

**PRELIMINARY PHYTOCHEMICAL ANALYSIS AND ANTIGOUT ACTIVITY OF  
AQUEOUS EXTRACTS OF SELECTED MEDICINAL PLANTS .  
( NEEM AND TULSI )**

**Running title : Anti gout activity of aqueous extracts of selected medicinal plants (Neem and  
Tulsi)**

**Type of study : original research**

**Srivarsha Ranjeet**

Saveetha Dental College and Hospitals

Saveetha Institute of Medical and Technical Sciences, Chennai

E-mail: [152001087.sdc@saveetha.com](mailto:152001087.sdc@saveetha.com)

**V. Vishnu Priya**

Department of Biochemistry, Saveetha Dental college and Hospitals

Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai.

E-mail: [vishnupriya@saveetha.com](mailto:vishnupriya@saveetha.com)

**Gayathri.R**

Department of Biochemistry, Saveetha Dental college and Hospitals

Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai.

Email: [gayathri.sdc@saveetha.com](mailto:gayathri.sdc@saveetha.com)

**Kavitha.S**

Department of Biochemistry, Saveetha Dental college and Hospitals

Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai.

E-mail: [kavithas.sdc@saveetha.com](mailto:kavithas.sdc@saveetha.com)

**Corresponding author**

**V. Vishnu Priya**

Professor

Department of Biochemistry, Saveetha Dental college and Hospitals

Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 600077

Tamilnadu, India

Email: [vishnupriya@saveetha.com](mailto:vishnupriya@saveetha.com)

**Abstract :**

**Introduction:** GOUT is a form of arthritis characterised by severe pain, redness, tenderness in joints. In gout, elevation of uric acid in the blood triggers the formation of crystals, causing joint pain. India is a country that is rich in medicinal plants. Therefore its flora might offer promising therapies for gout. Neem and Tulsi exhibit xanthine oxidase inhibitory activity. Neem and Tulsi have natural bioactive compounds which include flavonoids, proteins, amino acids, terpenoids, alkaloids, carbohydrates, saponins, steroids.

**Aim :** Aim of this study is to estimate the anti gout activity of aqueous extract of selected medicinal plants with standard drug (allopurinol).

**Materials and methods :** Phytochemical screening and anti gout activity of Neem and Tulsi were analysed and comparison with standard drug (allopurinol) is also done .

**Results :** NEEM and TULSI extract shows an increase in xanthine oxidase inhibition in tested concentration. Phytochemical analysis showed that neem contains proteins, amino acids, flavonoids, alkaloids, carbohydrates, saponins and tulsi contains terpenoids, flavonoids , alkaloids, carbohydrates, steroids.

**Conclusion:** From this study it is concluded that medicinal plants like Neem and Tulsi exhibit anti gout activity. The standard drug allopurinol showed greater percentage of inhibition of xanthine oxidase enzyme than that of tulsi , neem extracts in all given tested concentrations.

**Keywords:** Preliminary phytochemical analysis , Antigout activity , Innovative technique, Aqueous extract, Neem, Tulsi, novel method

**Introduction :**

**Gout** incidence has increased over the past 50 years, especially in developing countries (1). Gout is a type of inflammatory arthritis triggered by interactions of monosodium urate crystals and tissue(2).xanthine oxidase catalyses the oxidative hydroxylation of hypoxanthine to xanthine to uric acid, leading to painful inflammation (3).Gout has also been reported to cause joint deformities and kidney stones(4)).

Hyperuricemia , major etiological factor of gout, develops either due to overproduction caused by a metabolic disorder or due to under excretion of blood uric acid due to abnormal renal urate transport activity(5) ).Several risk factors for the development of gout have been established including hyperuricemia, age, genetic factors, dietary factors, alcohol consumption, metabolic syndrome, hypertension, obesity, diuretic use, cholesterol level, chronic renal diseases.(6)

Several drugs have been approved for the treatment of gout , including colchicine, steroids, non-steroidal anti - inflammatory drugs, inhibitors, allopurinol. Although these agents are effective , they also cause side effects such as skin allergies, fever, rashes, renal dysfunction, aseptic meningitis and hepatic dysfunction(7). Recently treating disease using medicinal plants is gaining new interest and research on medicinal plants has increased worldwide due to fewer side effects and lower cost(8). India is a country that is rich in medicinal plants. The 2 important medicinal plants in India are Neem and Tulsi which is used in the treatment of gout.(9)

