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Research Article

PHYTOCHEMICAL EVALUATION AND *IN-VITRO* ANTIOXIDANT POTENTIAL OF WHOLE PLANT OF *TANACETUM PARTHENIUM* (L)

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ABSTRACT

In the current scenario, exploration is aimed at scrutinizing the phytochemicals for total phenol content, total Flavonoid estimation, antioxidant potentials obtained from natural origin. The study was focused on evaluation of antioxidant potential of various extracts of *Tanacetum parthenium* whole plant based on polarity. The total phenolic content and flavonoid content of Ethanolic extract of plant was found to be 32.91 ± 0.629 mg and 67.55 ± 1.170 mg of GAE and Quercetin equivalents respectively. Different *in-vitro* assays such as 2-2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, Nitric oxide scavenging activity and reducing power estimation were studied for the various plant extracts and measured spectroscopically. The Ethanolic extract of plant showed the highest antioxidant activity as measured by DPPH, nitric oxide scavenging activity with IC_{50} values of 197.543 ± 0.659 and 266.449 ± 0.761 respectively. A strong correlation was observed between antioxidant capacities and their total phenolic content indicated that phenolic compounds were a major contributor to antioxidant properties of plant extract. These results suggest that the Ethanolic extract of *Tanacetum parthenium* can constitute a promising new source of natural compounds with antioxidants ability.

Keywords: *Tanacetum parthenium*, Antioxidant activity, DPPH, Nitric oxide scavenging activity, FRAP

INTRODUCTION

ROS causes oxidative damage to cellular compartment that leads to cell injury and death. Scavenging reactive oxygen species (ROS) are superoxide, hydrogen peroxide and hydroxyl radicals that cause lipid peroxidation or damage to DNA or protein. This phenomenon leads to various health problems like heart diseases, carcinogenesis. Antioxidants quench lipid peroxidation and prevent DNA damage. Oxidative damage can be prevented by increase intake of antioxidants through diet. Prolonged usage of synthetic antioxidants produces serious toxicity, So, Researchers are now looking for natural antioxidants which do not have any side effects on human health. The search is underway to find out newer, effective and safe antioxidants, in order to use them in foods and pharmaceutical preparations to replace the synthetic ones.

Feverfew (*Tanacetum parthenium* L.) belonging to the family Asteraceae is a daisy-like perennial plant found commonly in gardens and along roadsides and is used for the treatment of various diseases such as arthritis and migraine in traditional medicine. The name stems from the Latin word *febrifugia*, "fever reducer." The first-century Greek physician Discords prescribed feverfew for "all hot inflammations." Also known as "feather few," because of its feathery leaves.[1-3] It is a short, bushy, aromatic perennial that grows 0.3–1 m in height. Its yellow-green leaves are usually less than 8 cm in length, almost hairless and pinnate–bi pinnate (chrysanthemum-like). Its yellow flowers bloom from July to October, are about 2 cm in diameter. They resemble those of chamomile (*Matricaria chamomilla*), for which they are sometimes confused and have a single layer of white outer-ray florets.[4-6] This plant contains various antioxidant compounds such as sesquiterpene lactones and various flavonoids.[7] Therefore, this study was conducted with the aim of investigating the various chemical constituents and analyzing antioxidant potential of the effects of various extracts of *Tanacetum parthenium* using *in-vitro* antioxidant assays.

MATERIAL AND METHODS

Collection of Plant material and extraction

The whole plant of *Tanacetum parthenium* was collected from the village of Manala, Rajanna Siricilla District, situated in the state of Telangana (India) and shade dried and powdered mechanically. The plant specimen was authenticated by botanist of Osmania University and authenticated voucher specimen Number 453 of the plant has been preserved in department for future reference. The dried plant powder was extracted with various solvents based on polarity (Pet ether, Chloroform, Ethyl acetate, Ethanol) by hot continuous extraction in Soxhlet's apparatus and method of maceration for aqueous extract. The extracts were evaporated to dryness under vacuum, dried in vacuum desiccators and stored in refrigerator.

