PHYTOCONSTITUENTS AND ANTI-GOUT ACTIVITY ANALYSIS OF SELECTED MEDICINAL PLANTS

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Abstract:

Gout, a common rheumatic disease worldwide, is an inflammatory syndrome with pathogenic basis of hyperuricemia, which usually exceeds 390 µmol/L or 6.5 mg/dL of uric acid level in the serum and thereby leads to crystal formation of monosodium urate (MSU) in various tissues, especially in the joints. Compared with women, men have a four- to nine fold increased risk of developing gout Plants such as Andrographis paniculata, Cissus quadrangulari, Michelia champaca, Tinospora cordifolia, Zingiber officinale, Carica papaya and Tephrosia purpurea were subjected to phytoconstituents and anti-diabetic activity analysis. Tinospora cordifolia and Costus pictus was found to have positive result for alkaloids, flavonoids, polyphenolics, phytosterol, fixed oils and fats, carbohydrates amino acids and proteins. Andrographis paniculata, Cissus quadrangulari and Zingiber officinale have been found to have alkaloids, flavonoids, polyphenolics, phytosterol, fixed oils and fats, carbohydrates amino acids and proteins. The phytochemicals content varied from solvent to solvent used for extraction. Many types of highly useful phytoconstituents were found out from all the test plants and many of them were found to have many biological activities including antigout activity. This was proved in their anti-gout activity. Effect of plant extracts on serum uric acid level in normal and gout induced rats were tested along with xanthine oxidase inhibition activity. All the plants were identified to have anti-gout activity. This study has clearly showed that the plants after purifying the phytoconstituents could effectively be used as anti diabetic plant derived drug, which are free from side effects.

***Key words*:** Phytoconstituents, Anti-gout Activity, *Andrographis paniculata, Cissus quadrangulari, Michelia champaca, Tinospora cordifolia, Zingiber officinale, Carica papaya* and *Tephrosia purpurea*

I.INTRODUCTION

Gout, a common rheumatic disease worldwide, is an inflammatory syndrome with pathogenic basis of hyperuricemia, which usually exceeds 390 μmol/L or 6.5 mg/dL of uric acid level in the serum and thereby leads to crystal formation of monosodium urate (MSU) in various tissues, especially in the joints (Tausche *et al*., 2009). MSU crystals are potent proinflammatory stimuli and can initiate an innate immune inflammatory response in the joints with a key component of neutrophils migrating to the crystals' surface to cause acute gouty arthritis (Tausche *et al*., 2009). Identification of MSU in the joints is considered the gold standard for diagnosis of gout. In clinical practice, gout has been considered as a single disease with different stages, including acute gout (episodes of acute intensely painful and inflammatory arthritis), inter critical gout (the intervals between attacks of gout), and persistent clinical manifestations of chronic gout (Perez-Ruiz *et al*., 2014).

Mechanisms of Hyperuricemia

Compared with women, men have a four- to nine fold increased risk of developing gout (Annemans *et al*., 2005). Recent studies show that major urate loci are genetic variants of SLC2A9 and ABCG2, which encode secretory uric acid transporters. The SLC2A9 locus involves in renal and gut excretion of uric acid; whereas ABCG2 locus involves primarily in extra-renal uric acid under-excretion (Merriman *et al*., 2015). ABCG2 export dysfunction decreases intestinal urate excretion (Ichida *et al*., 2012). Apart from hereditary disorders associated with decreasing uric acid excretion and increased purine metabolism, the main causes of gout are conditions of high-level uric acid production mostly due to purine-rich food, alcohol consumption, and overweight (Choi *et al*., 2004). That is way the control of uric acid production has been widely considered as a key factor in the prevention and treatment of these diseases.

Western Hypouricemic Agents

Uricosuric drugs (i.e., probenecid and benzbromarone) could reduce the serum uric acid concentration by increasing the renal excretion of uric acid. However, they may lead to renal tubular aggregation of urate crystals and induce renal damage. Xanthine oxidoreductase (XOR), a cytoplasmic molybdenum-containing oxidoreductase, is the key enzyme in the catabolism of purines and uric acid production. XOR inhibitor (e. g., allopurinol) could decrease serum uric acid by inhabiting uric acid synthesis. XOR can also act as a source of reactive oxygen species (ROS) which may be involve in the pathogenesis of various degenerative diseases (Maia *et al*., 2007; Choi, 2008). That is why the inhibition of XOR activity may decrease the level of uric acid and ROS production, and can result in anti-hyperuricemic and anti-oxidative effects

Since ancient ages plants have served human beings as a natural source of treatments and therapies, among them medicinal herbs have gain attention because of its wide use and less side effects. In current scenario focus on plant research has increased throughout the world and a huge amount of evidences have been collected to show immense potential of medicinal plants used in various traditional systems. More than 15000 plants have been studied during the last 5

year period. Recently scientists are using these renewable resources to produce a new generation of therapeutic solutions. Many of the plant extracts have proven to posses pharmacological actions.

Traditional medicines derive the scientific heritage from rich experiences of early civilization (Shailajan et al., 2005).. Plants are the source of medication for preventive, curative, protective or promotive purposes (Sidhu et al., 2007). Plant derived foods help in the prevention of lifestyle associated diseases. Several groups of constituents in plants have been identified as potentially health promoting in animal studies, including cholesterol lowering factors, antioxidants, enzyme inducers and others (Dragsted et al.,2006). A thousand years ago an extensive use of plants as medicines have been reported and were initially taken in the form of crude drugs and other herbal formulations (Gullo et al., 2006). Toxicology is the important aspect of pharmacology that deals with the adverse effect of bio active substance on living organisms prior to be use as drug or chemical in clinical use (Aneela et al., 2011). As per the OECD guidelines, in order to establish the safety and efficiency of a new drug, toxicological studies are very essential in animals like mice, rat, pig, dog, rabbit, monkey etc under various conditions of drug. Toxicological studies help to make decision whether a new drug should be adopted for clinical use or not. OECD 401, 423 & 425 does not allows the use of drug clinically without its clinical trial as well as toxicity studies. Depending on the duration of drug exposure to animals toxicological studies may be three types such acute, sub-acute and chronic toxicological studies. Plants or drugs must be ensured to be safe before they could be used as medicines. A key stage in ensuring the safety of drugs is to conduct toxicity tests in appropriate animal models, and acute toxicity studies are just one of a battery of toxicity tests that are used (Kathryn Chapman, 2007). Following are the objectives addressed in the present investigation:

In this study phytochemical analysis of plants such as *Andrographis paniculata, Cissus quadrangularis Michelia champaca, Tinospora cordifolia, Zingiber officinale, Carica papaya* and effect of plant extracts on anti-gout activity in induced rats were tested

II. MATERIALS AND METHODS

Plant Material

Plants such *Andrographis paniculata, Cissus quadrangularis Michelia champaca, Tinospora cordifolia, Zingiber officinale, Carica papaya* were collected from area in and around Arcot and the they were confirmed by the P.G Research center of Botany, Thiyagarajar College, Madurai**.**

Alcoholic Extraction

The whole plants were collected and shadow dried. The shade-dried whole plants were subjected to pulverization to get coarse powder. The coarsely powder of whole plant was used for extraction with methanol in Soxhlet apparatus. The extract was evaporated to dryness under vacuum and dried in vacuum desiccator (15.5% w/w).

