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# Phytochemical Screening of *Tanacetum Parthenium* L. (Feverfew) Leaves: An Important Medicinal Plant

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**ABSTRACT**

*Tanacetum parthenium* L. (Feverfew) is a medicinal plant belongs to Asteraceae family and it is a rich source of various bioactive secondary metabolites which are responsible for an array of therapeutic properties. In the present study, *T. parthenium* leaves were collected from Kodaikanal hills, Tamil Nadu, India and they were extracted using different solvents such as petroleum ether, hexane, chloroform, acetone and methanol. Qualitative phytochemical analysis of the prepared plant extracts revealed the presence of carbohydrates, proteins, phenols, flavonoids, tannins, terpenoids, phlobatannins, coumarins, steroids, quinones and cardiac glycosides. The same extracts were used for quantitative analysis of phenols, flavonoids, tannins, terpenoids, carbohydrates and proteins. Of the five extracts used for phytochemical screening, the methanolic extract showed the presence of maximum number of bioactive compounds. Among the quantitative analysis of six bioactive compounds, terpenoids (53.65 mg/g) and phenols (52.01 mg/g) were found at maximum concentrations.

## 1. INTRODUCTION

Medicinal plants are used to sustain healthy life, prevent diseases and cure ailments due to presence of various bioactive compounds. They can be used directly or in extracted forms for the treatment of various diseases[1]. Plant products can be derived from barks, leaves, flowers, roots, fruits and seeds and they have been a part of phytomedicines since time immemorial[2]. It has been estimated that even today, 80% of the world population rely on herbal medicines for their primary health care[3]. In world, India is among the twelve mega diversity countries with a rich source of various medicinal plants. Out of thirty-four hotspots recognized, India has two major hotspots, namely the Eastern Himalayas and the Western Ghats. India is the largest producer of medicinal herbs and is called the botanical garden of the world[4] and also the globe is now looking towards India for new drugs to deal with diverse diseases due to its prosperous biodiversity of medicinal plants. In recent years, scientific populaces have been interested to investigate the pharmacological behavior of medicinal plants. The most important bioactive constituents of plants are alkaloids, tannins, flavonoids, steroids, terpenoids, carbohydrates and phenolic compounds[5] with different activities, including anti-inflammatory, anticancer, antiviral, antibacterial and cardio protective activities. Crude extract from medicinal plants are more biologically active than isolated compounds due to their synergistic effects[6]. *Tanacetum parthenium* L. which is commonly known as Feverfew and is belong to Asteraceae family. It has been used for centuries as a medicinal herb for the treatment of fever, migraine prophylaxis and arthritis[7, 8]. It is also used for the treatment of various diseases like epilepsy, stomach ache, toothache and insect bites[9]. According to our knowledge on phytochemical screening, primary and secondary metabolite contents of different crude extracts obtained from Indian *T. parthenium* have never been studied before. For this reason, the present work is designed to investigate the qualitative and quantitative phytochemical analysis of *T. parthenium* leaves native to India.

## 2. MATERIALS AND METHODS

### 2.1. Collection and identification of plant material

Fresh leaves of the plant, *Tanacetum parthenium* L. were collected from kodaikanal hills, Tamil Nadu, India. The taxonomic identity of the plant was confirmed by Botanical Survey of India,

Southern circle, Coimbatore, Tamil Nadu. The plant materials were rinsed under running tap water to eradicate the surface pollutants and the leaves were air dried under shade. The dried leaves were powdered and stored at 4°C.

**2.2. Preparation of plant extracts**

Twenty grams of powdered leaf sample was used for extraction by a soxhlet system with 250 ml of different solvents like petroleum ether, hexane, chloroform, acetone and methanol successively for 24 h. The obtained extract was filtered using Whatman No.1 filter paper. Extracts were subsequently concentrated under reduced pressure and stored at 4°C for future studies.

**2. 3. Qualitative phytochemical analysis**

The extracts were tested qualitatively to find out the presence of various bioactive compounds using standard methods[10, 11].

