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## *In vitro* antioxidant activity of whole plant extracts of *Thalictrum foliolosum* DC (pilijari)

Akhilesh<sup>\*1</sup>, Neeraj Kumar<sup>1</sup>, D.K. Sharma<sup>1</sup> and Manoj Bisht<sup>1</sup>

1.Devsthali Vidyapeeth College of Pharmacy Lalpur, Rudrapur, Uttarakhand, India

### ABSTRACT

Present study was design to investigated *In vitro* antioxidant activity of hydroethanolic (HEETF), methanolic (METF) and aqueous extracts (AQETF) of *Thalictrum foliolosum* family-Ranunculaceae. Traditionally used for jaundice, antimalarial, antipyretic, Diuretics, dyspepsia and febrifuge was found in scientific literature. The antioxidant activity (AA) was determined by the possible four complementary test assay methods namely total phenolic content, total flavonoid content, Inhibition of 2,2 diphenyl -1 picrylhydrazyl (DPPH) radicals and ABTS (2-2'-azinobis) radical scavenging activity or quenching activity .Total phenolic content were found in HEETF, METF and AQETF 43.64±24.2, 53.64± 32.8 and 55.82±30.6 respectively, result shows as mg/GAE/ g of extract. Total flavonoid content were found in HEETF, METF and AQETF 25.43±31.3, 33.42± 29.3 and 42.67±31.8 respectively, result shows as mg/Rutin /g of extract. In DPPH radical scavenging activity, IC<sub>50</sub> value were found in HEETF, METF and AQETF 4.270µg/ml, 4.90 µg/ml and 5.170 µg/ml respectively, in ABTS 2-2'- azinobis radical scavenging activity value were found in HEETF, METF and AQETF 3.35µg/ml, 3.58µg/ml and 4.86 µg/m respectively. The present study revealed that hydroethanolic extract shows significant Antioxidant, Thus it provides a platform for further research, it could be a potential candidate for further drug development

**Keywords:** *In vitro* antioxidant activity, Mamira, *Thalictrum foliolosum*, Pilijari, DPPH, ABTS, dyspepsia and febrifuge

\*Corresponding Author Email: [akhi.kotiyal482@gmail.com](mailto:akhi.kotiyal482@gmail.com)

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### INTRODUCTION

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Reactive oxygen species (ROS) including superoxide radicals, hydroxyl radicals, singlet oxygen and hydrogen peroxide are often generated as products of biological reaction or from exogenous factors. An inequality between oxidants and antioxidants is responsible for oxidative stress which is harmful for both plants and humans. Under physiological condition upto 1-3% of total oxygen absorbed in human's body is further converted into superoxide and other free radicals.<sup>1</sup>Free radicals can be elaborate as electron with odd number of atoms or group of atoms known as highly reactive chemical species and can be generate when oxygen interact with certain molecule. Although many crucial cellular function such as gene transcription, cell signaling, microbial killing, mitochondrial respiratory chain, phagocytosis, arachidonic acid metabolism, ovulation and fertilization have been performed by free radicals but they also destroy protein, lipids or DNA in the human body due to their high production.<sup>4</sup> However it also recorded those oxygen free radicals has been released during the recovery period from much pathological condition.<sup>2</sup> Excessive reactive oxygen species production induces unwanted oxidation which is responsible for membrane rupture, protein modification, DNA damage, necrosis and cell death. Free radical generation have a major role not only in the toxicity of xenobiotics but also in the pathophysiology of various diseases such as diabetes, cognitive dysfunction such as Alzheimer disease, heart related problem such as atherosclerosis, various types of cancer, cataract, chronic inflammatory disease of gastrointestinal tract, shock, deep injuries, production of nitric oxide by the vascular endotheliums, hepatotoxiciy etc.

