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Radical scavenging and antioxidant activities of ethanolic and aqueous extract from the leaves of feverfew (*Tanacetum parthenium* L.) and a synthetic compound parthenolide

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Abstract
The herb Feverfew (*Tanacetum parthenium* L.) (Asteraceae) is a medicinal plant traditionally used in the treatment of fevers, migraine headaches, rheumatoid arthritis, stomach aches, toothaches, insect bites, infertility and problems with menstruation. Parthenolide, a gemacranolide-type of sesquiterpene lactone, is the major constituent of feverfew leaves. In our present study the highest antioxidant and radical scavenging activities of the *Tanacetum parthenium* extracts and Parthenolide in terms of their free radical-scavenging activities have been studied. The DPPH radical showed maximum inhibition of aqueous extract at 67.90%, ABTS radical showed maximum inhibition of Parthenolide at 87.10% and the total Antioxidant Capacity of Parthenolide was found to be 4.57mM/L equivalent to ascorbic acid. Fe^{2+} chelating capacities of ethanolic extract were 0.135µg/ml which was highly effective with lower EC_{50} value. Superoxide radical generated by the photo-oxidation of methionine-riboflavin and their scavenging property showed maximum inhibition of aqueous extract at 68.88%. However, the efficiency of the extract differed against various free radicals depending on the specific assay methodology. The results obtained from this study reveal that Parthenolide, ethanolic and aqueous extract of *T. parthenium* showed effective free radical scavenging activity. Thus the results support its traditional use in ailments and as a source of natural antioxidants which protect cells against oxidative stress.

Keywords: Antioxidant, DPPH, ABTS, Feverfew, Parthenolide.

1. Introduction
Feverfew (*Tanacetum parthenium* L.) belonging to the family Asteraceae (daisies) is a daisy-like perennial plant found commonly in gardens and along roadsides. The name stems from the Latin word febrifugia, means “fever reducer.” The first-century Greek physician Dioscorides prescribed feverfew for “all hot inflammations.” It’ also known as “feather few,” because of its feathery leaves [1-3]. The herb feverfew (*Tanacetum parthenium* L., Asteraceae) has an ancient reputation as an effective anti-inflammatory, analgesic, antipyretic, and anti-asthmatic agent [4]. After its reemergence over the last 2 decades, it is recommended and accepted for migraine prophylaxis [5]. Parthenolide a gemacranolide-type sesquiterpene lactone, is the major constituent of European feverfew, (*Tanacetum parthenium* L.), and several other members of the Asteraceae and Magnoliaceae families [6].
The oxidation induced by ROS can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver injury and cardiovascular disease [7]. Although the body possesses such defence mechanisms, as enzymes and antioxidant nutrients, which arrest the damaging properties of ROS [8]. Continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radicals in the body beyond its capacity to control them, and cause irreversible oxidative damage [9]. Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and therapeutics of diseases.

2. Material and Methods
2.1 Plant materials
The entire experiment was carried out at the Central Research Laboratory (CRL), Nitte University, Mangalore, India.
The Ethanolic and Aqueous extract of the leaves of *T. Parthenium* was obtained from Organic Inc. China in March 2014. The leaf extracts are stored in air tight container for future reference. The synthethic compound parthenolide (98% min; HPLC graded) was obtained from Shanghai Better BioChem Co., Limited China.

2.2 Chemicals for Biological Activities
All the chemicals for the present study were obtained from Sigma Aldrich chemicals, USA.

2.3 Instrumentation
A Genesys UV/VIS spectrophotometer was used in all spectrophotometric assays

2.4 Phytochemical analysis
Test for various components like Carbohydrates, Aminoacids, Lipids, Alkaloids, Tannins, Saponins, Flavonoids, Terpinoids, Glycosides, Steroids/Quinones and Phenols were carried out in ethanolic and aqueous leaves extract of *T. parthenium* to identify various constitutes using standard methods of Sofowora, Trease and evans and Harbone with slight modifications [10].

2.5Antioxidant Activity
Antioxidant Activity was carried out for the ethanolic and aqueous extracts from the leaves of feverfew (*Tanacetum parthenium* L.) and the synthetic compound parthenolide.
The following free radical scavenging assays were carried out:

a. DPPH free radical scavenging assay [11]
DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay is a stable free radical with purple color, the intensity of which is measured at 510 nm spectrophotometrically. Antioxidant reduces DPPH to 1,1-diphenyl-2-picryl hydrazine, a colorless compound. The percentage inhibition value was calculated using the formula,

$$\frac{\text{Absorbance of Control- Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