Phytochemical Evaluation

Phytochemical investigation of alkaloids, saponins, Anthraquinones, carbohydrates, tannins, phenolics, flavonoids, proteins and amino acids, Terpenoids, Coumarins, steroids and

Quinones were carried out for various extracts of plant using standard protocols.

Total phenol content Estimation

The total phenolic content of *Tanacetum parthenium* was assessed using Foline-Ciocalteau phenol reagent method described by Singleton *et al*.[8] Briefly, 1.0 mL of the extract at various concentrations was mixed with 2.5 mL of 10% Foline-Ciocalteau reagent and 2.5 mL of 7.5% sodium carbonate. The contents were thoroughly mixed and allowed to stand for 30 minutes. The absorbance was read at 750 nm in a spectrophotometer. The total phenol content was expressed as gallic acid equivalents in milligram per gram of the extract.

Total flavonoid estimation

The flavonoid content of *Tanacetum parthenium* was determined using aluminium chloride colorimetric method described by Chang *et al*.[9] Briefly, 0.5 mL of the extract at various concentrations was mixed with 3 mL of 95% methanol, 0.1 mL of 10% (weight/volume) aluminium chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The reaction mixture was allowed to stand at room temperature for 30 minutes and absorbance was measured at 415 nm against a blank sample. A calibration curve was prepared using quercetin in methanol. The flavonoid content was expressed as quercetin equivalents in milligram per gram of the extract.

***In-vitro* antioxidant activity**

DPPH free radical scavenging activity

DPPH free radical scavenging activity is considered as one of the accurate and extensively used methods to find out the antioxidant potential of various natural products.[13] The antiradical potential of *Tanacetum parthenium* was determined spectrophotometrically as described by Ilahi *et al*.[10] Five different concentrations of various extracts of plant material (100, 200, 400, and 800 and 1000 µg/mL) were mixed with 100 µL of DPPH radical solution in a 96-well micro plate and incubated for 20 min at room temperature and absorbance of all samples were measured against blank at 517 nm by using Shimadzu UV-1800 spectrophotometer (Optima Tokyo, Japan). The absorbance of DPPH reagent alone was taken as control.

DPPH scavenging activity can be calculated by the following formula

$$\% \text{ free radical Scavenging activity} = (AControl - Asample)/(AControl) \times 100$$

Where *AControl* and *Asample* indicate the absorbance of the DPPH solution and the reaction mixture, respectively

The effective dose of plant extract needed to neutralize 50% of the DPPH radical solution (IC_{50}) was obtained from a plot comparing percent inhibition to extract concentration.

Nitric oxide scavenging activity

Nitric oxide radical scavenging activity was determined according to the method reported by Garrat.[11] Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess-Ilosvay reaction. 2 mL of 10 mM sodium nitroprusside in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of extract at various concentrations and the mixture incubated at 25°C for 180 min. From the incubated mixture 0.5 mL was taken out and added into 1.0 mL sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. finally, 1.0 mL naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min, the absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated.

Reducing power Determination

The Fe^{2+} reducing power of plant extract was determined by the method of Oyaizu[12] with slight modification. Various concentrations of plant extract (0.75 mL) was mixed with 0.75 mL of phosphate buffer (0.2 mole, pH 6.6) and 0.75 mL of potassium ferricyanide $K_3Fe(CN)_6$(1%w/v), followed by incubating at 50°C for 20 mins. The reaction was stopped by adding 2.5 mL of 10% (w/v) trichloroacetic acid followed by centrifugation at 3000 rpm for 10 min. Finally, 1.5 mL of the upper layer was mixed with 1.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%) and the absorbance was measured at 700 nm. Higher the absorbance of reaction mixture indicated greater the reducing power. Ascorbic acid is used as reference compound.

