. These plants are rich in antioxidant activity and other therapeutic values [1]. Medicinal plants are used for the treatment of various ailments [2]. For drug synthesis it serves as a starting material. *Moringa oleifera* belongs to the Moringaceae family. It is a native to Indian subcontinent [2,3]. The plant is widely grown in tropical and subtropical countries. *Moringa oleifera* is a highly valued plant for its nutrition and medication. Moringa trees are commonly known as drumsticks. It is a traditional medicinal source. The parts of the moringa trees like pods, seeds and leaves are edible. Moringa seeds are used in spice, cosmetic oils, food etc [4]. It is used as livestock feed and has several medicinal uses. The digestive ability of the plant is affected by the chemical composition of the fibre [5].

The nutritional value of plants depends on the environment, cultivation method, genetic background [4,6]. It shows antipyretic, analgesic and wound healing activities. The leaves contain 14 amino acids and leaves are the natural boosters, vitamin A,C,E, proteins, magnesium, calcium and carbohydrates [7]. *Moringa Oleifera* can serve as a wide variety of biological activity and antioxidants for humans and animals [8].

Gout is a type of arthritis which causes painful inflammation. Joint pain is caused due to elevation of uric acid in the blood which triggers the formation of crystals. Xanthine oxidase catalyses the oxidative hydroxylation of hypoxanthine to xanthine to uric acid [9]. The further catalysis is done by enzyme uricase which converts uric acid to highly soluble allantoin which is secreted in urine. Symptoms of gout can be intense joint pains, lingering discomfort, inflammation, and redness [10]. Development of gout has several risk factors including hyperuricemia, age, dietary factors, metabolic syndrome, diuretic, chronic renal disease [11]. *Alternanthera sessilis* belongs to amaranthaceae family. It is a perennial herb and an aquatic plant. It is found in sterms, wetlands, marshy areas, reservoirs, and river banks. It is also used for gastrointestinal problems and the branches grow from the root upto 50cms long [8,12]. It is used for the treatment of skin disease, fever, cuts, gonorrhoea, diarrhoea, burning sensation, liver & spleen disease [8]. Aerial part

of the plant is the source of vitamins, minerals and antioxidants. It contains bio active phytoconstituents. It is rich in nutrition, and contains iron and vitamin A, serves as food and is also used in medicine [13]. There is not much research done on antioxidant and anti gout properties of the plant. Our team has extensive knowledge and research experience that has translate into high quality publications [14-33]. The main aim of the study is to analyse anti gout activities of ethanolic extract of *Alternanthera sessilis* and *Moringa oleifera*.

2. MATERIALS AND METHODS

2.1 Phytochemical Screening Test

2.1.1 Test for phlobatannin

1 ml of the extract was treated with 1ml of 1% HCl and boiled for 10 mins. The formation of red color precipitate indicates the presence of phlobatannin.

2.1.2 Test for carbohydrates

Three to five drops of Molisch reagent was added with 1 mL of the extract and then 1 mL of concentrated sulphuric acid was added carefully through the side of the test tube. The mixture was then allowed to stand for two minutes and diluted with 5 mL of distilled water. The development of a red or dull violet ring at the junction of the liquids showed the presence of carbohydrates.

2.1.3 Test for flavonoids

Few drops of 1% liquid ammonia were taken in a test tube and along with it 1 ml of the extract was added resulting in the formation of yellow color thereby indicating the presence of flavonoids.

2.1.4 Test for alkaloids

2 ml of sample was mixed with 2 ml of HCl. Then 6 drops of HCN was added and further 2 drops of picric acid was added that resulted in a creamish pale yellow ppt indicating the presence of alkaloids.

2.1.5 Test for terpenoids

2 ml of sample along with 2 ml of chloroform and 3 ml of con. H₂SO₄ was added. Red color ppt obtained indicates the presence of terpenoids.

2.1.6 Test for proteins

One milliliter of ninhydrin was dissolved in 1 mL of acetone and then a small amount of extract was added with ninhydrin. The formation of purple colour revealed the presence of protein.

2.1.7 Detection of saponins

Foam test: A fraction of the extract was vigorously shaken with water and observed for persistent foam.

2.1.8 Test for steroids

One milliliter of chloroform was mixed with 1 mL of extract and then ten drops of acetic anhydride and five drops of concentrated sulphuric acid were added and mixed. The formation of dark red colour or dark pink colour indicates the presence of steroids.