b. ABTS radical scavenging assay [12]
ABTS(2, 2'-azinobis-3-ethylbenzothiozoline- 6-sulphonic acid) radical cations (ABTS+) were produced by reacting ABTS solution (7mM) with 2.45mM potassium persulphate. The mixture was allowed to stand in the dark at room temperature for 12-16 hours before use. Aliquots (0.5ml) of the three different extracts were added to 0.3ml of ABTS solution and the final volume was made up to 1ml with ethanol. The absorbance is measured immediately at 734 nm. The percentage inhibition value was calculated using the formula,

$$\frac{\text{Absorbance of Control- Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

c. Determination of total antioxidant capacity
This quantitative assay [13] is based on the conversion of Molybdenum(Mo VI) by reducing agents likes to molybdenum (Mo V), which further reacts with phosphate under acidic pH resulting in the formation of a green coloured complex, the intensity of which can be read spectrophotometrically at 695 nm and results were expressed in mM/L equivalent to Ascorbic acid.

d. Reducing power (FRAP) assay
The Ferric Reducing Antioxidant Power was determined using 2, 4, 6-tripyridyl–S-triazine (TPTZ) in a spectrophotometer at 595nm. The results were expressed in terms of EC_{50}, the concentration at a particular absorbance at which it is exactly half of the absorbance of the control. The Fe^{3+}-reducing power of the extract was determined. All tests were performed in duplicates. A higher absorbance of the reaction mixture indicated greater reducing power. Ascorbic acid was used as a positive control.

e. Superoxide Anion Radical Scavenging Assay
The method of Ganga *et al.* [15] was followed with slight modification. The superoxide Radical generated by the photo-oxidation of methionine-riboflavin and their scavenging property was determined spectrophotometrically at 560nm using Nitro Blue Tetrazolium (NBT) as the chromogenic substrate.

2.6 Statistical analysis
For all assays, samples were analysed in triplicate and the results were expressed as mean ± SD. Student t test was used for statistical evaluation; $p < 0.05$ was considered statistically significant.

2. Results and Discussion
Biological molecules such as DNA and proteins are subject to pro-oxidative stress induced by free radicals, which can result in various diseases such as cancer, cataract and aging [16, 17]. Therefore, antioxidants that can quench free radicals may be implicated in the prevention of these diseases. Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The generation of free radical can bring about thousands of reactions and thus cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radicals. Antioxidants may offer resistance against oxidative stress by scavenging the free radicals. The free radicals are involved in various acute and chronic diseases including cancer, atherosclerosis, ageing etc [18].
Natural antioxidants, therefore, have gained importance. Phytochemical analysis of ethanolic and aqueous extract of feverfew leaves showed the presence of Carbohydrates, Flavonoids, Terpinoids, Glycosides and Steroids. Potent antioxidant activity of ethanolic and aqueous extract of leaves were analysed by making use of four different methods. However, the efficiency of the extract differed against various free radicals depending on the specific assay methodology, which reflects the complexity of the mechanisms and diversity of the chemical nature of the synthetic compound, ethanolic and aqueous extract of *T. parthenium*.

Table 1: Results of Phytochemical Analysis

Test Result	Aqueous leaf extract of *T. parthenium*	Ethanolic leaf extract of *T. parthenium*
Test for carbohydrate		
I. Fehlings Test	+	+
II. Benedict's Test	+	+
III. Iodine Test	-	-
Test for Proteins		
I. Millon's Test	-	-
II. Biuret Test	-	-
Test for Lipids		
Saponification Test	+	+
Test for Alkaloid		
I. Mayers Test	+	+
II. Wagner's Test	+	+
III. Dragendorff Test	+	+
Test for Tannins	-	-
Test for Saponins	+	+
Test for Flavonoids	+	+
Test for Terpenoids	+	+

Tests for glycosides		
I. Borntrager's Test	+	+
II. Keller- Kiliani Test	+	+
Tests for steroids/quinones	+	-
Test for Phenols	+	+

Note: "-"symbol represents absence of component where as "+" symbol represents presence of compound.

According to the results obtained the ethanolic and aqueous extract of the leaves of *T. parthenium* found to be a rich source of Carbohydrates, Flavonoids, Terpinoids, Glycosides, Steroids and phenolic contents.

a. DPPH radical scavenging assay
DPPH test provides simplified version to detect the antioxidant properties of various molecules present in the extracts. A DPPH solution is decolourized when the odd electron becomes paired off in the presence of a free radical scavenger. The colour becomes light yellow from deep violet. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating activity [19]. Higher % inhibition indicates better scavenging activity. The percentages of radical scavenging property of the ethanolic, aqueous extract and Parthenolide in this assay were 63.53±13.07%, 67.90±11.05% and 26.09± 16.18% respectively. The results of the assay are given in the figure 1.The synthetic compound fraction demonstrated the lowest antioxidant potential compared to the rest of the fractions. The effect of ethanolic extract with that of aqueous extract were statistically not significant with (P>0.05).The comparison of the ethanolic, aqueous extract with that of parthenolide were statistically significant with (P<0.05).