Tulsi is a traditional plant which is widely used as a herbal tea, commonly used in ayurveda . Tulsi promotes a healthy heart, anti aging, treats kidney stones, relieves headache, fights acne, relieves fever, eye health and oral health.(10) It has anti-inflammatory properties. It lowers rheumatoid arthritis. Chewing of tulsi cures cold , cough , sore throat.(11)

Neem is an antifungal, anti diabetic, anti bacterial, contraceptive sedative. Neem products are used selectively in controlling pests in plants . Neem is considered a part of ayurvedic medicine. All parts of neem are used for preparing many different medicines especially for skin disease((12). Neem leaves are used in the treatment of leprosy, eye disorders, bloody nose, skin ulcers, cardiovascular disease, diabetes , liver problems. The leaf is also used for birth control and to cause abortions. Our team has extensive knowledge and research experience that has translate into high quality publications (13),(14),(15),(16),(17),(18),(19),(20),(21),(22),(23), (24),(25),(26),(27),(28),(29),(30),(31),(32)

The aim of the study is to estimate the anti gout activity of aqueous extract of selected medicinal plants ( neem, tulsi ) with standard drug (allopurinol).

**Materials and methods:**

**Phytochemical screening test :**

**Test for phlobatannin:**

1 ml of extract was treated with 1ml of 1% of HCL and boiled for 10min the formation of red colour precipitate indicates the presence of phlobatannin

**Test for carbohydrates:**

3 to 5 drops of molisch reagent was added with 1 ml of the extract concentrated. Sulphuric acid was added carefully through the side of the test tube. The mixture was allowed to stand for 2 minutes and diluted with 5ml of distilled water. The development of red or dull violet ring at the junction of the liquid showed the presence of carbohydrates

**Test for flavonoids:**

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

**Test for alkaloids:**

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

**Test for terpenoids:**

2ml of sample along with 2ml of chloroform and 3ml of conc H<sub>2</sub>SO<sub>4</sub> was added . The red colour ppt obtained indicates the presence of terpenoids.

**Test for proteins:**

1mm of ninhydrin was dissolved in 1ml of acetone and the small amount of extract was added with anhydrin. The formation of purple colour revealed the presence of proteins.

**Direction of saponins:**

FOAM TEST - a fraction of the extract was vigorously shaken with water and observed for persistent foam.

**Test for steroids :**

1 ml of chloroform was mixed with 1ml of extract and then 10 drops of acetic anhydride and 5 drops of concentrated sulphuric acid were mixed. The formation of dark red colour or dark pink colour indicates the presence of steroids.

**Invitro xanthine oxidase inhibitory activity of Neem and Tulsi extract :**

In vitro xanthine oxidase inhibitory activity of the extract was assessed as per the method of (14, 15). Briefly the assay mixture consisted of 1ml of the fraction (0.1 to 0.5g/ml), 2.9ml/g phosphate buffer (pH 7.5) and 0.1ml of xanthine oxidase enzyme solution (0.1 units/ml in phosphate buffer pH 7.5) which was prepared immediately before use. After pre-incubation at 25c from 15 mins , the reaction was initiated by the addition of 2ml of substrate solution (150 M xanthine in the same buffer). The assay mixture was incubated at 25c for 30 mins. The reaction was then stopped by addition by 1ml of 1N hydrochloric acid and the absorbance was measured at 290nm using a spectrometer. Allopurinol (0.1 to 0.5mg/dl) a known inhibitor of xo is defined as the amount of enzyme required to produce 1 mm of the uric acid 1 min at 25c. Xol activity was expressed as the percentage inhibition of xo in the above assay calculated as percentage of inhibited as follows:

$$\text{Inhibitory activity (\%)} = (1 - \text{AS/AC}) * 100 \text{ where}$$

AS - Absorbance in presence of test substance.  
 AC - Absorbance of control.

**Statistical analysis :**

The data were subjected to satisfied 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  $p < 0.05$ .

