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ORIGINAL ARTICLE

Phytochemical Stability and Anti-Oxidant Efficacy of Feverfew (*Tanacetum parthenium*) Leaves with Therapeutic Implications for Migraine

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ABSTRACT

Oxidative stress and inflammatory mechanisms are significant causes in the development of migraine, a crippling neurological condition. Herbal drugs with antioxidant properties are being studied more and more as possible supplemental treatments because they are safe and work on multiple targets. Feverfew (Tanacetum parthenium) has been used for a long time to cure migraines, but pharmaceutical standards necessitate a full evaluation of its phytochemical stability and antioxidant efficiency. This study examined the phytochemical contents, stability profile, and in vitro antioxidant activity of feverfew leaf ethanolic extract. The dried feverfew leaves were macerated in ethanol, and then the extract was concentrated. Qualitative phytochemical screening and organoleptic analysis were done to find the key bioactive components. Stability studies are performed to assess the impact of humidity and temperature on phytochemical integrity under storage conditions recommended by the ICH and WHO. The DPPH free radical scavenging assay was applied to measure antioxidant activity, and ascorbic acid was used as the reference standard. Phytochemical study of the ethanolic extract found sesquiterpene lactones, flavonoids, phenolic acids, volatile oils, sterols, tannins, alkaloids, and glycosides. Stability tests showed that phytochemical stability can only be maintained in controlled storage conditions. When compared to ascorbic acid, the extract had a low anti-oxidant capacity and DPPH radical scavenging activity that depended on the concentration. The antioxidant effect is due to the presence of phenolic compounds and flavonoids that can neutralise free radicals. The results indicate feverfew ethanolic extract has strong antioxidant activity and stable phytochemical constituents, which supports its pharmaceutical significance in the development of herbal formulations for migraine prevention.

Keywords: *Tanacetum parthenium*; Feverfew; Antioxidant activity; DPPH assay; Phytochemical stability; Migraine

INTRODUCTION

Migraine is a persistent neurological condition indicated by frequent episodes of modest to severe headache, repeatedly associated by nausea, vomiting, photophobia and phonophobia[11, 12]. Because of its high prevalence, repeated nature, and substantial impact on quality of life, it constitutes a major public health concern[11, 12]. Although there are a number of pharmaceutical treatments for migraines, their long-term usage is frequently restricted by side effects, inconsistent effectiveness, high cost, and the possibility of medication overuse headaches[11, 12]. These difficulties have prompted research into safer and more potent alternative treatments, especially herbal remedies[1-3, 6].

PLANT DESCRIPTION

Feverfew (*Tanacetum parthenium L.*), a perennial plant that mimics daisies and is frequently found in gardens and at the sides of roads, belongs to the Asteraceae family[10, 11]. Febrifugia, which means "fever reducer," is a Latin word[10,

[11]. In the first century AD, the Greek physician Dioscorides prescribed feverfew for "all hot inflammations[10, 11]." It is additionally referred to as "featherfew" owing to its feathery leaves. It is a little, bushy, fragrant perennial that mature to a height of 0.3-1m[10, 11]. Like chrysanthemums, its yellow-green, nearly hairless leaves are pinnate to bipinnate and usually measure less than 8 centimeters in length[10, 11]. From July to October, it produces a yellow bloom with a diameter of 2 cm[10, 11].

TAXONOMICAL CLASSIFICATION

Kingdom: plantae (plants)

Subkingdom: Trachiobionta (vascular plants)

Superdivision: spermatophyta (seed plants)

Division: Mangliophyta (flowering plants)

Class: Magnoliopsida (Dicotyledons)

Subclass: Asteridae

Family: Asteraceae (Aster family)

Order: Asterales

Genus: Tanacetum (tansy)

Species: *Tanacetum parthenium* (Feverfew)

ETHNOMEDICAL APPLICATIONS OF FEVERFEW

Feverfew, or *Tanacetum parthenium*, has traditionally been used in European and mediterranean medicine, especially to treat migraines and persistent headaches. In order to avoid headache episodes, the leaves were frequently eaten either fresh or dried. In the past, feverfew was also used as a febrifuge to lower fever and as an anti-inflammatory treatment for ailments including rheumatism and arthritis. It is also used to manage toothaches, menstruation problems, and digestive issues. It has also been applied externally to treat skin irritation and insect bites. Current research on its antimigraine, anti-inflammatory, and antioxidant properties is based on these ethnomedical uses.