2.2 DPPH Free Radical Scavenging Activity of *Alternanthera sessilis* and *Moringa oleifera*

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical was assessed by the method of Hatano et al, (1989). The DPPH solution (1.0 ml) was added to 1.0 ml of extract at different concentrations (0.1 to 0.5 mg/ml). The mixture was kept at room temperature for 50 minutes and the activity was measured at 517 nm. Ascorbic acid at the same concentrations was used as standard. The capability to scavenge the DPPH radical was calculated and expressed in percentage (%) using following formula:

$$\text{DPPH radical scavenged (\%)} = (\text{Control OD} - \text{Sample OD} \times 100) / \text{Control OD}$$

2.3 In-vitro Xanthine Oxidase Inhibitory Activity of *Alternanthera sessilis* and *Moringa oleifera*

In vitro Xanthine oxidase inhibitory of the extract was assessed as per the method of (Nguyen et al, 2004; Umamaheswari et al., 2007). Briefly, the assay mixture consisted of 1 ml of the fraction (0.1 to 0.5 g/ml), 2.9 ml of phosphate buffer (pH 7.5) and 0.1 ml of xanthine oxidase enzyme solution (0.1 units/ml in phosphate buffer, pH 7.5), which was prepared immediately before use. After preincubation at 25°C for 15 min, the reaction was initiated by the addition of 2 ml of the substrate solution (150 M xanthine in the same buffer). The assay mixture was

incubated at 25°C for 30 min. The reaction was then stopped by the addition of 1 ml of 1N hydrochloric acid and the absorbance was measured at 290 nm using a UV spectrophotometer. Allopurinol (0.1 to 0.5mg/ml), a known inhibitor of XO, was used as the positive control. One unit of XO is defined as the amount of enzyme required to produce 1 mmol of uric acid/min at 25°C. XO activity was expressed as the percentage inhibition of XO in the above assay system calculated as percentage of inhibition as follows.

$$\text{Inhibitory activity (\%)} = (1 - \text{As}/\text{Ac}) \times 100$$

Where,

As – absorbance in presence of test substance,

Ac – absorbance of control

2.4 Statistical Analysis

The data were subjected to statistical analysis using one – way analysis of variance (ANOVA) and Duncan's multiple range test to assess the significance of individual variations between the groups. In Duncan's test, significance was considered at the level of $p < 0.05$.

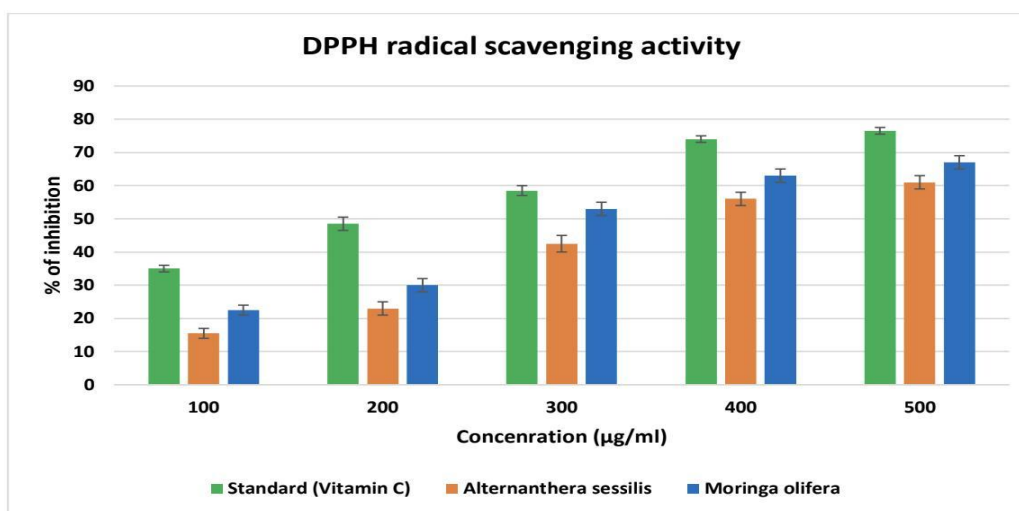
3. RESULTS AND DISCUSSION

The phytochemical screening of the ethanolic extract of *Alternanthera sessilis* and *Moringa oleifera* showed the presence of different phytoconstituents such as Amino acid, Flavonoids, Alkaloids, Terpenoids, Saponins, steroids and proteins. In *Alternanthera sessilis* amino acids, flavonoids, alkaloids, terpenoids are richly present whereas saponins and steroids are fairly present. In *Moringa oleifera* proteins, amino acids, flavonoids, saponins, steroids are fairly present and alkaloids, terpenoids are richly present.