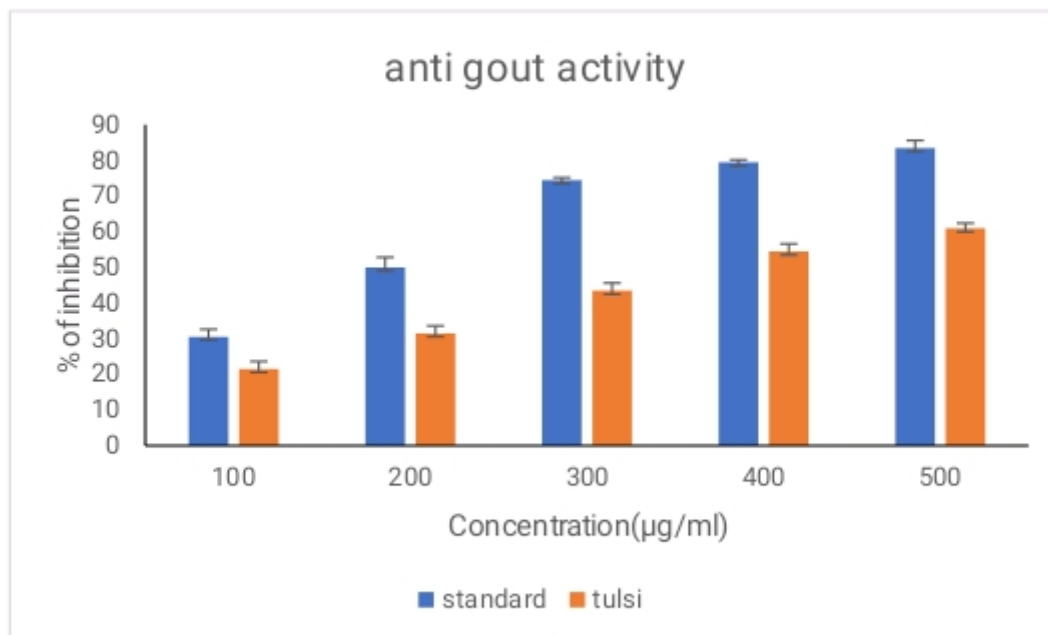
**Results:**

The main constituents found in the extract were flavonoids , phenols, coumarins, steroids , terpenoids and quinones. Secondary metabolites such as alkaloids, quinones and phenols present in neem, tulsi extract showed it is effective in treating various disorders.

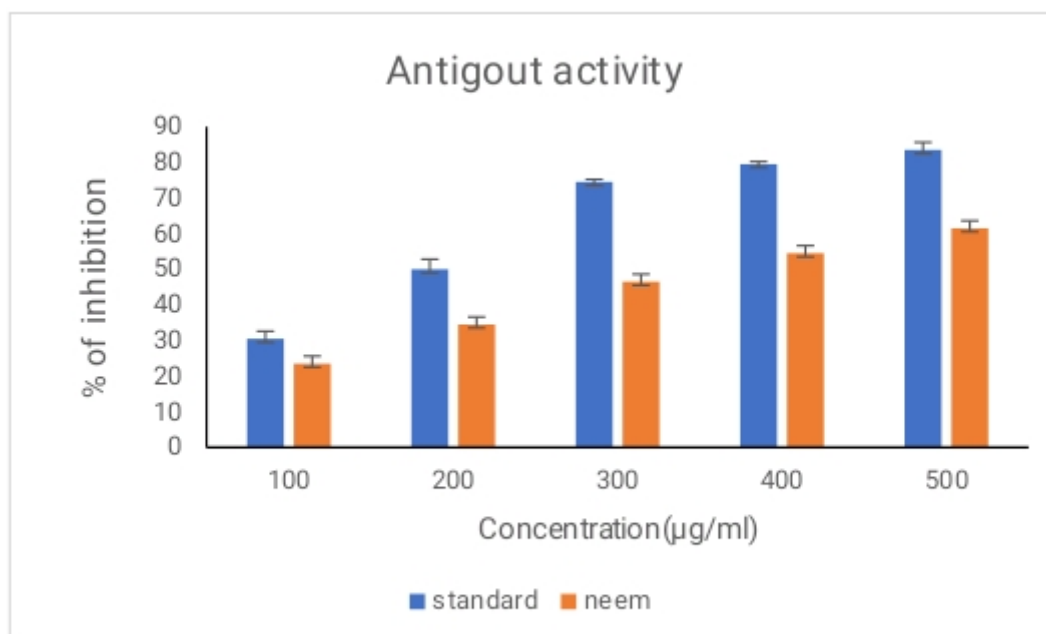
The results of qualitative phytochemical analysis of neem and tulsi extract are given in the below given table . The results revealed the presence of terpenoids, alkaloids, carbohydrates, steroids in tulsi extract and protein, amino acid , alkaloids , saponins, carbohydrates in neem extract.