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Preliminary phytochemical analysis

Qualitative and Quantitative estimation of phytoconstituents

Extraction procedure

The plant's stem was washed with fresh water and dried under shade at room temperature, cut into small pieces and the juice was taken using a mixer grinder. Then this juice (100ml) was mixed with solvents such as methanol (85%), chloroform, Ethyl acetate and hexane extracts for overnight at room temperature (Grouch *et al.,* 1992; Matanjun *et al.,* 2008). The extracts were subjected to phytochemical screening for the presence of amino acids, proteins, saponins, triterpenoids, flavonoids, carbohydrates, alkaloids, phytosterols, glycosidal sugars, protein, tannins, phenols and furanoids using the method of (Harborne 1973). The same method was also followed using Soxhlet extraction procedure.

Soxhlet extraction

Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity that is insoluble in solvent. If the desired compound has a high solubility in a solvent, then a simple filtration can be used to separate the compound from the insoluble substance. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. This method cannot be used for thermolabile compounds as prolonged heating may lead to degradation of different compounds (Roopashree *et al.,* 2008).

Phytochemical screening: Phytochemical examinations were carried out for all the extracts, as per the standard methods (Audu *et al.,* 2007; Roopashree *et al.,* 2008; Obasi *et al.,* 2010)

DETECTION OF ALKALOIDS: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

b) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). The formation of a brown / reddish precipitate indicates the presence of alkaloids.

c) Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of a red precipitate indicates the presence of alkaloids.

d) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids was confirmed by the formation of a yellow coloured precipitate.

DETECTION OF CARBOHYDRATES: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

b) Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

c) Fehling's Test: Filtrates were hydrolyzed with dil. HCl neutralized with alkali and heated with Fehling's A & B solution. Formation of a red precipitate indicates the presence of reducing sugars.

DETECTION OF GLYCOSIDES: Extracts were hydrolyzed with dil. HCl, and then subjected to test for glycosides.

a) Modified Borntrager's Test: Extracts were treated with a Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammoniacal layer indicates the presence of ethanol glycosides.

b) Legal's Test: Extracts were treated with sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

DETECTION OF SAPONINS

a) Froth Test: Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. A formation of 1 cm layer of foam indicates the presence of saponins.

b) Foam Test: 0.5 gm of the extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

DETECTION OF PHYTOSTEROLS

a) Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

b) Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. The formation of brown ring at the junction indicates the presence of phytosterols.

DETECTION OF PHENOLS

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

DETECTION OF TANNINS

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

DETECTION OF FLAVONOIDS

a) Alkaline Reagent Test: Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on the addition of dilute acid, indicates the presence of flavonoids.

b) Lead acetate Test: Extracts were treated with a few drops of lead acetate solution. Formation of a yellow colour precipitate indicates the presence of flavonoids.

DETECTION OF PROTEINS AND AMINOACIDS

a) Xanthoproteic Test: The extracts were treated with a few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

b) Ninhydrin Test: To the extract, 0.25% w/v Ninhydrin reagent was added and boiled for a few minutes. Formation of blue colour indicates the presence of amino acid.

DETECTION OF DITERPENES

Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Quantitative Analysis

The hexane, chloroform, ethyl acetate, 85% Methanol extract *of Andrographis paniculata, Cissus quadrangulari, Michelia champaca, Tinospora cordifolia, Zingiber officinale, Carica papaya* and *Tephrosia purpurea* was analyzed for some of the biochemical constituents by standard procedures is followed.

Total alkaloids were measured by the method of Manjunath, (2012). Flavonoid was extracted and estimated by the method of Cameron, (1993). The amount of total phenols in the plant tissues was estimated by the method proposed by Mallick and Singh, (1980). The phytosterol content (TPC) weas calculated as β-sitosterol (g %) using the photometric standard equation to calculate steroids proposed by Kim and Goldberg (1969) : TPC = C_s x Aa/As, Where: Cs= Standard Concentration; Aa= Absorbance of the sample; As =Absorbance of the standard.

Saponin quantitative determination was carried out using the method reported by Ejikeme *et al*., (2014) and Obadoni and Ochuko (2002). Ant it was determined by a formula % of Saponin = Weight of Saponin / Weight of Sample x 100. The Free fatty acid content was estimated by the method of Horn and Mehanan, (1981). The total carbohydrate content was estimated by the method of Hedge and Hofreiter, (1962). The total protein content was estimated by the method of Lowry (1951).

ANTI- GOUT ACTIVITY STUDIES

Animals

Wistar albino rats (8–10 weeks) of both sexes were obtained from the animal house of Nizam Institute Of Pharmacy, Deshmukhi, Ramoji Film City, Hyderabad. Before and during the experiment, rats were fed with standard diet (Gold Moher, Lipton India Ltd). After randomization into various groups and before initiation of experiment, the rats were acclimatized

for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 hours ad libitum.

EXPERIMENTAL DESIGN

Experimental design

For control I: Water with routine food.

For inducing gout in animals II: **Pottasium oxonate** (250 mg/kg body weight, IP) for 3months

For test animal III: potassium oxonate + ***Andrographis paniculata*** for 3months.

For test animal III: potassium oxonate + ***Cissus quadrangularis*** for 3months

For test animal IV: potassium oxonate + ***Michelia champaca*** L for 3months

For test animal VI: potassium oxonate + ***Tinospora cordifolia*** L for 3months

For test animal VIII: potassium oxonate + ***Zingiber officinale*** L for 3months

For test animal IX: potassium oxonate + ***Carica papaya***L for 3months

For test animal IX: potassium oxonate + ***Tephrosia purpurea*** L for 3months

For test and treatment with allopathy: potassium oxonate +Allopurinol (5 mg/kg body weight, PO) for 3months

Whole plant extracts and standard drug Allopurinol (5 mg/kg) and saline were administered with the help of feeding cannula. Group I serve as normal control, which received saline for 14 days. Group II to Group V are gout control rats. Group III to Group V (which previously received potassium oxonate) are given a fixed dose of whole plants extract (300 mg/kg, p.o), (500 mg/kg, p.o) and standard drug Allopurinol (5 mg/kg) for 14 consecutive days.