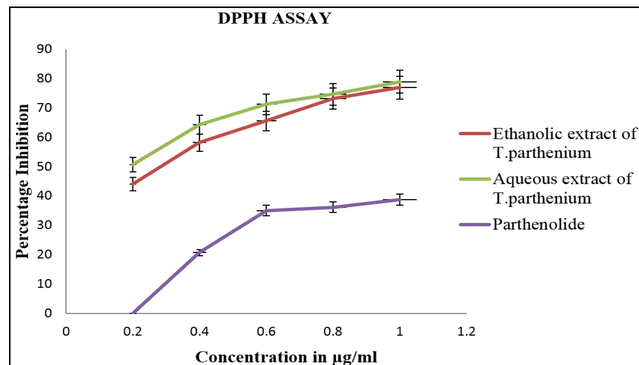

Fig 1: Graphical representation of DPPH Assay

b. ABTS radical scavenging assay
The antioxidant activity of standard, ethanolic extract, aqueous extract and synthetic compound was determined by decolourisation of ABTS, a protonated radical, has characteristic absorbance maxima at 734 nm which decreases with the scavenging of the proton radicals [20]. On comparative basis parthenolide showed better activity in quenching ABTS with an percentage inhibition value of87.10±0.100% followed by aqueous extract with 86.55±.162% and ethanolic extract of 86.011±.185% are shown in Figure2. The Parthenolide has shown higher antioxidant activity (% inhibition) as compared to ethanolic and aqueous extracts. There was high significant difference (P< 0.001)***in comparison of Ethanolic extract with that of parthenolide. The comparison of aqueous extract with that of Parthenolide and ethanolic extract were statistically significant (P<0.05).

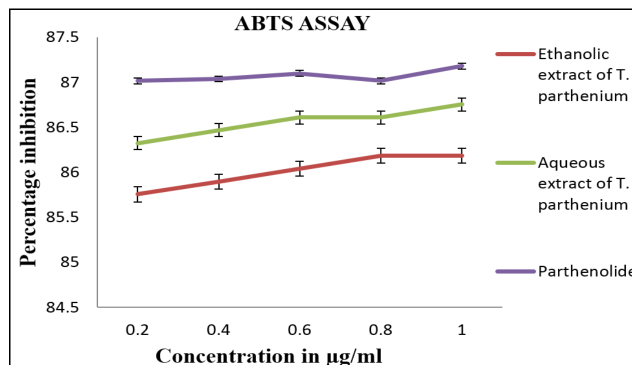

Fig 2: Graphical representation of ABTS Assay

c. Total antioxidant capacity
The total antioxidant capacity of ethanolic and aqueous extract of the leaves of feverfew is expressed as the number of equivalents of ascorbic acid as shown in Figure 4. The Parthenolid e and *T. parthenium* extracts demonstrated electron donating capacity and thus they may act as radical chain terminators, transforming reactive free radical species into stable non-reactive products [21]. The total antioxidant capacity was found to be aqueous extract at 1.316±7.60mM/L, ethanolic extract at 2.22±1.133mM/L and Parthenolide at 4.57±2.23mM/L equivalent to ascorbic acid. However in comparison the ethanolic, aqueous extract and parthenolide were statistically non-significant (P>0.05).