phytochemical	Neem	Tulsi
Protein	+	-
Amino acid	+	-
Terpenoids	-	+
Flavonoids	+	+
Alkaloids	+	+
Carbohydrates	+	+
Saponins	+	-
Steroids	-	+

**Table 1:** represents qualitative phytochemical analysis of neem and tulsi extract .



**Figure 1 :** represents Anti gout activity of aqueous extract of selected medicinal plants ( Tulsi ). Each bar represents  $\pm$  mean SD of three independent observations. Significance at the level of  $P < 0.05$



**Figure 2 :** represents Anti gout activity of aqueous extract of selected medicinal plants (Neem ) . Each bar represents  $\pm$  mean SD of three independent observations. Significance at the level of  $P < 0.05$

As dose dependent xanthine oxidase inhibitory activity was observed for the extracts and drug. In the present study, the standard drug allopurinol showed greater percentage of inhibition of xanthine oxidase enzyme than that of tulsi, neem extracts in all given tested concentrations.

Neem extracts showed increased xanthine oxidase inhibition than the tulsi in all tested concentrations. (Graph 1 & 2) Allopurinol is the standard drug used for the treatment of gout.

The result revealed that standard drug allopurinol is the most important potential drug of all the three bioactive molecules observed in the study. The neem extract has more potential than tulsi. The presence of phytonutrients like terpenoids, flavonoids, carbohydrates, steroids which was present in neem and tulsi extract might have resulted in xanthine oxidase inhibitory activity.

#### **Discussion :**

xanthine oxidase inhibition assay is considered a gold standard to study anti gout potential of medicinal plants (neem and tulsi). Some plants and their phytochemicals are worthy of exploration as they can act as xanthine oxidase inhibitors. These compounds are also safe if taken in the appropriate amount and have less side effects. The different parts of the same plants can contribute differently to effect on uric acid levels, which was shown in some previous studies ((33)

Medicinal plants contain many bio active and antioxidants that can be used as alternative medicine to treat gout. Approximately 65% - 80% of people in developing countries use plants as remedies. Plants are also an important source of medicine in all the countries. (34)

Method of extraction is considered an important factor that affects xanthine oxidase inhibition. Based on the results of xanthine oxidase inhibitory analysis neem and tulsi showed 85% activity.  $IC_{50}$  values, the concentration at which half the xanthine oxidase activity is inhibited, were determined in some previous studies (35)(36). Phytochemical screening of the extracts also revealed their major constituents which includes alkaloids, flavonoids and saponins that may act as xanthine oxidase inhibitors (37).

Allopurinol is the standard drug and common drug used in the treatment of gout approved by the US FDA for dose upto 800 mg/d(7)). In this study the plant extracts are compared with standard drugs and found to have efficient xanthine oxidase inhibitory potential. Furthermore, for anti gout there is a very limited number of clinical studies especially for xanthine oxidase inhibitor mechanisms. Many pharmacologists and clinical investigators conducted larger randomized clinical trials for a long time in order to determine the efficiency of plant based drugs in the treatment of gout. The dose of the plant extract, method of extract preparation and extraction solvent must also be taken in consideration for further studies.

#### **Limitations of study:**

Active compounds can be isolated and experimental analysis can be done.

#### **Future scope:**

As most of the people need to know about anti gout activity of Neem and Tulsi extract. It is more advisable to do more comparisons between medicinal plant extract and standard drug to know which are all the medicinal plants that exhibit anti gout activity through which many of the drugs can be developed for treatment of gout.

#### **Conclusion:**

From this we may conclude that aqueous extract of neem and tulsi exhibits anti gout activity. Further in vivo studies and detailed molecular mechanisms may be required for developing these extracts into commercially available drugs for treatment of gout.

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#### **Author contribution:**

Ms.Srivarsha Ranjeet : Literature search, survey, data collection, analysis, manuscript writing.

Dr.V.Vishnupriya, Dr.R.Gayathri, Dr.S.Kavitha : Study design, data verification, manuscript drafting, manuscript correcting.

**Conflict of interest:**

None to declare

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