Specimen collection

After 3 months the animals were sacrificed by cervical decapitation, for the analysis of biochemical estimation, by applying local anesthesia using chloroform. The blood sample were collected and then transferred in to tubes. The tubes of the blood sample and the vials of serum sample were marked similarly for identification.

1. The blood samples were centrifuged at 1500 rpm for10 minutes.
2. Serum sample collected was transferred into the vials.
3. The specimens collected were used for the biochemical estimations.
4. Biochemical estimations were follows.

Biochemical analysis

Determination of serum uric acid

Uricase acts on uric acid to produce allantion, carbon dioxide and hydrogen peroxide . Hydrogen peroxide in the presence of peroxidase reacts with a chromogen (amino – antipyrine and dichloro – hydroxybenzen sulfnate) to yield quinoneimine, a red colored complex .The absorbance measured at 520 nm (490 – 530) is proportional to the amount of uric acid in the specimen (Tietz,.,1999) .

Xanthine oxidase inhibitory activity assay

The inhibitory effect on XO was measured spectrophotometrically at 295 nm under aerobic condition, with some modifications, following the method reported by Umamaheswari, et al [8]. A well known XOI, allopurinol (100 µg/ml) was used as a positive control for the inhibition test. The reaction mixture consisted of 300 µl of 50 mM sodium phosphate buffer (pH 7.5), 100 µl of sample solution dissolved in distilled water or DMSO, 100 µl of freshly prepared enzyme solution (0.2 units/ml of xanthine oxidase in phosphate buffer) and 100 µl of distilled water. The assay mixture was pre-incubated at 37°C for 15 min. Then, 200 µl of substrate solution (0.15 mM of xanthine) was added into the mixture. The mixture was incubated at 37°C for 30 min. Next, the reaction was stopped with the addition of 200 µl of 0.5 M HCl. The absorbance was measured using UV/VIS spectrophotometer against a blank prepared in the same way but the enzyme solution was replaced with the phosphate buffer. Another reaction mixture was prepared (control) having 100 µl of DMSO instead of test compounds in order to have maximum uric acid formation.

The inhibition percentage of xanthine oxidase activity was calculated according to the formula =(A control-A sample) / A control × 100% [9]. Naseem *et al*., 2006

Estimation of Urea in Serum was carried out using Diacetyl Monoxime (DAM) Method (Martinek, 1969). Estimation of creatinine in serum was carried out using Alkaline Picrate Method (Bones *et al.,* 1945) and Estimation of SGOT (ASAT) and SGPT (ALAT) in serum using modified IFCC Method (Bergmeyer e*t al.,* 1986). Apart from those tests Lipid Profile including Estimation of total Cholesterol and, estimation of HDL using (Wybenga And Pileggi et al., 1970) and test for Triglycerides, test For Low Density Lipoprotein (LDL) was calculated using a formula LDL = Cholesterol- $\left[\frac{TG}{5} - HDL\right]$.

Statistical Analysis

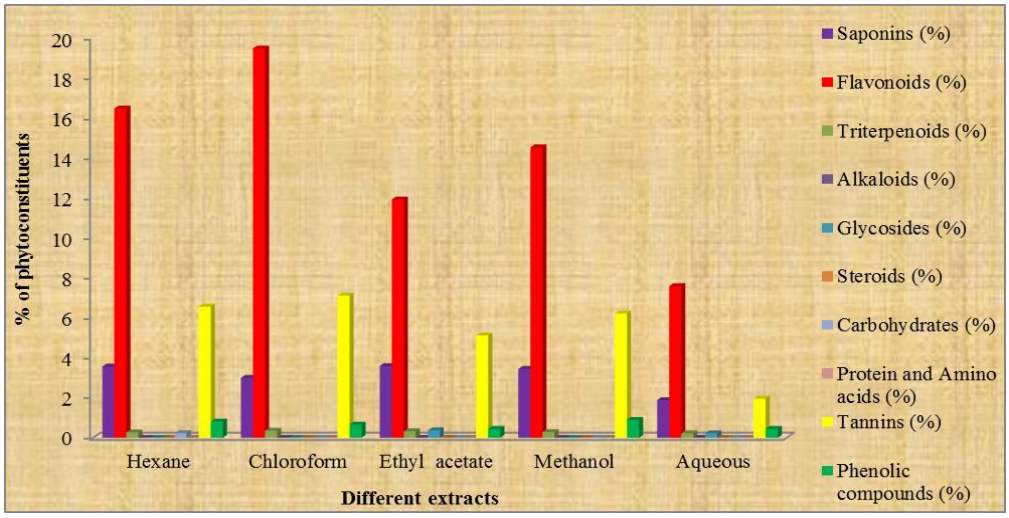
All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean± standard error of mean (S.E.M.) and analyzed for ANOVA and post hoc Dunnet's -test. Differences between groups were considered significant at $P < 0.1$ levels.

III - RESULTS

Phytochemicals	Quantitative Phytochemical Estimation (mg/g)				
	Hexane	Chloroform	Ethyl acetate	Methanol	Aqueous
Saponins (%)	3.55±0.09	2.99±0.17	3.58±0.13	3.44±0.22	1.88±0.00
Flavonoids (%)	16.44±0.4	19.44±0.30	11.90±0.02	14.50±0.0	7.57±0.11
Triterpenoids (%)	0.27± 0.14	0.35± 0.22	0.32± 0.10	0.28± 0.2	0.22± 0.30
Alkaloids (%)	0.00	0.00	0.00	0.00	0.00
Glycosides (%)	0.00	0.00	0.37±0.00	0.00	0.22±0.00
Steroids (%)	0.00	0.00	0.00	0.00	0.00
Carbohydrates (%)	0.23± 0.16	0.00	0.00	0.00	0.00
Protein and Amino acids (%)	0.00	0.00	0.00	0.00	0.00
Tannins (%)	6.55±0.48	7.10±0.55	5.11±0.65	6.20±0.27	1.95±0.36
Phenolic compounds (%)	0.81±0.13	0.65±0.02	0.44±0.14	0.89±0.11	0.44±0.15

Table 1- Quantitative analysis of phytochemicals in the different extracts of *Andrographis paniculata*