Antioxidant enzymes such as superoxide dismutase, hydroxyl peroxidase, catalase and radical scavengers like gallic acid, ascorbic acid, tocopherols, rutin etc an antioxidant defence barrier against the damage induced by the free radicals.<sup>3</sup> However this antioxidant system is also elevated in certain malaise to keep the coordination between antioxidants and prooxidants in the body. Antioxidant defense system can be improved by consuming the antioxidant rich diet mainly obtain from fruits and vegetables which contain vitamin C, carotenoids, flavanons and polyphenolics. Naturally and synthetically sources of antioxidant. Various domestic available and currently application of synthetic antioxidants are BHT, rutin, TBHQ etc. According to the recent published data about the synthetic antioxidants have indicated that chronic human consumption of these produces possible toxic effects such as liver damage and mutagenesis, There are two major sections of antioxidants such as enzymatic antioxidants which produce endogenously such as superoxide dismutase, catalase and glutathione peroxidase and nonenzymatic antioxidants such as ascorbic acid, tocopherols, flavonoids, carotenoids and tannins which obtained from natural plant sources. A series of defense mechanism has been developed by the organism upon exposure to free

radicals. The first line of defense is provided by the antioxidants which inhibit oxidation and counteract the damaging effects of oxidation in body tissues. They prevent damage caused by free radicals. The defense mechanism against free radical induced oxidative stress include: Repair mechanism, preventive mechanism, repair mechanism, physical and antioxidant defenses.<sup>4</sup>

*Thalictrum foliolosum* have worldwide distribution in both tropical and temperate regions including Asia, Africa but leads mainly in mountain area of the South-West of Uttarakhand in India where it is known under the local name of Pilihari in Garhwal region and Naga guining Meadow rue and Mamira in Kumaun region where it is widely distributed in climatically moderate zones of Northern Hemisphere.<sup>5</sup> It is a slow growing, dump forming, rhizomatous perennial plant a traditional herb which found wildy in the forest region of eastern and western India. It is a sub erect, tall prickly herb, heavily branched which rise up to 0.9-1.2 m in height.<sup>6</sup> Stem is branched smooth and pale due to this reason it is known as Pilihari.<sup>7</sup> The cladodes are thread like and soft many frequent, 5-20 filiform, straight, ascending or recurred or erect 8-12 mm long. The spines are straight thorns measuring 1-1.5 cm in length.<sup>8</sup> Traditionally used for jaundice while antipyretic,<sup>9</sup> antimalarial<sup>10</sup> Diuretics, antivenom, dyspepsia, febrifuge<sup>11-12</sup> and as antimicrobial agent are scientifically validated.<sup>13</sup> Aim of the study the present study was investigate to potentiate antioxidant activity of herbal medicine

## MATERIALS AND METHOD

### Instruments, Drugs & Chemicals

UV- Vis spectrophotometer Shimadzu, Japan, Vacuum Rotatory Evaporator Scientech, Rutin, Gallic acid, DPPH, Folin- ciocaltaeu reagent and ABTS, were purchased from Yarrow Chem. Mumbai, India. The solvent and other chemical was used of analytical grade. The whole plant *T. foliolosum* was collected during September to October in 2015 from the wild region of Rudraprayag, Uttarakhand, India, and authenticated by botanist Professor S.K. Srivastava, Botanical Survey of India, Dehradun, Uttarakhand, India with a Specimen number of plant is 115900. The whole plant was shade dried at room temperature and extracted with hydroethanolic, methanolic and aqueous solvents, using hot soxhlet apparatus and extracts were dried at 50°C on water bath and the % yield of HEETF, METF and AQETF were found to be 11.16%, 12.8% and 9.58% respectively. Phytochemical screening of whole plant extracted with different solvents revealed the presence of various secondary plant metabolites (Table 1).

### Total Phenolic Content:

UV spectrophotometric method was used to estimate the total phenolic content about 5ml of Folin-ciocalteu's phenol reagent was mixed with 5 ml of sample and 60 ml of water. After 5 min. 15ml of 10% Na<sub>2</sub>CO<sub>3</sub> solution was mixed and the volume was adjusted to 100 ml with deionised water. The whole mixture was incubated for 2 hrs at 23 °C and the absorbance was measured at 517 nm. The total phenol content was expressed as milligrams of Gallic acid equivalent per g of dried sample. (Lechaman *et al.*, 2000).<sup>14</sup>

#### **Total flavanoid content:**

Total flavonoid content was estimated by method described by (Eom *et al.*)<sup>15</sup> In a glass test tube, the minimum amount of extract 0.3 was mixed with exact amount of 0.15 ml of 0.5 M NaNO<sub>2</sub> and 0.15 ml of 0.3 M AlCl<sub>3</sub>.6H<sub>2</sub>O and 3.4 ml of 30% methanol. Then 1ml of 1M NaOH was added after 5 min and mixed thoroughly. Further the absorbance of the solution was measured at 506 nm. Rutin was used as standard. Flavonoid content was expressed as milligrams of rutin equivalents per g of dried fraction.