RESULT AND DISCUSSION

Phytochemical Constituents

Phytochemical screening was carried out for various extracts (Pet ether, Chloroform, Ethyl acetate, Ethanol and aqueous) by conducting preliminary tests. Based on results, Pet ether extract contains carbohydrates. Chloroform extract shows the presence of Alkaloids, carbohydrates, steroids and Tannins. Ethyl acetate shows the presence of Alkaloids, Phenols, steroids, Coumarins and Tannins Ethanolic extract shows the presence of Alkaloids, Phenols, Flavonoids, Terpenoids and Coumarins. Aqueous extract shows the presence of Carbohydrates and Coumarins. Results were shown in Table 1. '+' indicate the presence and '-' indicates the absence of phytoconstituents.

Table 1: Phytochemical Constituents in various extracts of *Tanacetum parthenium*

	Pet Ether	Chloroform	Ethyl acetate	Ethanol	Aqueous
Alkaloids	-	+	+	+	-
Saponins	-	-	-	-	-
Anthraquinones	-	-	-	-	-
Carbohydrates	+	+	-	-	+
Phenols	-	-	+	+	-
Flavonoids	-	-	-	+	-
Steroids	-	+	+	-	-
Proteins and Amino acids	-	-	-	-	-
Terpenoids	-	-	-	+	-
Coumarins	-	-	+	+	+
Quinones	-	-	-	-	-
Tannins	-	+	+	-	-

Total phenol content Estimation

Phenolic compounds are the key phytochemicals with high free radical scavenging activity. It has generated a great interest among the scientists for the development of natural antioxidant compounds from plants. In the current work, phenolic content of the Ethyl acetate and Ethanolic extracts of *Tanacetum parthenium* were estimated at 25.33 ± 0.877 and 32.91 ± 0.629 respectively. Extracts of *Tanacetum parthenium* were measured and listed in (Table 2). The Ethanolic extract of *Tanacetum parthenium* showed higher number of phenolic compounds when compared to other extracts. The concentration of the phenolic compounds was increased with an increase in the dose. The results are expressed as Gallic acid equivalents in mg /gm extract (GAE) (Figure 1).

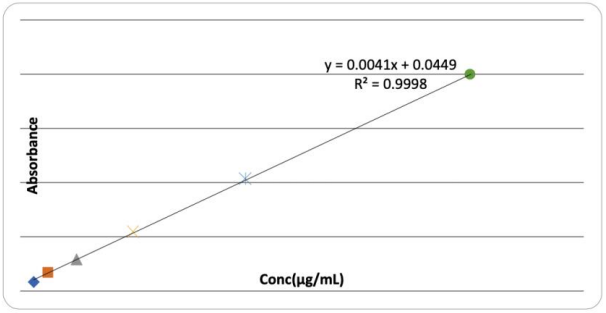

Figure 1: Calibration Curve of Gallic acid

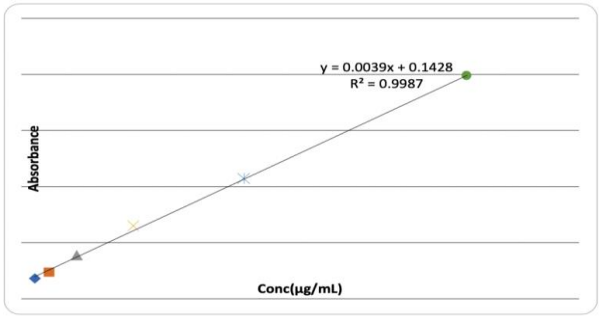

Figure 2: Calibration curve of Quercetin

Total Flavonoid estimation

The flavonoid content of various extracts of *Tanacetum parthenium* was determined using aluminium chloride colorimetric method. In the current study, the total flavonoid content was measured and shown in Table 2. The total flavonoid content was found only in Ethanolic extract, as Flavonoids were not seen in other extracts. The flavonoid content was expressed as quercetin equivalents in milligram per gram of the extract (Figure 2).