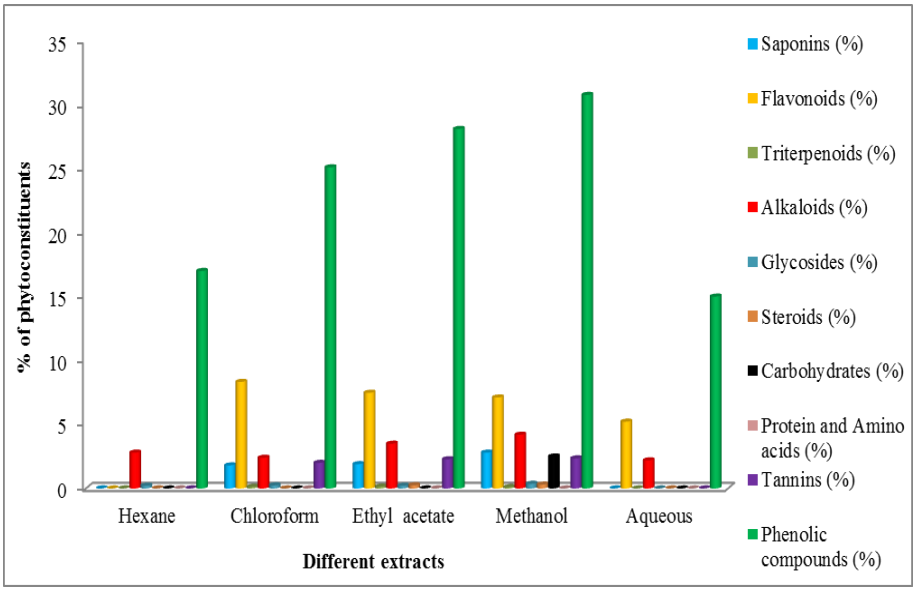
Figure 1- Quantitative analysis of phytochemicals in the different extracts of *Andrographis paniculata*



Phytochemicals	Quantitative Phytochemical Estimation (mg/g)				
	Hexane	Chloroform	Ethyl acetate	Methanol	Aqueous
Saponins (%)	0.00	1.80± 0.10	1.90± 0.07	2.80± 0.16	0.00
Flavonoids (%)	0.00	8.33± 0.10	7.48± 0.11	7.11± 0.10	5.22±0.10
Triterpenoids (%)	0.00	0.12± 0.22	0.14± 0.31	0.10± 0.07	0.00
Alkaloids (%)	2.80±1.20	2.40±0.01	3.50 ± 0.02	4.20±0.03	2.20±0.03
Glycosides (%)	0.21± 0.30	0.24± 0.11	0.21±0.00	0.36± 0.18	0.00
Steroids (%)	0.00	0.00	0.25±0.02	0.30± 0.02	0.00
Carbohydrates (%)	0.00	0.00	0.00	2.50±0.20	0.00
Protein and Amino acids (%)	0.00	0.00	0.00	0.00	0.00
Tannins (%)	0.00	2.00± 0.0	2.28± 0.00	2.35± 0.01	0.00
Phenolic compounds (%)	17.0±1.40	25.10±1.50	28.10±2.01	30.76 ± 1.0	15.00±1.80

Table 2- Quantitative analysis of phytochemicals in the different extracts of *Cissus quadrangularis*

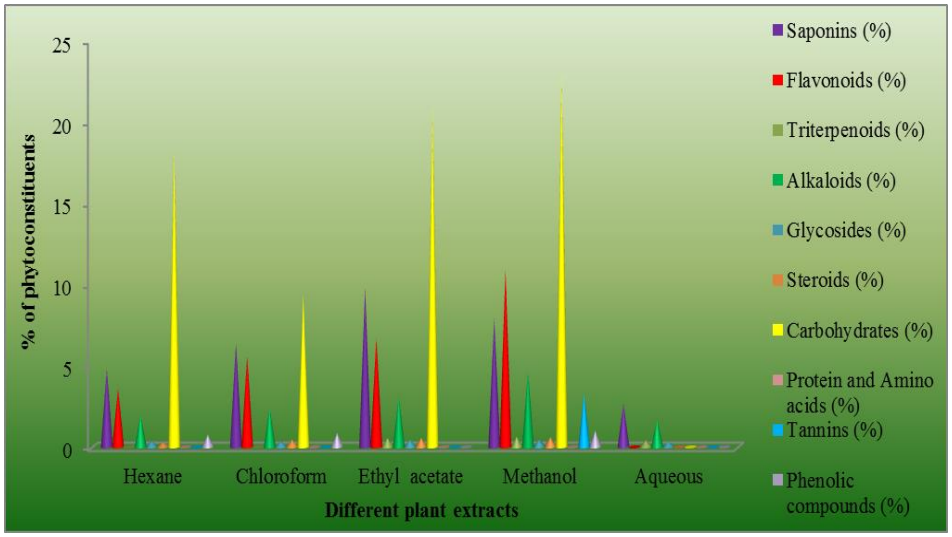
Figure 2- Quantitative analysis of phytochemicals in the different extracts of *Cissus quadrangularis*



Phytochemicals	Quantitative Phytochemical Estimation (mg/g)				
	Hexane	Chloroform	Ethyl acetate	Methanol	Aqueous
Saponins (%)	4.8±0.37	6.4±0.40	9.8±0.33	7.9±0.44	2.68±0.20
Flavonoids (%)	3.6±0.20	5.6±0.10	6.7±0.05	10.9±0.55	0.00
Triterpenoids (%)	0.00	0.00	0.56±0.10	0.63±0.00	0.42±0.10
Alkaloids (%)	1.99± 0.01	2.40±0.01	3.10±0.03	4.66±0.02	1.70±0.30
Glycosides (%)	0.35±0.00	0.35±0.01	0.45±0.21	0.44±0.10	0.32±0.00
Steroids (%)	0.28±0.00	0.45±0.00	0.55±0.01	0.61±0.01	0.00
Carbohydrates (%)	18.20±1.30	90.40±1.90	20.80±1.20	22.91±1.20	0.00
Protein and Amino acids (%)	0.00	0.00	0.00	0.00	0.00
Tannins (%)	0.00	0.00	0.00	3.30± 0.1	0.00
Phenolic compounds (%)	0.76±0.01	0.85±0.01	0.00	0.99±0.11	0.00

Table 3- Quantitative analysis of phytochemicals in the different extracts of *Tinospora cordifolia*