PHYTOCHEMISTRY

Tanacetum parthenium comprises a variety of bioactive phytoconstituents that contribute to its pharmacological characteristics, especially when it pertains to treating migraines. Sesquiterpene lactones, flavonoids, phenolic compounds, volatile oils, and other secondary metabolites are among the main ingredients. Parthenolide, a sesquiterpene lactone, is the most significant and distinctive component of feverfew and is regarded as the primary bioactive marker. There have also been reports of similar sesquiterpene lactones as chrysanthemonin, costunolide, and artecanin. The anti-inflammatory, anti-oxidant, neuroprotective characteristics of these substances were well recognized.

Additionally, feverfew is rich in flavonoids, including apigenin, luteolin, quercetin, and kaempferol, which have anti-oxidant and free radical scavenging activities. Some phenolic acids that boost its antioxidant power are ferulic acid, caffeic acid, and chlorogenic acid. The plant also has volatile oils that may make its pain relieving and anti-inflammatory effects stronger. Camphor, borneol, chrysanthenyl acetate, and β-caryophyllene make up most of these oils. Feverfew has also been found to have small amounts of sterols, tannins, glycosides, and alkaloids.

Anti-oxidant activity and phytochemical stability of *Tanacettum parthenium*

The bioactive components of Tanacetum parthenium (feverfew), like Parthenolides, flavonoids, and Phenolic acids, are what make the plant excellent for medicine. They are very good at fighting inflammation and free radicals. Many phytochemicals can break down chemically and lose some of their biological activity because they are sensitive to changes in the environment and how they are processed, such as temperature, light, humidity, and how they are stored. Losing stability may make feverfew antioxidant activity less effective. This is an important way that it lowers oxidative stress related neuroinflammation that is associated to the development of migraines.

In vitro experiments including DPPH, ABTS, FRAP, and hydrogen peroxide scavenging reveal that feverfew antioxidant activity is strongly related to the well-being of these phytochemicals. Feverfew protects against oxidative damage in many ways.

It works by getting rid of reactive oxygen species, stopping lipid peroxidation, binding to transition metals, and making the body's own antioxidant enzymes work better. To make sure that herbal remedies based on feverfew work all the time, we need to test both the stability of the phytochemicals and the effectiveness of the antioxidants. This will give us a good reason to make them all the same and put them together.

MATERIALS AND METHODS

Collection of Plant Material

The whole Feverfew plant came from a nursery in Tadepalli, Vijayawada, Andhra Pradesh. A botanist cleaned, shade dried and checked the plant material before it was utilised in more studies.

Drying of Plant Material

The fresh leaves of Tanacetum parthenium were thoroughly rinsed with water after cleaning to remove any extraneous material. They were then arranged in a single layer and left to dry in the shade at room temperature, ensuring adequate air circulation. This method protected the thermolabile phytoconstituents from direct sunlight. The drying process continued until the leaves attained the desired weight. Subsequently, the dried leaves were stored in airtight containers for future use.

Blending

We used a clean mechanical blender to grind fully dried feverfew leaves into a coarse powder that was all the same. To keep the plant material's phytochemical integrity, care was taken to keep the heat from getting too high while mixing. They put the mixed mixture into containers that were airtight and left it there until they could do something else with it.

Extraction of plant material

A precise amount of 100 g of dried, powdered feverfew leaves were macerated and put in a clean, amber-colored glass container. We soaked the leaf powder in 500 mL of ethanol (1:5 w/v) to fully extract the active ingredients. The container was tightly sealed and kept at room temperature (25-28°C) for 72 hours, with occasional gentle shaking 8-12 hours to enhance solvent penetration. After the maceration phase Whatman No. 1 filter paper was used to filter the mixture so as to separate the liquid extract from remaining plant material (marc). In order to eliminate the ethanol and produce a semi-solid or dry extract, the filtrate was further condensed under low pressure at a low temperature using a rotary evaporator. The finished extract was stored in airtight amber containers at 4°C until more phytochemical analysis or experimental use could be done.

Organoleptic evaluation of feverfew powder

Organoleptic evaluation is a basic and very important way to check the quality of herbal materials for identification and standardisation. Before doing more in-depth investigation, it helps to check the powder's legitimacy and purity by looking at its sensory properties with human senses. The fundamental sensory profile of feverfew herb powder was ascertained in the present study by the assessment of its organoleptic properties. The dried plant was pulverised into a fine powder and looked at in a normal lab setting.