**2.4. Quantification of primary metabolites**

**2.4.1. Total carbohydrate content**

The total carbohydrate content was measured by Hedge and Hofreiter method[12]. To 0.5 mL of plant extract or standard, added 0.5 mL of distilled water and 4 mL of anthrone reagent. The mixture was heated for 8 min in boiling water bath and cooled. The green color developed was read at 630 nm using UV/visible spectrophotometer. The carbohydrate content of the plant extract was calculated from the calibration curve of glucose and the results were expressed as 1g of glucose equivalents per mg of plant extract.

**2.4.2. Total protein content**

Protein content of the prepared extracts was measured by Lowry's method[13].To 1 mL of the plant extract or standard, added 5 mL of alkaline copper sulphate reagent, mixed well and allowed to stand for 10 min and then added 0.5 mL of Folins-Ciocalteau's reagent and mixed well. The reaction mixture was kept at room temperature under dark for 30 min. The blue color developed was read at 660 nm using UV/visible spectrophotometer. The protein content of the plant extract was calculated from the calibration curve of Bovine Serum Albumin (BSA) and the results were expressed as 1g of BSA equivalents per mg of plant extract.

## 2.5. Quantification of secondary metabolites

### 2.5.1. Total phenol determination

The total phenolic content was determined according to McDonald *et al* (2001)[14]. To 1 mL of plant extract or standard, 5 mL of Folin Ciocalteau reagent and 4 mL of sodium carbonate were added. The mixture was allowed to stand for 15 min under room temperature. The blue color formed was read at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated by calibration curve of gallic acid and the results were expressed as gallic acid equivalent (mg/g).

### 2.5.2. Total flavonoid determination

The total flavonoid content was determined according to Chang *et al* (2002)[15]. To 0.5 mL of plant extract or standard was mixed with 4.5 mL of methanol. 0.1 mL of 10% aluminium chloride and 0.1 mL of 1M sodium acetate were added to the mixture. Then the reaction mixture was kept at room temperature for 30 min. Then the absorbance at 415 nm was read by UV/visible spectrophotometer. The flavonoid content was calculated by calibration curve of quercetin and the results were expressed as quercetin equivalent (mg/g).

### 2.5.3. Total tannin determination

The total tannin content was determined by the method of Schanderl (1970)[16]. To 1 mL of the plant extract or standard was mixed with 0.5 mL Folin's phenol reagent and then added 5 mL of 35% sodium carbonate and the mixture was allowed to stand for 5 min at room temperature. The blue color produced was read at 640 nm using UV/visible spectrophotometer. The tannin content was calculated by calibration curve of gallic acid and the results were expressed as gallic acid equivalent (mg/g).

### 2.5.4. Total terpenoid determination

Total terpenoid content was determined by the method of Ghorai *et al* (2012)[17]. To 1 mL of the plant extract, 3 mL of chloroform was added. The sample mixture was thoroughly vortexed and left for 3 min and then 200 µl of concentrated sulfuric acid ($H_2SO_4$) was added. Then it was incubated at room temperature for 1.5h-2h in dark condition and during incubation a reddish brown precipitate was formed. Then carefully and gently, all supernatant of reaction mixture was decanted without disturbing the precipitation. 3 mL of 95% (v/v) methanol was added and

vortexed thoroughly until all the precipitation dissolve in methanol completely. The absorbance was read at 538 nm using UV/visible spectrophotometer. The total terpenoid content was calculated by calibration curve of Linalool and the results were expressed as Linalool equivalent (mg/g).

### 2.6. Statistical analysis

All measurements were carried out in triplicate and the results were presented as mean values ± SD. Statistical analyses were performed using a one-way analysis of variance ANOVA test and the significance of the difference between means was determined by Duncan's multiple range test. Differences at $P < 0.05$ were considered statistically significant. The SPSS 20.0 (Chicago, Illinois, USA) was used to perform statistical analysis.

## 3. RESULTS AND DISCUSSION

### 3.1. Phytochemical screening

Preliminary phytochemical screening of different solvent extracts of *T. parthenium* showed the presence of many types of chemical constituents such as steroids, flavonoids, carbohydrates, proteins, tannins, terpenoids, quinones, coumarins, steroids, phlobatannins, cardiac glycosides and phenols and the results are depicted in Table 1. Alkaloids, anthraquinones and saponins were absent in all the extracts. Preliminary analysis of phytochemicals is essential for the quantitative estimation of pharmacologically active chemical compounds[18].