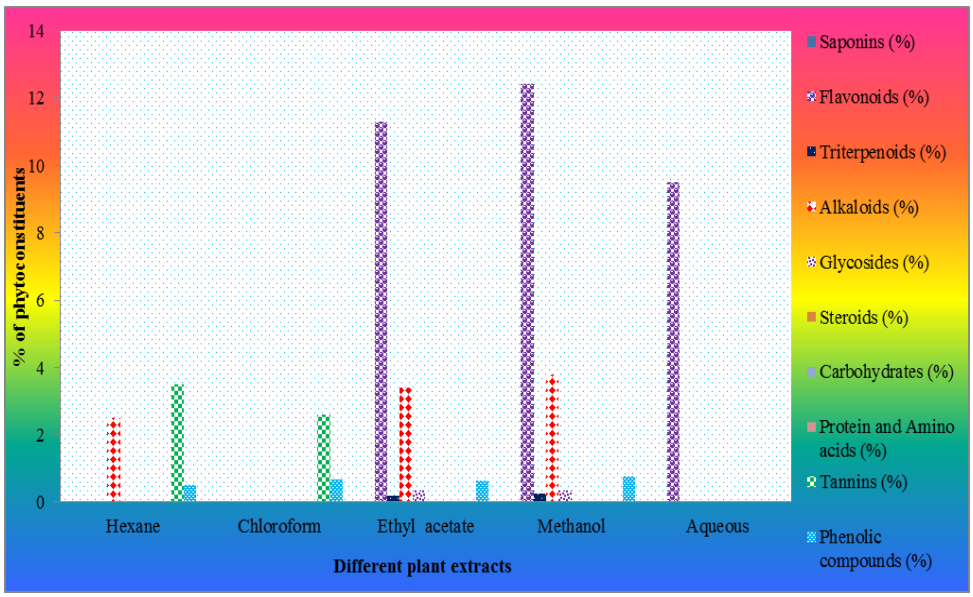
Figure 3- Quantitative analysis of phytochemicals in the different extracts of *Tinospora cordifolia*



Phytochemicals	Quantitative Phytochemical Estimation (mg/g)				
	Hexane	Chloroform	Ethyl acetate	Methanol	Aqueous
Saponins (%)	0.00	0.00	0.00	0.00	0.00
Flavonoids (%)	0.00	0.00	11.30±0.20	12.44±0.40	9.50±0.10
Triterpenoids (%)	0.00	0.00	0.20±0.00	0.25±0.01	0.00
Alkaloids (%)	2.50±0.00	0.00	3.40±0.02	3.80±0.01	0.00
Glycosides (%)	0.00	0.00	0.34±0.00	0.35±0.01	0.00
Steroids (%)	0.00	0.00	0.00	0.00	0.00
Carbohydrates (%)	0.00	0.00	0.00	0.00	0.00
Protein and Amino acids (%)	0.00	0.00	0.00	0.00	0.00
Tannins (%)	3.50±0.20	2.60±0.00	0.00	0.00	0.00
Phenolic compounds (%)	0.52±0.00	0.66±0.00	0.65±0.01	0.77±0.02	0.00

Table 4 -Quantitative analysis of phytochemicals in the different extracts of *Michelia champaca*

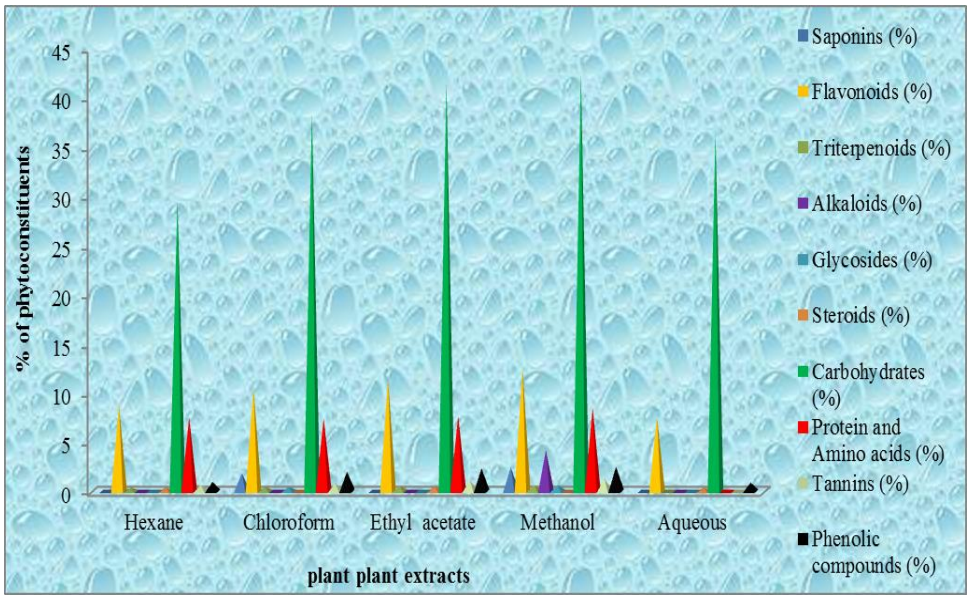
Figure 4-Quantitative analysis of phytochemicals in the different extracts of *Michelia champaca*



Phytochemicals	Quantitative Phytochemical Estimation (mg/g)				
	Hexane	Chloroform	Ethyl acetate	Methanol	Aqueous
Saponins (%)	0.00	1.85±0.01	0.00	2.45±0.02	0.00
Flavonoids (%)	8.66±0.20	10.44±0.10	11.43±0.20	12.5±0.25	7.54±0.22
Triterpenoids (%)	0.36±0.00	0.44±0.01	0.55±0.02	0.65±0.01	0.00
Alkaloids (%)	0.00	0.00	0.00	4.20±0.20	0.00
Glycosides (%)	0.00	0.45±0.01	0.00	0.66±0.00	0.00
Steroids (%)	0.35±0.01	0.00	0.45±0.01	0.00	0.35±0.00
Carbohydrates (%)	29.56±0.3	38.35±0.20	41.58±0.30	42.5±0.20	36.50±0.10
Protein and Amino acids (%)	7.50±0.11	7.44±0.10	7.66±0.10	8.50±0.20	0.00
Tannins (%)	0.66±0.00	0.8±0.00	1.10±0.10	1.28±0.00	0.00
Phenolic compounds (%)	0.95±0.00	2.00±0.10	2.33±0.10	2.50±0.20	0.90±0.00

Table 5 -Quantitative analysis of phytochemicals in the different extracts of *Zingiber officinale*