The inspection checked the item's colour, taste, texture, smell, and overall appearance. We looked at the powder in good light to see if the colour was consistent, and if there were any signs of fading, it could mean it was damaged or mixed with something else. We carefully smelt the sample to determine whether it had a distinctive aroma compared to the others, noting whether it was sweet or strong. As part of our routine safety checks, we tasted small amounts of the paste to identify any unpleasant or unusual flavours. We rubbed a small quantity of the powder between our fingers to assess its texture, observing whether it felt rough, smooth, or fine. Additionally, we examined the entire sample to ensure it was not wet, lumpy, or contained any foreign substances. This organoleptic test provides valuable insights into the quality of herbal medicines, aiding in their identification, standardisation, and quality assessment. The findings from this evaluation could serve as a basis for further research and allow for comparisons with established regulations or pharmacopoeial standards.

PHYTOCHEMICAL SCREENING

Qualitative phytochemical tests

Test for Parthenolides

Salkowski Test (for sesquiterpene lactone): In a cleaned test tube pour 2 ml of extract and Put in 2 ml of chloroform and shake it gently. Carefully add 1 ml of strong sulphuric acid to the side of the test tube without shaking it. Let the mix sit for a bit. There was a reddish-brown or yellowish-brown layer at the interface that showed parthenolides were there.

Baljet Test (specially for lactone ring): To the 2 ml of extract add 1 ml of sodium picrate solution. After combining let it stand for 2-3 minutes. The colour changes from orange to reddish. Confirms the presence of molecules that include lactone, which helps find parthenolides.

Legal's Test: Add 1 ml of pyridine and 1 ml of sodium nitroprusside solution to the 1 ml of extract and shake it gently. Add two to three drops of a 10% sodium hydroxide solution to make the medium alkaline. A pink or red colour means that parthenolide (an unsaturated lactone nucleus) is present.

Test for Flavonoids

Shinoda Test: Add a little amount of magnesium turnings and 2 drops of strong hydrochloric acid to 2 ml of extract. Let the mixture sit for 2 to 3 minutes so that it can fully react. The pink, red, or orange colour shows that flavonoids (flavones and flavone's) are present.

Alkaline reagent Test: Put 2–3 drops of sodium hydroxide solution into 1 ml of extract. Watch how the hue changes. Put in a few drops of hydrochloric acid that has been watered down. NaOH causes color changes to yellow. Adding acid makes the colour go away. Confirms that there are flavonoids.

Lead acetate Test: Put 1 mL of the extract into a test tube and add a few drops of a 10% lead acetate solution. Precipitate that is either white or yellow in color. It reveals that there are flavonoids.

Zinc-Hydrochloride Reduction Test: Put a small amount of zinc dust into the 2ml of extract. Wait five minutes after adding the diluted hydrochloric acid. The red colour shows that flavonoids are present.

Table 1: Stability studies

Intended Storage Conditions	Types of Stability Studies	Storage Conditions					
		ICH			WHO		
		Temperature (°C)	RH (%)	Time (Months)	Temperature (°C)	RH (%)	Time (Months)
Room temperature	Long Term	25±2℃	60±5%	12	30±2℃	65±5%	12
	Intermediate	30±2℃	65±5%	6	--	--	
	Accelerated	40±2℃	75±5%	6	40±2℃	75±5℃	6
Refrigerator	Long Term	5±3℃	--	12	5±3℃	--	12
	Accelerated	25±2℃	60±5%	6			
Freezer	Long Term	-20±5℃	--	12	-20±5℃		12

Test for Phenolic acids

Ferric chloride Test: Put 1–2 ml of extract into a clean test tube. place in 2 to 3 drops of freshly produced 5% ferric chloride solution. Blend gently. Development of blue, green or violet color indicates the existence of phenolic acids.

Test for Volatile oils

Spot (Grease) Test: Put a few drops of feverfew extract on a clean, dry piece of filter paper. Let the paper dry at room temperature. Put the paper close to a heat source so it warms up slowly. There is typically a clear spot that doesn't last long. When you warm up, the spot fades away. It shows that there are some unstable oils there.

Test for Sterols

Liebermann-Burchard Test: Put 2ml of extract in a test tube that is dry. Add 2 ml of chloroform to the extract to make it completely dissolve. Mix with 1 ml of acetic anhydride and shake gently. Carefully add 1 ml of strong sulphuric acid to the side of the test tube. Do not disturb the mixture. A bluish-green, blue, or green colour shows that triterpenoids and sterols are present.

Sulphur powder Test: Put a little bit of sulphur powder into the extract. Shake it up well and let it warm up slowly. Sulphur tends to sink when sterols are present.

Test for Tannins

Ferric chloride Test: Put 2–3 drops of the freshly made 5% ferric chloride solution into 2 ml of extract. Stir gently, then look at the colour right away. The blue black colour shows that there are hydrolysable tannins present. You can see condensed tannins in either a greenish-black or a green colour.