**Table 1. Preliminary phytochemical screening of *Tanacetum parthenium* leaf extracts**

| Phytochemical constituents | Tests | Various solvent extracts | | | | |
|---|---|---|---|---|---|---|
| | | PE | HE | CH | AC | ME |
| Phenolics/tannins | $FeCl_3$ test<br>Lead acetate test | ++ | + | + | ++ | +++ |
| Flavonoids | Shinoda test | + | + | ++ | ++ | +++ |
| Alkaloids | Mayers test<br>Wagners test | - | - | - | - | - |
| Proteins/amino acids | Ninhydrin test<br>Biuret test | + | + | + | ++ | +++ |
| Carbohydrates | Molisch's test<br>Fehling's test | + | + | +++ | + | ++ |
| Steroids | Salkowski test<br>Libermann's test | - | + | + | ++ | ++ |
| Saponins | Foam test | - | - | - | - | - |

| | | | | | | |
|---|---|---|---|---|---|---|
| Terpenoids | Salkowski test | ++ | + | ++ | + | +++ |
| Cardiac glycosides | Keller-Killiani test | + | + | - | + | +++ |
| Quinones | Acid test | ++ | + | - | + | + |
| Anthraquinones | Borntrager's test | - | - | - | - | - |
| Coumarins | Base test | + | - | ++ | + | ++ |
| Phlobatannins | Precipitate test | - | - | + | + | + |

PE=petroleum ether extract; CH=Chloroform extract; HE= Hexane extract; AC=acetone extract; ME =methanol extract
+++= Copiously present; ++= moderately present; + =slightly present; - = absent.

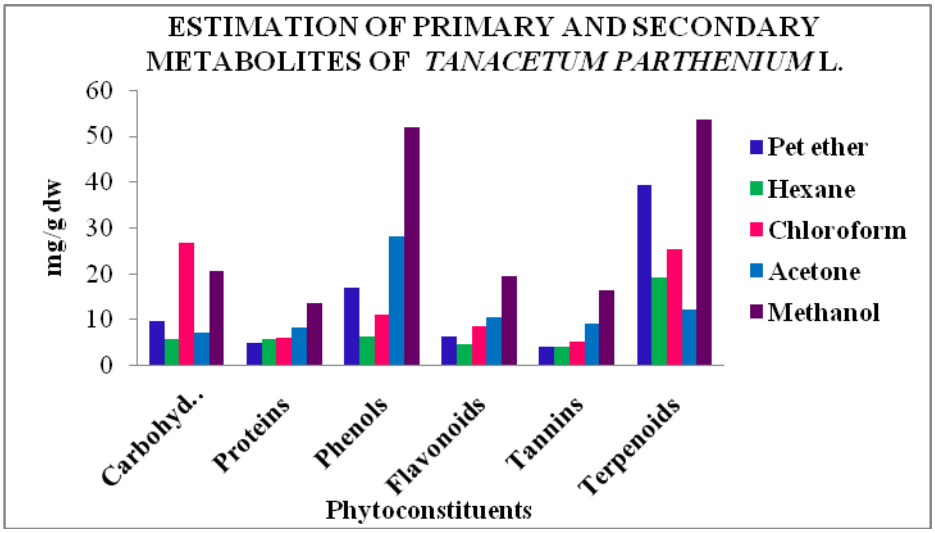
Among different solvents used for extraction, methanol was found an excellent extractor for majority of the bioactive compounds present in feverfew leaves. It indicates that methanol is effective solvent to extract various polar biological compounds due to its high polarity nature.