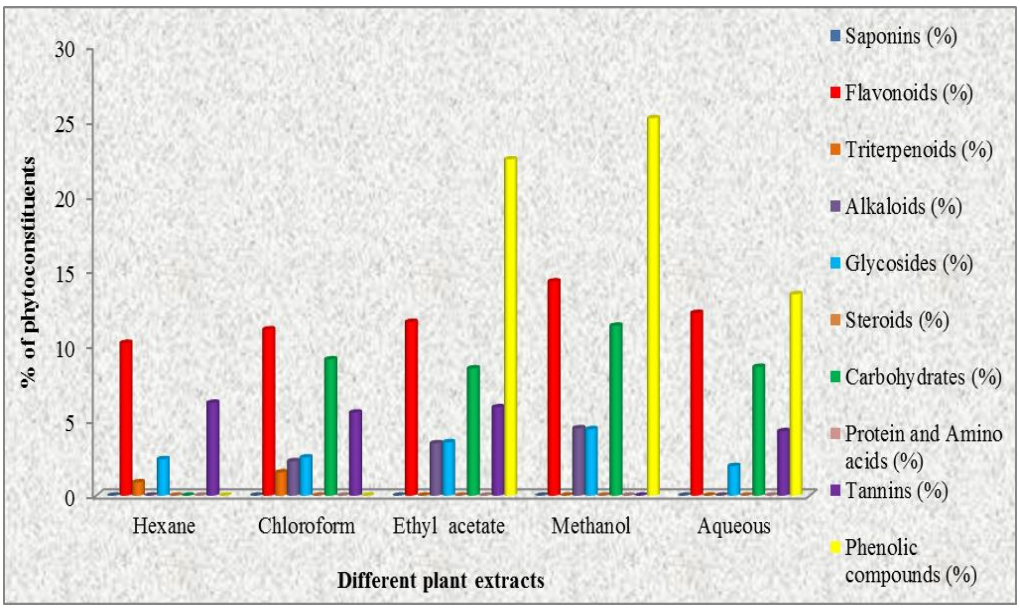
Figure 5- Quantitative analysis of phytochemicals in the different extracts of *Zingiber officinale*



Phytochemicals	Quantitative Phytochemical Estimation (mg/g)				
	Hexane	Chloroform	Ethyl acetate	Methanol	Aqueous
Saponins (%)	0.00	0.00	0.00	0.00	0.00
Flavonoids (%)	10.20±0.02	11.10±0.10	11.60±0.80	14.30±1.0	12.20±1.00
Triterpenoids (%)	0.89±0.01	1.55±0.00	0.00	0.00	0.00
Alkaloids (%)	0.00	2.30±0.10	3.50±0.10	4.50±0.20	0.00
Glycosides (%)	2.44±0.00	2.55±0.04	3.56±0.00	4.44±0.30	1.98±0.10
Steroids (%)	0.00	0.00	0.00	0.00	0.00
Carbohydrates (%)	0.00	9.10±0.00	8.50±0.30	11.35±0.30	8.6±0.00
Protein and Amino acids (%)	0.00	0.00	0.00	0.00	0.00
Tannins (%)	6.20±0.20	5.55±0.10	5.90±0.20	0.00	4.30±0.10
Phenolic compounds (%)	0.00	0.00	22.45±0.30	25.20±0.20	13.45±0.10

Table 6 -Quantitative analysis of phytochemicals in the different extracts of *Carica papaya*

Figure 7- Quantitative analysis of phytochemicals in the different extracts of *Carica papaya*



Phytochemicals	Quantitative Phytochemical Estimation (mg/g)				
	Hexane	Chloroform	Ethyl acetate	Methanol	Aqueous
Saponins (%)	0.00	0.00	0.00	0.00	0.00
Flavonoids (%)	15.22±0.00	16.6±0.40	16.50±0.30	22.45±0.40	9.50±0.20
Triterpenoids (%)	1.46±0.00	1.89±0.20	2.50±0.00	2.90±0.30	0.98±0.00
Alkaloids (%)	3.50±0.30	3.60±0.20	3.80±0.20	4.55±0.20	2.20±0.00
Glycosides (%)	0.50±0.00	0.65±0.10	0.80±0.00	1.40±0.20	0.40±0.00
Steroids (%)	21.10±1.60	23.40±1.0	26.32±2.50	27.53±0.82	14.20±1.20
Carbohydrates (%)	0.00	0.00	0.00	0.00	0.00
Protein and Amino acids (%)	0.00	0.00	0.00	0.00	0.00
Tannins (%)	3.65±0.00	3.80±0.20	4.55±0.40	5.65±0.20	2.55±0.00
Phenolic compounds (%)	0.00	0.00	0.90±0.00	1.25±0.10	0.55±0.00

Table 8 -Quantitative analysis of phytochemicals in the different extracts of *Tephrosia purpurea*

Figure 8-Quantitative analysis of phytochemicals in the different extracts of *Tephrosia purpurea*

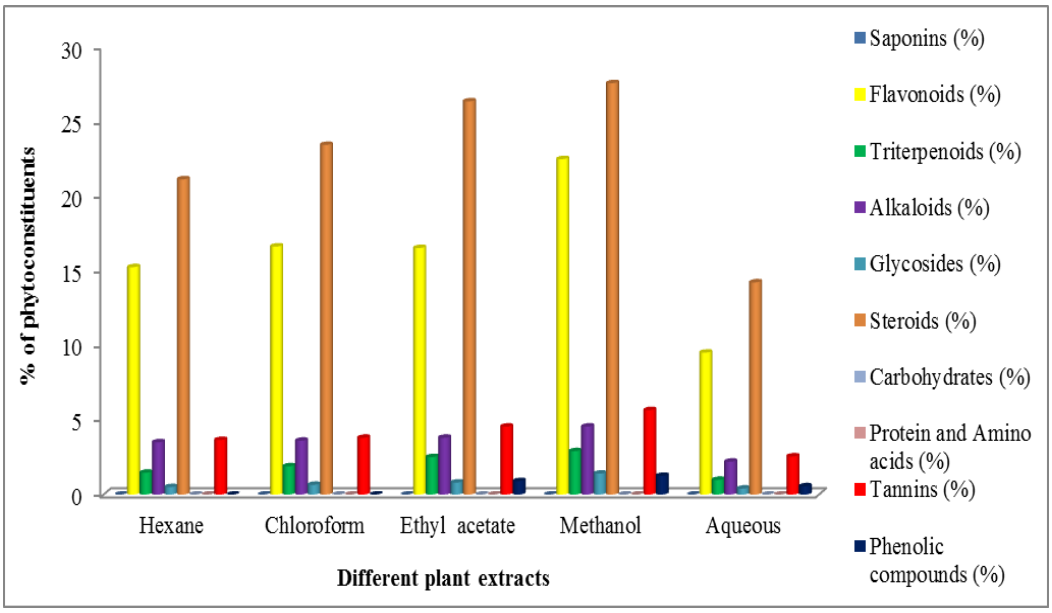


Table 9 – Study on Uric Acid Level in mg/dl

S.No.	Plants	URIC ACID LEVEL IN mg/dl.				
		Hexane extract	Chloroform extract	Ethyl acetate	Methanol	Aqueous
1	Control	1.2	1.2	1.2	1.2	1.2
2	Induced	3.4	3.4	3.4	3.4	3.4
3	*Andrographis paniculata*	1.4	1.8	1.5	1.4	1.7
4	*Cissus quadrangularis*	1.4	1.4	1.5	1.5	1.4
5	*Michelia champaca*	1.5	1.8	1.4	1.4	1.8
6	*Tinospora cordifolia*	1.4	2.1	1.9	1.7	1.4
7	*Zingiber officinale*	1.7	1.8	1.4	1.6	1.8
8	*Carica papaya* and	1.4	1.8	1.7	1.8	1.4
9	*Tephrosia purpurea*	1.9	1.4	1.4	1.4	1.7
10	allopurinol (100 µg/ml)-positive control	0.8	0.8	0.8	0.8	0.8