Gelatin Test: Mix 1 mL of extract with 1 mL of a 1% gelatin solution that has sodium chloride in it. Shake it gently and let it stand. The formation of white precipitate shows that tannins are there.

Test for Alkaloids

Dragendroff's Test: Add 2–3 drops of diluted hydrochloric acid to 2 mL of the extract and shake thoroughly. Add 2–3 drops of Dragendorff's reagent to this. Existence of alkaloids was confirmed the formation of reddish-brown or orange precipitate.

Mayer's Test: Add diluted HCl to the 1 ml of extract to make it acidic. Then add2-3 drops of mayers reagent. Alkaloidal ingredients are approved by the development of pale yellow or cream precipitate.

Test for Glycosides

Keller-Killiani Test: To the 2 ml of extract add 1 ml of glacial acetic acid in a clean test tube which have tiny bit of ferric chloride in it. Carefully add 1 ml of concentrated sulphuric acid down the edge of test tube without shaking. Brown or reddish-brown ring is formed at the junction of 2 layers confirms the glycosides detected.

Borntrager's Test: Mix 2 ml of the extract with some sulphuric acid. Warm up slowly, then cool down. Use chloroform to get rid of the organic layer, then throw it away. Add a mild solution of ammonia to the chloroform layer. The pink to red colour of ammonical layer shows that glycosidic parts are there.

Antioxidant activity assay

DPPH Free Radical Scavenging Assay

Principle of the DPPH Assay:

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a quick, easy, and popular in-vitro technique for assessing plant extracts' capacity to scavenge free radicals. The stable free radical DPPH has a strong violet/purple color. Strong absorbance is seen at 517 nm. Antioxidants donate hydrogen atoms or electrons to DPPH. This turns DPPH from violet to yellow by reducing it to diphenylpicrylhydrazine. The

extract's antioxidant capacity is indicated by the extent of discolouration.

Chemicals and reagents:

- DPPH(2,2-diphenyl-1-picrylhydrazyl)
- Ethanol (analytical grade)
- Powdered feverfew ethanolic extract
- Common antioxidants: Ascorbic acid/gallic acid
- Distilled water

Preparation of DPPH Solution:

- DPPH is prepared at 0.1mM concentration in ethanol
- The solution should appear deep violet
- Prepared freshly and protected from sunlight

Experimental procedure:

1. Prepare different concentrations of feverfew ethanolic extract (for example: 20,40,60,80,100µg/ml)
2. Take 1ml of each extract concentration
3. Add 1 ml of DPPH solution (0.1mM)
4. For control:

   - 1 ml DPPH + 1 ml ethanol (without extract)

5. For standard:

   - Replace extract with standard antioxidant at similar concentrations

6. Incubate all samples at room temperature for 30 minutes in dark conditions
7. Measure absorbance at 517nm using UV-Visible spectrophotometer.

Calculation of anti-oxidant activity

Percentage radical scavenging activity (%RSA)

$$\% \text{ DPPH Scavenging activity} = \frac{A_{control} - A_{sample}}{A_{control}}$$

$A_{control}$ = Absorbance of the DPPH solution without the test sample (control)

A_{sample} = Absorbance of the DPPH solution with the test sample (e.g., feverfew extract)

Determination of IC_{50} Value

- IC_{50} = Concentration of extract required to inhibit 50% of DPPH Radicals.
- Plot: 0 Concentration (µg/ml) on X-axis; 0 % scavenging activity on Y-axis.
- Lower IC_{50} value= higher antioxidant activity.

RESULTS

Table 2: Phytochemical characteristics of feverfew extract

Phytochemical constituent	Ethanol extract	Water extract
Parthenolide	+	+
Flavonoids	+	+
Phenolic acids	+	-
Volatile oils	+	-
Sterols	+	-
Tannins	+	-
Alkaloids	+	+
Glycosides	+	+

DPPH scavenging activity

The feverfew extract showed considerable radical scavenging activity at lower dosages (20 µg/mL), which got stronger as the concentration went up. The highest inhibition observed at 100 µg/mL suggests the presence of bioactive phytoconstituents capable of donating hydrogen atoms or electrons to neutralise DPPH free radicals.

The reference standard, ascorbic acid, demonstrated much greater scavenging activity than the feverfew extract at all tested dosages. But a similar behaviour showed that the extract has antioxidant properties.

The feverfew ethanolic extract exhibited an above-average IC_0 value compared to ascorbic acid, signifying modest yet significant antioxidant activity. Flavonoids, phenolic compounds, and sesquiterpene lactone may be responsible for why feverfew have the scavenging effect that was detected.