#### **DPPH radical scavenging activity:**

The stable by 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) was used for determination of free radical scavenging activity of different extract. Different aliquot of each extract hydroethanolic, methanolic and aqueous extracts as per the method described by (Shen *et al.*, 2010).<sup>16</sup> In 100 ml of methanol dissolve 24 mg DPPH at 20°C making stock solution. The solution used for studying was obtained by dilution of methanol and the absorbance was adjusted to 0.98±0.02 at 517nm. A 3ml aliquot of this solution was mixed with 100 µl of the sample at various concentration (10-500 µg/ml). The mixture was then shaken and kept in dark for incubation at room temperature. Then absorbance was taken at 517 nm. The percentage of DPPH radical scavenging activity is calculated as the following equation.

$$\text{Scavenging effect (\%)} = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100$$

#### **ABTS radical scavenging assay:**

Free radical scavenging activity of *T. foliolosum* was determined as per the method described by (Haung *et al.*, 2011).<sup>17</sup> The ABTS (2,2'-azinobis (3-ethyl benzthiazoline -6- sulphuric acid ) cation was measured spectrophotometrically reaction of simple mixing with ABTS (7mm) and potassium persulfate (2.45mm) to make dark green colored solution contain ABTS solution and keeping it overnight in dark. The ABTS radical cation was priorly diluted with 50% methanol and adjusted to an initial absorbance of about 0.70±0.02 at 745 nm and kept in temperature of 30°C. The radical scavenging activity was assessed next day by mixing previously prepared ABTS solution with 300

$\mu$ l of test solution in micropipette. The decrease in absorbance after mixing test solution with ABTS solution was measured within 1 min.

Scavenging effect % = (control absorbance - sample absorbance)/control absorbance  $\times$  100

## RESULTS AND DISCUSSION

### Preliminary Phytochemical Screening:

The distribution of different phytochemical constituents in methanol, hydroethanol and aqueous extracts of whole plant of *T. foliolosum* were evaluated qualitatively. The presence of phytochemicals such as alkaloids, Carbohydrates flavanoids, phenols, Proteins, Tannins, and saponins, the following table summarizes the results of phytochemical screening in different extracts of *T. foliolosum*.

**Table 1: phytochemical screening in different extracts of T. foliolosum.**

S.No	Test	Methanol	Hydroethanol	Aqueous
1.	<b>Alkaloids</b>	++	++	++
	Dragendroff's test	++	++	++
	Mayer's test	-	++	-
	Wagner's test	++	++	++
	Hager's test	++	++	++
2.	<b>Carbohydrates</b>			
	Molisch test	++	++	++
	Fehling test	++	++	++
	Benedict test	++	++	++
3.	<b>Saponins</b>			
	Haemolysis test	-	++	-
	Foam test	-	++	-
4.	<b>Proteins</b>			
	Biurets test	++	++	++
	Millon's test	-	++	++
	Xanthoproteins	++	++	-
5.	<b>Phenolic Compounds</b>			
	FeCl <sub>3</sub> test	++	++	++
6.	<b>Tannins</b>			
	Lead acetate test	-	++	++

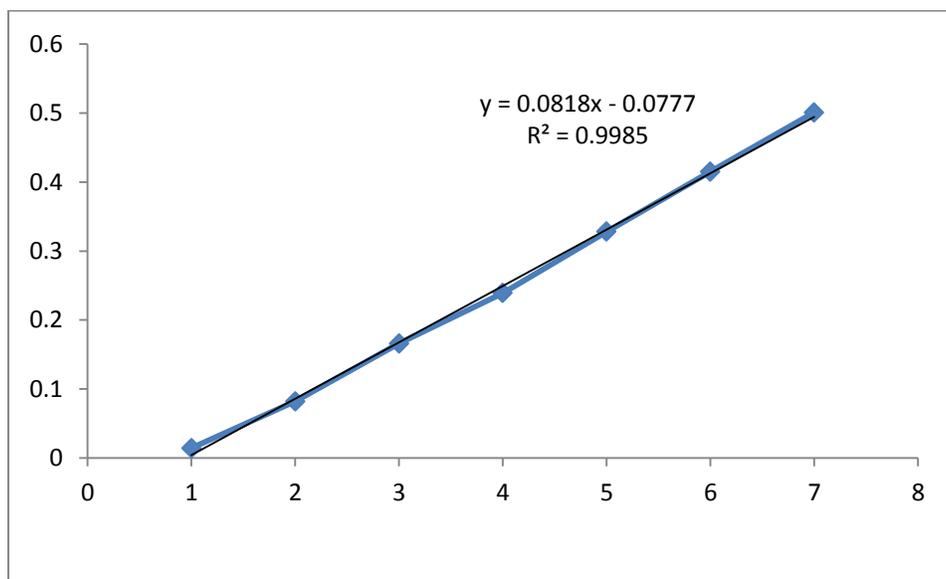
### Total phenolic content:

The total phenolic content of, hydroethanol, methanolic and aqueous extracts of the whole plant of *T. foliolosum* were estimated as milligrams of gallic acid equivalent (mg GAE) per gram plant extract and the data are shown in Table 2.

**Table 2: Total phenolic content of different extracts of *T. foliolosum***

S.No	Extracts of <i>T. foliolosum</i>	Total phenolic content (mg GAE/g of extracts) $\pm$ SD
1	Hydroethanolic extract	43.64 $\pm$ 24.2
2	Aqueous extract	53.64 $\pm$ 32.8
3	Methanolic extract	55.82 $\pm$ 30.6

Statistical significance was determined by one way ANOVA followed by dunnnett test values are expressed as mean  $\pm$  SD, n=3.

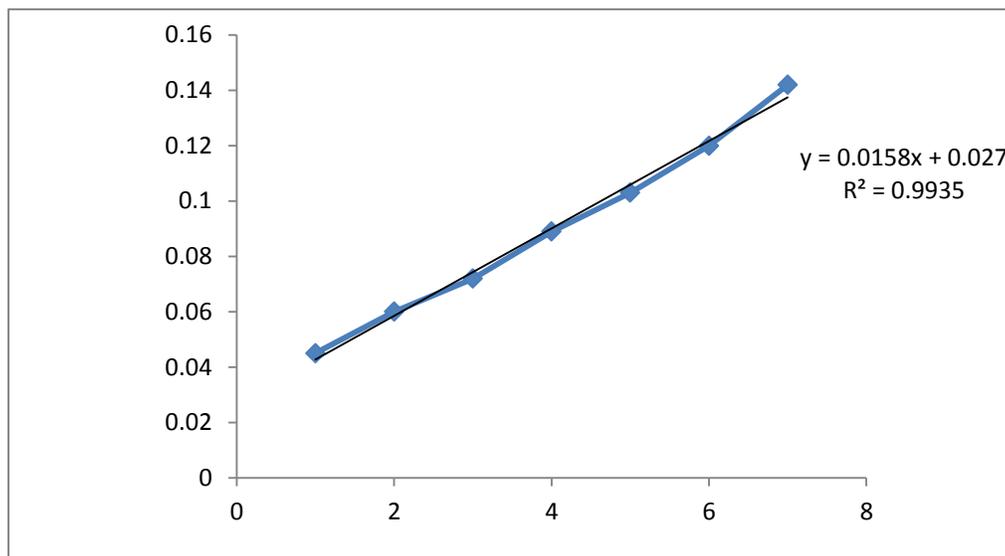
**Figure 1: Standard line equation curve of Gallic acid of total phenolic content****Total flavonoid content:**

The naturally occurring substance flavonoid has been confirmed to be responsible for the antioxidant activity in plants. The total flavonoid content of different extracts of *Thalictrum foiliolosum* were examined in this study and are summarized in Table 3

**Table 3: Total flavonoid content of different extract of *T. foliolosum***

S. No	Extracts of <i>T. foliolosum</i>	Total flavonoid content (mg rutin /g of extracts) $\pm$ SD
1	Hydroethanolic extract	25.43 $\pm$ 31.3
2	Aqueous extract	33.42 $\pm$ 29.3
3	Methanolic extract	42.67 $\pm$ 31.8

Statistical significance was determined by one way ANOVA followed by dunnnett test values are expressed as mean  $\pm$  SD, n=3.