Figure 9 – Study on Uric Acid Level in mg/dl

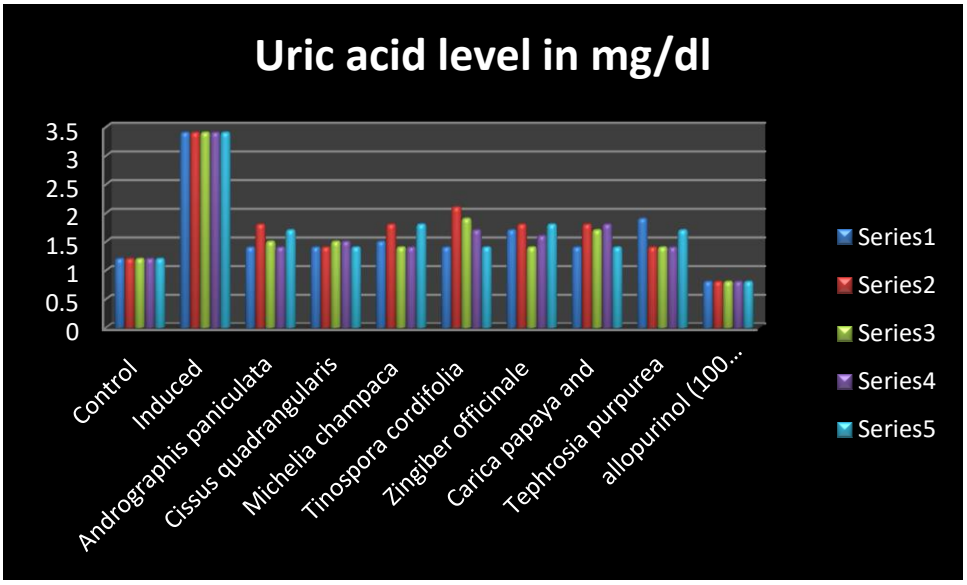
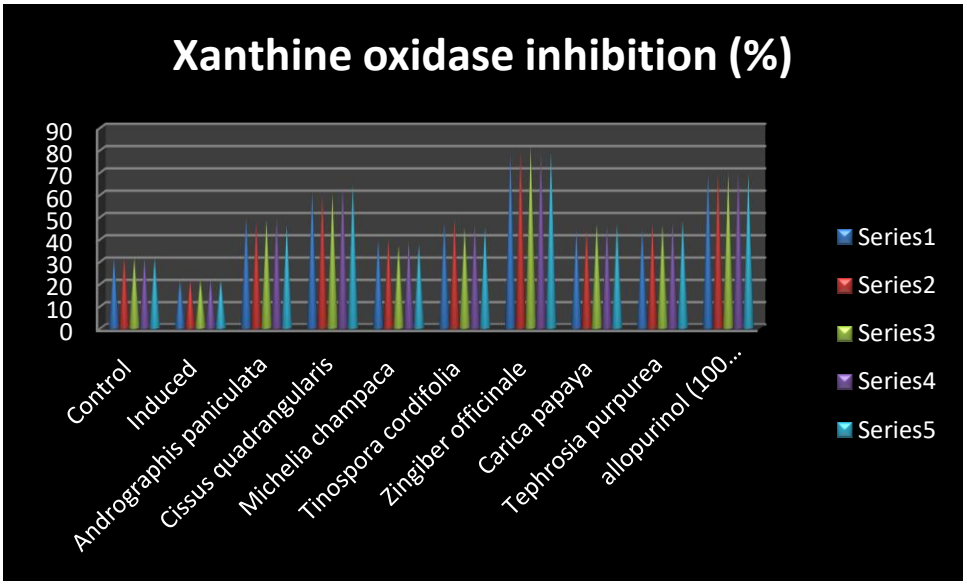


Table 10 – Study on Xanthine oxidase inhibitory activity (%)

S.No.	Plants	Xanthine oxidase inhibitory activity (%)				
		Hexane extract	Chloroform extract	Ethyl acetate	Methanol	Aqueous
1	Control	31.46	31.46	31.46	31.46	31.46
2	Induced	21.46	21.46	21.46	21.46	21.46
3	*Andrographis paniculata*	49.56	47.36	48.53	49.50	46.06
4	*Cissus quadrangularis*	61.16	59.26	60.00	63.55	64.06
5	*Michelia champaca*	39.66	40.00	36.90	39.06	37.50
6	*Tinospora cordifolia*	47.34	49.13	45.12	46.43	45.33
7	*Zingiber officinale*	77.86	79.63	81.50	78.36	79.06
8	*Carica papaya*	43.60	44.05	46.69	45.85	46.49
9	*Tephrosia purpurea*	44.44	46.65	46.00	47.05	48.40
10	allopurinol (100 µg/ml)- positive control	69.33	69.33	69.33	69.33	69.33

Graph 10– Study on Xanthine oxidase inhibitory activity (%)



IV-DISCUSSION

Gout is an emerging and common metabolic disorder closely related to hyperuricemia, the treatment of which aims to relieve acute gouty attacks and to prevent recurrent gouty episodes. Therapeutic approaches for treating gout include applications of anti-inflammatory agents for symptomatic relief, as well as selective inhibition of the terminal steps in uric acid biosynthesis for chronic gout (Emmerson, 1996). Combination of the relevant therapies, such as lowering uric acid levels, inhibiting inflammatory responses, and modifying dietary behaviors, was suggested for the treatment of gout (Cannella and Mikuls.,2005). Suppression of XO activity is one of the therapeutic strategies to reduce blood uric acid levels. However, only a few XO inhibitors, e.g., allopurinol and febuxostat, have been clinically used (Neogi, 2011).Although most of the Vietnamese traditional remedies for curing gout disease contain hy-thiem (NIMM,1993;, Do,2012), pharmacological research of this medicinal plant associated with the treatment of this metabolic and inflammatory disorder has been underexplored. XO, a form of oxidoreductase that generates reactive oxygen species (ORS), which affect kidney arteries and increase blood pressure and ultimately damage kidney cells (Serafi et al., 2011), is an enzyme that catalyzes the oxidation of hypoxanthine to xanthine and can further catalyze the oxidation of xanthine to uric acid.