**Table 2.Quantitative phytochemical estimation of *T. parthenium* leaf extracts (mg/g)**

| Solvent extracts | Carbohydrates | Proteins | Phenols | Flavonoids | Tannins | Terpenoids |
|---|---|---|---|---|---|---|
| Petroleum ether | $9.87 \pm 0.25^{c}$ | $5.07 \pm 0.28^{e}$ | $16.93 \pm 0.10^{c}$ | $6.51 \pm 0.20^{d}$ | $4.29 \pm 0.24^{d}$ | $39.45 \pm 0.23^{b}$ |
| Hexane | $5.75 \pm 0.23^{e}$ | $5.81 \pm 0.19^{d}$ | $6.28 \pm 0.20^{e}$ | $4.73 \pm 0.23^{e}$ | $4.06 \pm 0.12$d | $19.35 \pm 0.48^{d}$ |
| Chloroform | $26.73 \pm 0.37^{a}$ | $6.14 \pm 0.31^{c}$ | $11.08 \pm 0.14^{d}$ | $8.60 \pm 0.17^{c}$ | $5.35 \pm 0.15^{c}$ | $25.33 \pm 0.31^{c}$ |
| Acetone | $7.28 \pm 0.22^{d}$ | $8.33 \pm 0.15^{b}$ | $28.21 \pm 0.44^{b}$ | $10.56 \pm 0.06^{b}$ | $9.32 \pm 0.22^{b}$ | $12.26 \pm 0.25^{e}$ |
| Methanol | $20.64 \pm 0.21^{b}$ | $13.62 \pm 0.13^{a}$ | $52.01 \pm 0.27^{a}$ | $19.64 \pm 0.15^{a}$ | $16.53 \pm 0.32^{a}$ | $53.65 \pm 0.15^{a}$ |

Mean values ± standard deviations with the same letters within the same column are not significantly different at p> 0.05.

Table 2 summarizes the results of various quantitative phytochemical analysis of different solvent extracts of *T. parthenium* leaves. The level of carbohydrate was found higher in chloroform extract (26.73 mg/g) and lower in hexane extract (5.75 mg/g). The statistical data indicates that protein concentration is higher in methanol (13.62 mg/g) extract. Carbohydrate and proteins were playing a vital role in the nutrient supplement for plant growth and herbivores. Plants defend themselves against microbial pathogens by various defends responses including production of antimicrobial proteins[19].




Polyphenols are heterogeneous group of secondary plant metabolites and it is an important constituent of human diet[20]. Phenol content was higher in methanol (52.01 mg/g) and lower in hexane (6.28 mg/g). The phenolic compounds are one of the major aromatic plant secondary metabolites which possess mainly free radical scavenging ability and also having several biological properties such as antiapoptosis, anti-aging, anti-cancer, anti-inflammation and anti-atherosclerosis[21]. The methanolic extract had recorded the highest content for flavonoids (19.64 mg/g) and lower content of flavonoids were noted in hexane extract (4.73 mg/g). Due to their free radical scavenging capacity, flavonoids might be useful for prevention and treatment of various human diseases [22]. The methanolic extract had recorded the highest content for tannin (16.53 mg/g) where as low content had recorded in hexane extract (4.06 mg/g). Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and cancer[23]. In the case of terpenoids, methanolic extract (53.65 mg/g) showed the maximum level. Terpenoids includes triterpenes, sesquiterpenes and diterpenes have been used as antibiotics, insecticidal, anthelmintic and antiseptic in pharmaceutical industry[24]. The earlier workers of *T. parthenium* estimated only two bioactive compounds such as phenols and flavonoids. The level of phenols (31 mg/g) [25] and flavonoids (1.05 mg/g) [26] were found to be lower when compared to the present investigation. The reason may be the extract they have used (hydroalcoholic extract) and they were estimated only these two phytocompounds quantitatively. In the present investigation, we found the higher concentrations of phenols and flavonoids in methanolic extract. In addition we have done fourteen qualitative tests and six quantitative tests.

## 4. CONCLUSION

The results of qualitative and quantitative phytochemical screening of *Tanacetum parthenium* revealed that a methanolic leaf extract is a potential source of various active phytoconstituents present in it and which contribute medicinal as well as physiological properties of the plants. Therefore, methanol extract from this plant could be seen as a good source for production of useful drugs in future. Before put into drug form the crude extract should be evaluated against various diseases.

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