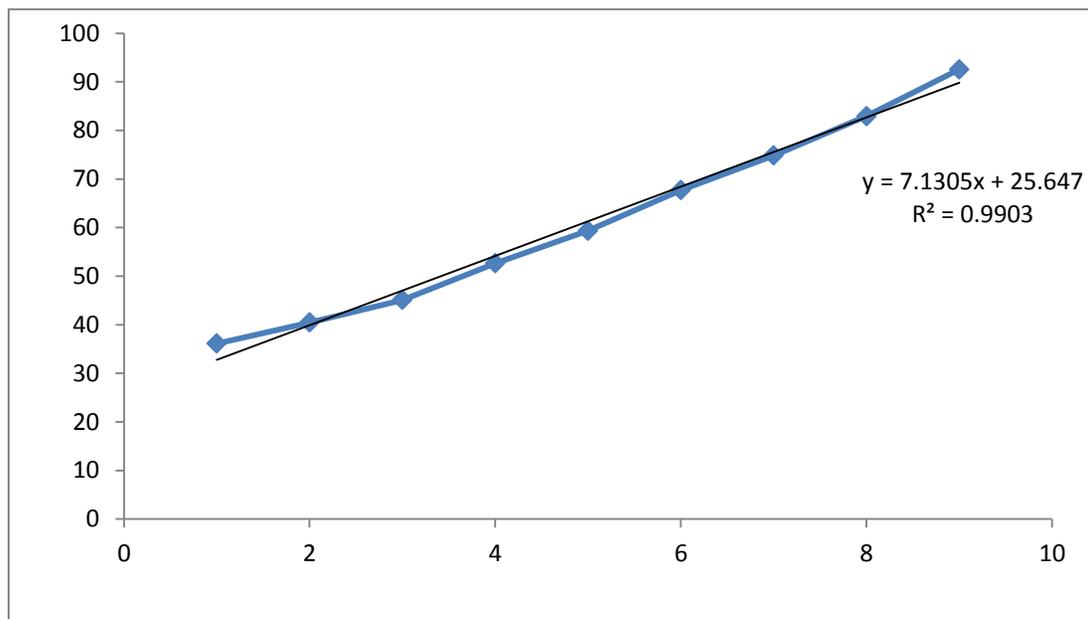
**Figure: 2 Standard line equation curve of Rutin for Total flavonoid content**

### DPPH quenching activity or scavenging activity

DPPH radical scavenging activity of hydroethanol, methanol and aqueous extracts of the whole plant of *T. foliolosum* is shown in table 4. The scavenging effect increases with the increase in the concentration of the extracts and standard respectively. Among the solvents tested, hydro ethanol extract exhibited the highest DPPH radical scavenging activity, with an IC<sub>50</sub> of 4.270 µg/ml followed by aqueous extract with an IC<sub>50</sub> of 4.90 µg/ml & methanolic extract with IC<sub>50</sub> of 5.170µg/ml when compared with standard ascorbic acid with an IC<sub>50</sub> of 3.140 µg/ml

**Table 4: IC<sub>50</sub> values of free radical scavenging effect by DPPH method of various extracts of *Thalictrum foliolosum*.**

Concentration	HEETF	AQETF	METF	Standard
10 µg/ml	34.59%	26.08%	13.14%	36.11%
20 µg/ml	41.24%	32.60%	21.48%	40.43%
30 µg/ml	42.62%	37.11%	34.41%	45.11%
50 µg/ml	47.68%	43.68%	38.76%	52.62%
100 µg/ml	50.21%	53.59%	43.74%	59.34%
200 µg/ml	57.56%	59.59%	56.58%	67.70%
300 µg/ml	64.82%	60.90%	70.44%	74.85%
400 µg/ml	71.12%	68.13%	71.88%	82.93%
500 µg/ml	77.94%	73.65%	78.58%	92.56%
IC <sub>50</sub> µg/ml	4.270 µg/ml	4.90 µg/ml	5.170 µg/ml	3.140 µg/ml