Pharmacological uses Antioxidant and free radical scavenging activity Methanol extract of *Cissus quadrangularis* exhibits strong antioxidant and free radical scavenging activity in vitro and in vivo systems mainly due to the presence of β-carotene (Palu *et al*., 2010). Anti-microbial and antibacterial activity Methanol extract (90%) and dichloromethane extract of stems possess antibacterial activity against *S. aureus, E. coli,* and *P. aeruginosa* and mutagenicity against *Salmonella* microsome. Antimicrobial activity has also been reported from stem and root extract. The alcoholic extract of aerial part was found to possess antiprotozoal activity against Entamoeba histolytica. Alcoholic extract of the stem showed activity against *E. coli*. Methanol and dichloromethane extract of whole plant were screened for in vitro antiplasmodial activity (Rajpal, 2005). Pharmacological uses Antioxidant and free radical scavenging activity Methanol extract of *Cissus quadrangularis* exhibits strong antioxidant and free radical scavenging activity in vitro and in vivo systems mainly due to the presence of β-carotene (Palu *et al*., 2010).

The possible reason for these results may be due to the presence of active constituents of *T. purpurea* which may be polar or non-polar compound like coumarins, flavonoids, flavanones, isoflavones, rotenoides, etc. The kinetic analysis using Lineweaver–Burk plot revealed that the root extracts of *T. purpurea* displayed high inhibitory activity. The pattern of inhibition is a type of non competitive type of inhibition in presence of *T. purpurea* were in Vmax is decreased and Km appears to be unaltered with respect to Xanthine as substrate. It indicates that the binding of extract may occur with the free enzyme or the enzyme–substrate complex. The significant inhibition of XO by root extracts of *T. purpurea* may suppress the production of active oxygen

species or uric acid *in vivo* under the conditions that xanthine oxidase works. (Baskar *et al*., 2008).

Young *et al.,* observed both analgesic and anti-inflammatory effect of 6-gingerol, one of the major phytochemical constituents of *Zingiber officinale* (Young *et al*., 2005). Acetic acid writhing and formalin induced licking tests were carried out to evaluate anti-inflammatory effect whereas carrageenan induced paw edema experiment was run to observe analgesic effect in male ICR mice. Both analgesic and anti-inflammatory effects of ethanol extract of dried *Zingiber officinale* were observed by Ojewole(2006). In another study aqueous extract of *T. cordifolia* showed a significant anti-inflammatory effect in the cotton pellet granuloma and formalin induced arthritis model, it's effect was comparable with indomethacin and its mode of action appeared to resemble that of nonsteroidal anti-inflammatory agent. The dried stem of *T. cordifolia* produced significant anti-inflammatory effect in both acute and subacute models of inflammation. *T. cordifolia* was found to be more effective than acetylsalicylic acid in acute inflammation, although in subacute inflammation, the drug was inferior to phenylbutazone (Jana, 1999).

Phytochemical screening of various extracts of *Michelia champaca* L. was studied by Kodongala, (2010). The phytochemical test reveals the presence of Triterpenoids and Steroids in petroleum ether extract and absence of Alkaloids, Carbohydrates, Flavanoids, Glycosides, Resins, Saponins and Tanins in all extracts. Further, Geetha, (2011), investigated the preliminary Pharmacognostical study on leaves and flowers of *Michelia champaca* L. in which they performed the preliminary phytochemical screening. The results showed the presence of alkaloids, saponins, tannins, glycosides, carbohydrates, amino acids, flavonoids and sterols in both leaves and flowers. Extracts used were acetone, ethanol, petroleum ether, chloroform and aqueous

We demonstrated in this study that CEE of hy-thiem significantly reduced uric acid levels in oxonate-induced hyperuricemia rats. In addition, in vitro inhibitory activity of the CEE on XO was observed. The most potent activity was detected for the *n*-BuOH-soluble portion. Among the fractions resulting from activity-guided fractionation, the BuOH fraction presented XO inhibitory activity even more potent than that of the whole crude extract (data not shown), and showed no acute and sub-chronic toxicity. Therefore, transference of active components from the CEE to the BuOH fraction was suggested. The BuOH fraction of hy-thiem showed its hypouricemic effect at dose of 120 mg/kg. Subsequent in vivo studies at the same dose also revealed that the BuOH fraction remarkably inhibited liver XO activity in rats. These observations suggested that the hypouricemic effect of hy-thiem is caused by, at least in part, its inhibitory potency on XO, a key enzyme in the biosynthetic pathway of uric acid. Furthermore, the BuOH fraction also displayed a notable anti-inflammatory and antinociceptive activities in the carrageenan-induced animal model, as shown previously with the crude extract of *S.*

orientalis [Zhang et al., 2006; Hong et al., 2014). Finally, deposition of urate crystals, an important initiation factor in the inflammatory process of gout, has been taken into consideration in our experiments. The BuOH fraction was found to have anti-inflammatory effect in the urate-induced synovitis model, which represents acute gouty attacks, confirming that the inhibition of XO is associated with anti-inflammatory responses (Neogi, 2011).

Therapeutic potency of crude extract was compared with the phytochemical constituent gingerol and its derivatives. They found that the individual phytochemicals showed considerable effect. Interestingly, the crude extract containing essential oils and more polar compounds exhibited better activities by preventing joint inflammation and bone destruction. They concluded that not only gingerol but also non-gingerol compounds of *Zingiber officinale* had considerable anti-arthritic activity. Another study by Sharma's group showed both anti-arthritic and anti-inflammatory strong effects of ginger oil (Sharma, 1994).

V. CONCLUSIONS

Although numerous synthetic drugs were developed for the treatment of gout but the safe and effective treatment paradigm is yet to be achieved. Plants such as *Andrographis paniculata, Cissus quadrangulari, Michelia champaca, Tinospora cordifolia, Zingiber officinale, Carica papaya* and *Tephrosia purpurea* were subjected to phytoconstituents and anti-gout activity analysis. Phytoconstituents such as alkaloids, flavonoids, polyphenolics, phytosterol, saponins, fixed oils and fats, carbohydrates, amino acids and proteins. Many types of highly useful phytoconstituents were found out from all the test plants and many of them were found to have many biological activities including anti-gout activity. Moreover, during the past few years many phytochemicals responsible for anti-gout effects have been isolated from the plants. Several phytoconstituents such as alkaloids, glycosides, flavonoids, and saponins, obtained from various plant sources that have been reported as potent anti-gout agents. The selected plants were also d identified to have one or more of these anti-gout chemicals. Many types of highly useful phytoconstituents were found out from all the test plants and many of them were found to have many biological activities including anti-gout activity. This was proved in their anti-gout activity. This study has clearly showed that the plants after purifying the phytoconstituents could effectively be used as anti-gout plant derived drug, which are free from side effects.

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