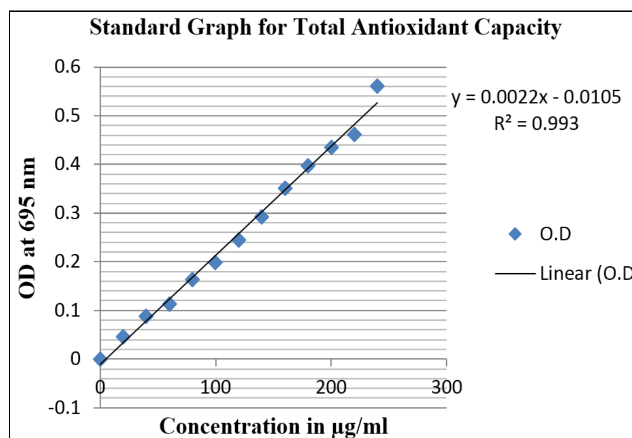

Fig 3: Graphical representation of Standard Graph for Total Antioxidant activity

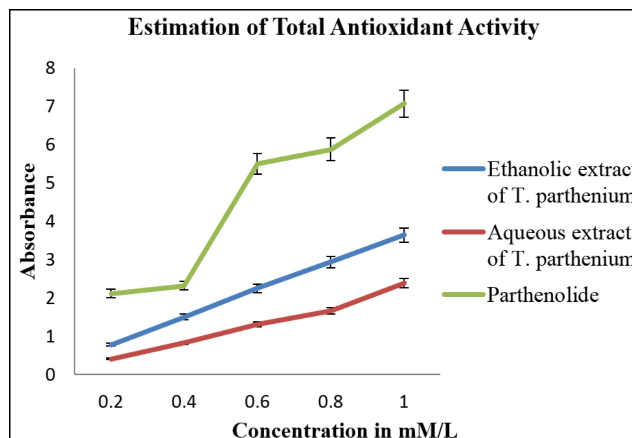

Fig 4: Graphical representation of Total Antioxidant activity

d. Reducing power assay

FRAP assay measures the change in absorbance at 595 nm owing to the formation of a blue colored FeII-tripyridyltriazine compound from color less oxidized FeIII form by the action of electron donating [22]. Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity [23]. The extract showed potent antioxidant power by reducing power ability. Results of reducing power assay are shown in Figure 5. The EC_{50} value of ethanolic extract, aqueous extract and Parthenolide were found to be 0.135±0.09 µg/ml, 0.141±0.09µg/ml and 0.154±0.10µg/ml (0.2 µg/ml-1.0µg/ml) respectively. In this study the ethanolic extracts showed higher scavenging activity when compared to that of aqueous extract and parthenolide. The results obtained were statistically not significant with (P> 0.05).

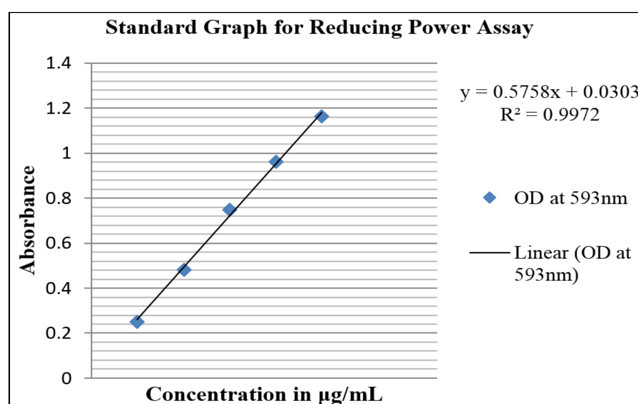

Fig 4: Graphical representation of Standard Graph for Reducing power Assay

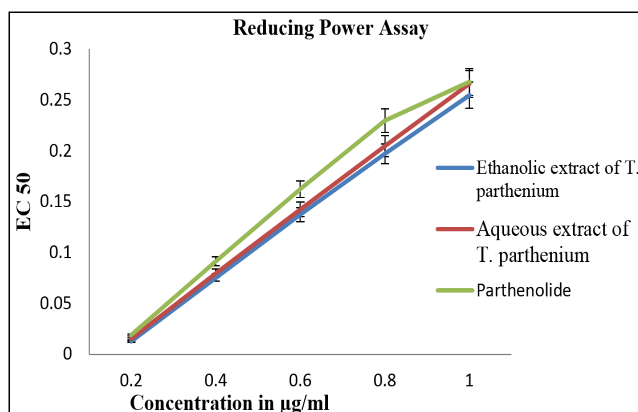

Fig 5: Graphical representation of Reducing power Assay

e. Superoxide anion radical scavenging Assay

Superoxide anion radical is one of the strongest ROS among the free radicals and get converted to other harmful reactive oxygen species such as hydrogen peroxide and hydroxyl radical, damaging biomolecules which results in chronic diseases [24]. The Aqueous extract exhibited an highest percentage inhibition value of 68.88±3.40µg/ml. Parthenolide and ethanolic extract exhibited a value of 66.96±8.44µg/ml and 59.16±3.78 µg/ml respectively. Among this aqueous extract was found to be an effective scavenger of superoxide radical generated by photo reduction of riboflavin. The comparison of parthenolide, ethanolic and aqueous extracts were statistically not significant (P>0.05).

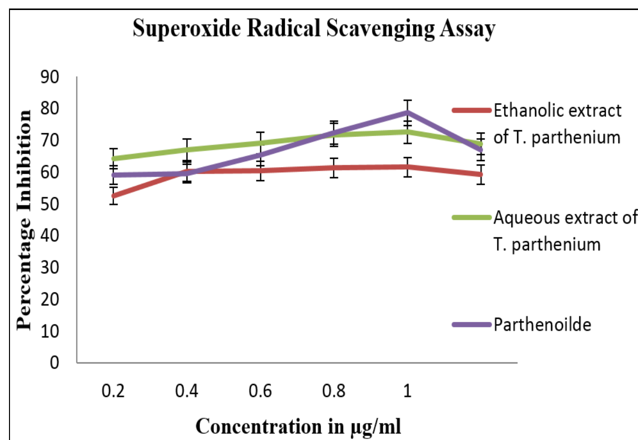

Fig 5: Superoxide radical scavenging Assay

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