Table 2: Total Phenolic and Total Flavonoid content in various extracts of *Tanacetum parthenium*

S. No.	Extract	Total Phenolic Content (Gallic acid equivalents in mg /gm extract)	Total Flavonoid Content (Quercetin equivalents in mg/ gm extract)
1	Pet Ether	----------	---------
2	Chloroform	----------	----------
3	Ethyl Acetate	25.33 ± 0.877	----------
4	Ethanol	32.91 ± 0.629	67.55 ± 1.170
5	Aqueous	----------	----------

DPPH free radical scavenging activity

DPPH antioxidant assay is based on the ability of DPPH, a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. Upon reaction with suitable reducing agent, it decolorizes stoichiometrically with the number of electrons which is measured spectrometrically at 517 nm. DPPH radical scavenging activity of various extracts of *Tanacetum parthenium* in comparison with ascorbic acid are reported in (Figure 3). IC_{50} values for the various extracts were calculated using Graph Pad Prism 8.3.1 and mentioned in (Table 3) and (Figure 4). Ethanolic Extract showed better antiradical activity than the remained extracts.

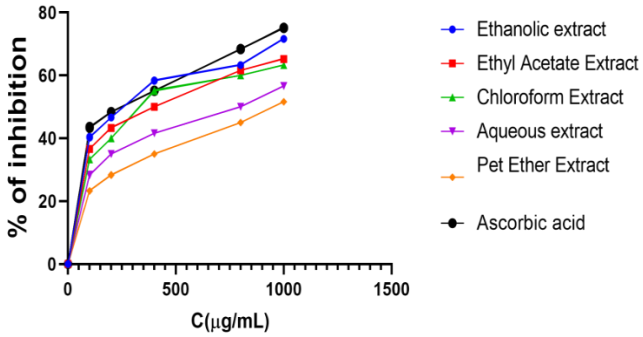

Figure 3: DPPH free radical scavenging activity of various extracts of *Tanacetum parthenium*

Nitric Oxide scavenging activity

Nitric Oxide is a potent pleiotropic mediator of physiological processes playing vital role in various biological systems. The nitric oxide scavenging activity of various extracts of *Tanacetum parthenium* in comparison with ascorbic acid are reported in (Figure 5). IC_{50} values for the various extracts were calculated using Graph Pad Prism 8.3.1 and mentioned in (Table 3) and (Figure 6).

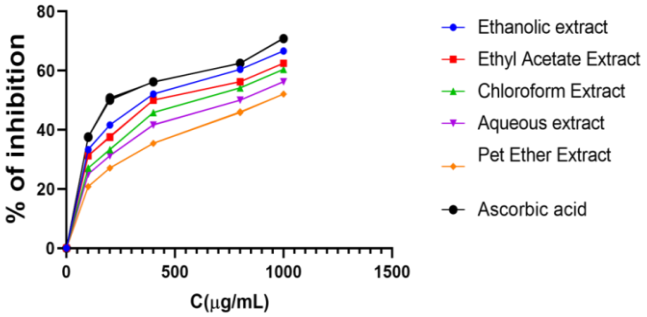

Figure 5: The nitric oxide scavenging activity of various extracts of *Tanacetum parthenium*

Table 3: IC_{50} values for the various extracts of *Tanacetum parthenium*

S. No.	Extract	DPPH Method	Nitric oxide scavenging activity
1	Pet Ether	947.975 ± 0.320	936.112 ± 1.457
2	Chloroform	262.640 ± 1.220	708.246 ± 0.252
3	Ethyl Acetate	255.656 ± 0.883	331.645 ± 2.663
4	Ethanol	197.543 ± 0.659	266.449 ± 0.761
5	Aqueous	885.561 ± 0.364	838.942 ± 0.740
6.	Ascorbic acid	194.99 ± 1.258	193.420 ± 2.099