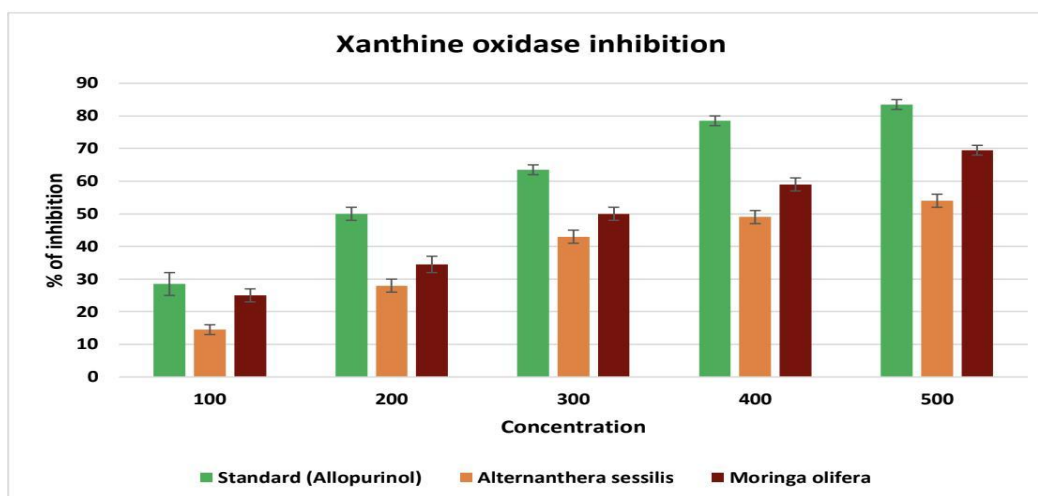
The percentage of radical scavenging activity of plant extract was evaluated at different concentrations. The IC_{50} value of *Alternanthera sessilis* was found to be 380 µg/ml and the IC_{50} value of *Moringa oleifera* was estimated as 290 µg/ml, which is significantly higher than *Alternanthera sessilis*. xanthine oxidase inhibitory potential of *Alternanthera sessilis* and *Moringa Oleifera* was analysed. *Moringa oleifera* showed higher xanthine oxidase inhibitory activity ($IC_{50} = 300$ µg/ml) than *Alternanthera sessilis*.

Chart 1. The table depicts the phytoconstituents of *Alternanthera sessilis* and *Moringa oleifera*

Phytoconstituents	<i>Alternanthera sessilis</i>	<i>Moringa oleifera</i>
Protein	Absent	+
Amino acid	++	+
Flavonoids	++	+
Terpenoids	++	++
Sapponents	+	+
Steroids	+	+
Alkaloids	++	++



Graph 1. The bar graph depicts the *in vitro* antioxidant activity of *Alternanthera sessilis* and *Moringa oleifera*. The X axis represents the concentration and the Y axis represents the percentage of inhibition. Blue represents the activity of *Moringa oleifera* and orange represents the activity of *Alternanthera sessilis*. Each line represents mean \pm SEM of 3 independent observations. Significance at $p \leq 0.05$



Graph 2. The bar graph depicts the *in vitro* xanthine oxidase inhibitory activity of *Alternanthera sessilis* and *Moringa oleifera*. The X axis represents the concentration and the Y axis represents the percentage of inhibition. Blue represents the activity of *Moringa oleifera* and orange represents the activity of *Alternanthera sessilis*. Each line represents mean \pm SEM of 3 independent observations. Significance at $p \leq 0.05$

Medicinal plants contain many bioactive compounds and antioxidant potential. Herbal extracts can be used as alternative medicines for the treatment of gout. Xanthine oxidase inhibitory assay is considered as standard to study the anti gout potential of medicinal plants. Some plants and their phytochemicals can act as xanthine oxidase inhibitors [34,35]. The phytochemical screening of *Alternanthera sessilis* and *Moringa oleifera* also revealed the presence of various phytonutrients.

The previous study revealed the presence of strong antioxidant potential in both *Moringa oleifera* and *Alternanthera sessilis* extract [34]. Xanthine oxidase inhibitory activity was observed for the extracts and compared with the standard drug allopurinol. Though the extracts exhibited a lesser anti gout potential, side effects exhibited by the synthetic drugs needs to be considered. The most prevalent side effects of the synthetic drugs are skin allergies, fever, rashes etc. Thus, further research is required to utilize these herbal extracts into drug formulations.

4. CONCLUSION

Phytochemical screening of ethanolic extract of *Alternanthera sessilis* and *Moringa oleifera* showed the presence of phytoconstituents. Both the plant extracts exhibited antioxidant activity. Xanthine oxidase inhibitory activity was observed for the extracts and the standard drug allopurinol. It is concluded that the extracts of *Alternanthera sessilis* and *Moringa oleifera* showed antigout activities and thus it can be used as a natural phytochemical in the treatment of gout.

SOURCE OF FUNDING

The present study was supported by the following agencies.

- Saveetha Institute of Medical and Technical Sciences (SIMATS)
- Saveetha Dental College
- Saveetha University
- Dhanesh bankers, Adambakkam

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

The authors would like to thank Saveetha Dental College and Hospitals, Saveetha Institute of medical and technical Sciences, Saveetha University for providing research laboratory facilities to carry out the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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