The IC_{50} value of the erthanolic extract of Tanacetum parthenium was calculated to be approximately 53.5 µg/mL, whereas the reference standard ascrorbic acid exhibited an IC_{50} value of approximately 30.0 µg/mL. The lower IC_{50} value of ascorbic acid indicates a stronger free radical scavenging activity than the feverfew extract.

Table 3: Stability study results

Intended Storage Conditions	Types of Stability Studies	Storage Conditions						Observation
		ICH			WHO			
		Temperature (°C)	RH (%)	Time (Months)	Temperature (°C)	RH (%)	Time (Months)	
Room temperature	Long Term	25±2°C	60±5%	12	30±2°C	65±5%	12	Minor degradation, stable, within acceptable limits
	Intermediate	30±2°C	65±5%	6	--	--		Moderately Stable
	Accelerated	40±2°C	75±5%	6	40±2°C	75±5°C	6	Significant degradation, unstable under stress conditions
Refrigerator	Long Term	5±3°C	--	12	5±3°C	--	12	Highly stable, negligible degradation
	Accelerated	25±2°C	60±5%	6				Slight degradation, stable
Freezer	Long Term	-20±5°C	--	12	-20±5°C		12	Extremely stable

Table 4: DPPH radical scavenging activity of ethanolic extract of *Tanacetum parthenium* compared with ascorbic acid

Concentration (µg/ml)	% Inhibition (*Tanacetum parthenium* extract)	% Inhibition (Ascorbic acid)
20	22.5±1.2	41.2±1.0
40	38.7±1.5	58.9±1.3
60	55.4±1.8	72.3±1.6
80	68.9±2.1	85.6±1.9
100	79.6±2.4	94.1±2.0

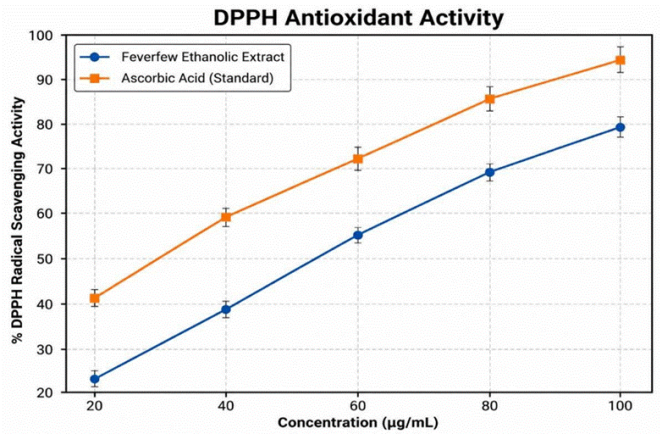


Fig. 1: This graph illustrates how feverfew ethanolic extract's concentration-dependent DPPH radical scavenging activity compares to ascorbic acid

However, the extract demonstrated appreciable antioxidant activity in a concentration-dependent manner, supporting the presence of bioactive phytoconstituents such as flavonoids and phenolic compounds.

Feverfew extract: 50% inhibition lies between 40 µg/mL (38.7%) and 60 µg/mL (55.4%).

Calculated $IC_{50} \approx 53.5$ µg/mL.

Ascorbic acid: 50% inhibition lies between 20 µg/mL (41.2%) and 40 µg/mL (58.9%).

Calculated $IC_{50} \approx 30.0$ µg/mL.

DISCUSSION

The results show that the ethanolic extract of feverfew leaves has a lot of different phytochemicals, like flavonoids, phenolic acids, sesquiterpene lactones, tannins, alkaloids, glycosides, sterols, and volatile oils. This means that it has a lot of compounds that are good for your health. Stability studies demonstrated that phytochemicals remain stable solely under controlled temperature and humidity conditions. This shows how important it is to put things away in the right way. The DPPH test showed that the substance could get rid of free radicals, but not as well as vitamin C. This means that it can fight free radicals to some extent, mostly because it has phenolic and flavonoid compounds. These findings substantiate the notion that feverfew serves as a natural source of antioxidants that may alleviate conditions associated with oxidative stress, such as migraines.

CONCLUSION

The present study demonstrates that the ethanolic extract of feverfew leaves contains diverse bioactive phytoconstituents, such as flavonoids, phenolics, and sesquiterpene lactones, which augment its antioxidant activity. The extract showed that it could scavenge free radicals in a concentration-dependent way, but not as well as the standard. Research on stability shows that active compounds can become less stable when the temperature or humidity changes. Overall, these results support the idea that feverfew could be a natural source of antioxidants. The results also support its traditional use with scientific evidence and point to the need for more research on how to make it and use it as a treatment for conditions related to oxidative stress.

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