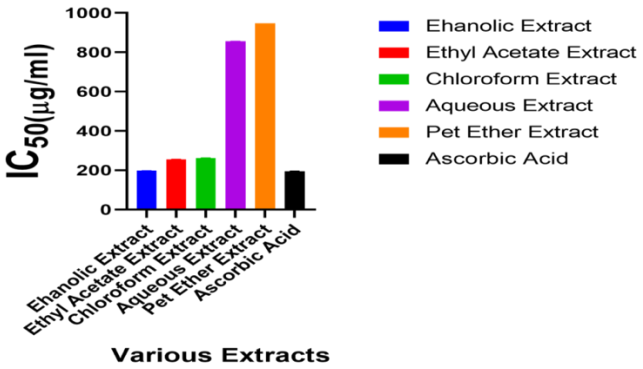

Figure 4: IC_{50} values for the various extracts of *Tanacetum parthenium* by DPPH free radical scavenging activity

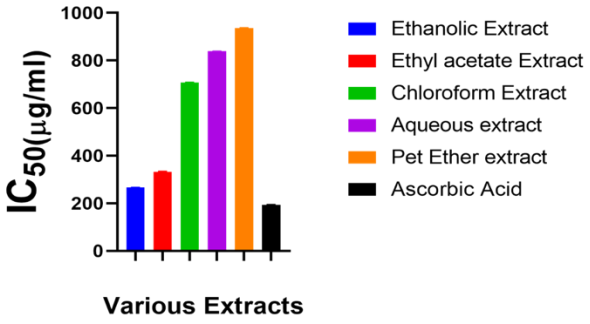

Figure 6: IC_{50} values for the various extracts of *Tanacetum parthenium* by Nitric oxide scavenging activity

Reducing Power

Reducing power experiment is a good reflector of antioxidant activity of the plant. The plant having high reducing power generally reported to carry high antioxidant potential too. In this experiment, Ferric ions are reduced to ferrous ions which are identified by colour change from yellow to bluish green. The results for ferric reducing power activity of various extracts of *Tanacetum parthenium* in comparison with ascorbic acid are reported in (Figure 7). Ethanolic extract showed high reducing power than that of other extracts. Reducing power potential of extracts increase with the dose, however the extracts exhibited low reducing power than that of ascorbic acid.

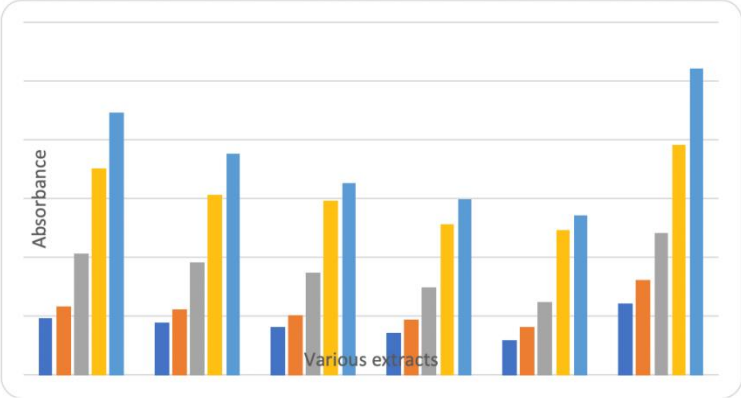

Figure 7: Reducing power of Various Extracts of *Tanacetum parthenium*

CONCLUSION

Antioxidant activity is a complex procedure usually happening through several mechanisms and is influenced by many factors, which cannot be fully described with one single method. Therefore, it is essential to perform more than one type of antioxidant capacity measurement to take into an account the various mechanisms of antioxidant action. In this study, three complementary tests were used to assess the antioxidant activity of *Tanacetum parthenium.* The findings obtained in this study support the traditional uses of plant species as therapeutic agents. The higher antioxidant potential of *Tanacetum parthenium* is conferred by their high phenolic and Flavonoid content. Ethanolic extract of *Tanacetum parthenium* has shown the highest TPC, TFC and antioxidant capacity values. Inaddition, there exists a good correlation between phenolic content, Flavonoid content and antioxidant capacity of the Ethanolic extract. These results suggest that the Ethanolic extract of *Tanacetum parthenium* constitutes a valuable source of antioxidant metabolites.

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