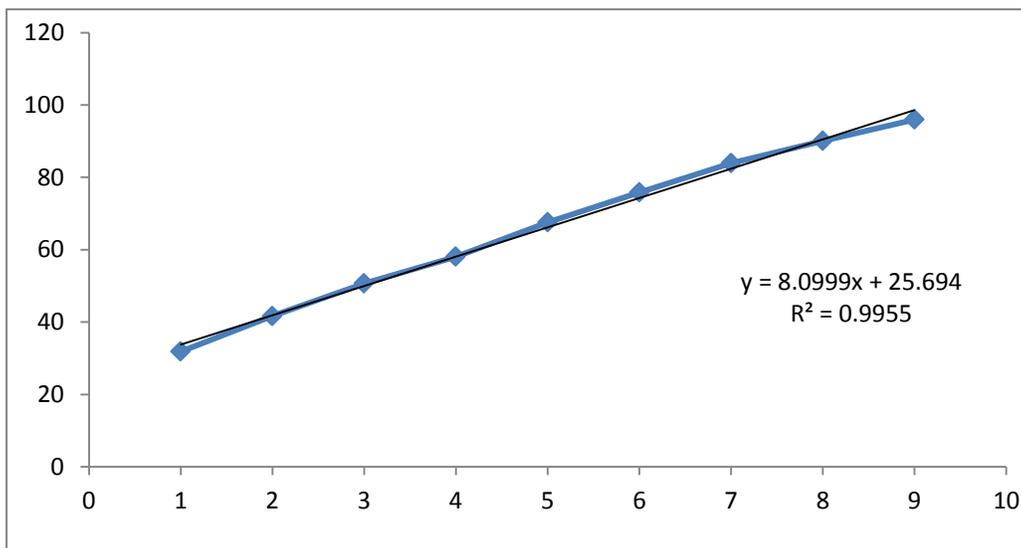
**Figure: 3 Standard line equation curve of ascorbic acid antioxidant using DPPH free radical scavenging activity.**

**ABTS 2-2'-azinobis) radical scavenging activity:**

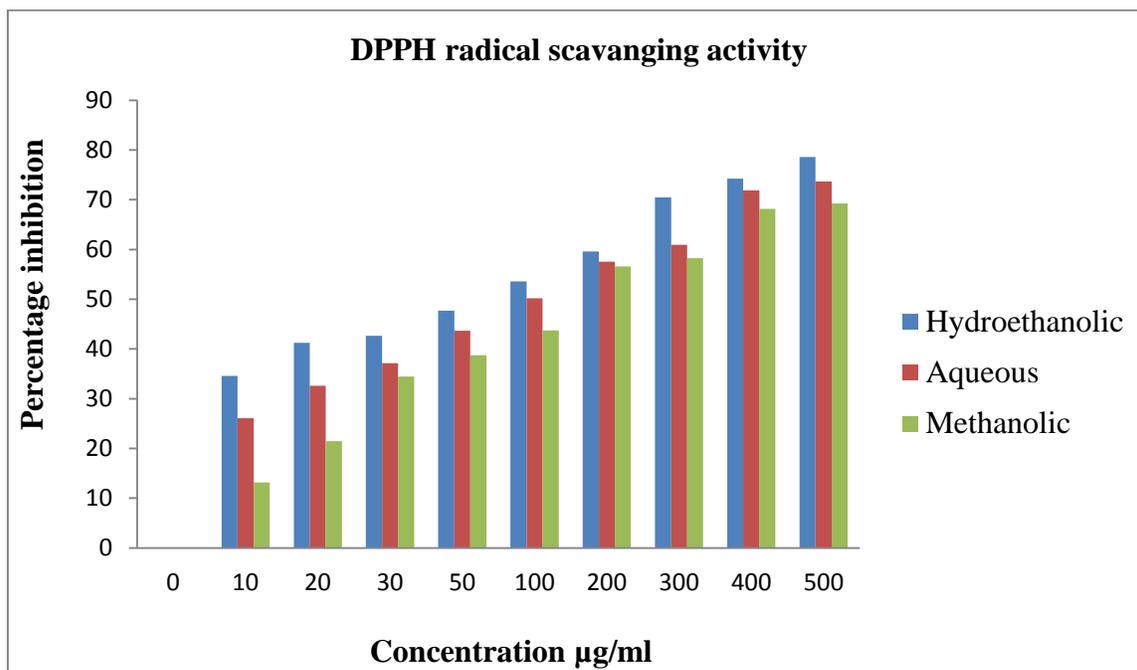
By reducing the ABTS and potassium persulfate, this assay generates a blue/green ABTS chromophore. All the extracts of *Thalictrum foliolosum* neutralized ABTS radical in a concentration dependent way (10- 500 µg/ml). The free radical scavenging activity of different extracts on ABTS radicals were in the following order- HEETF > AQETF > METF > The IC<sub>50</sub> value of the standard was found to be 3µg/ml. The results for ABTS free radical scavenging activity have been summarized in order of the highest antioxidant activity in Table 5.

**Table 5: IC<sub>50</sub> values of free radical scavenging effect by ABTS method of various extracts of *T. foliolosum*.**

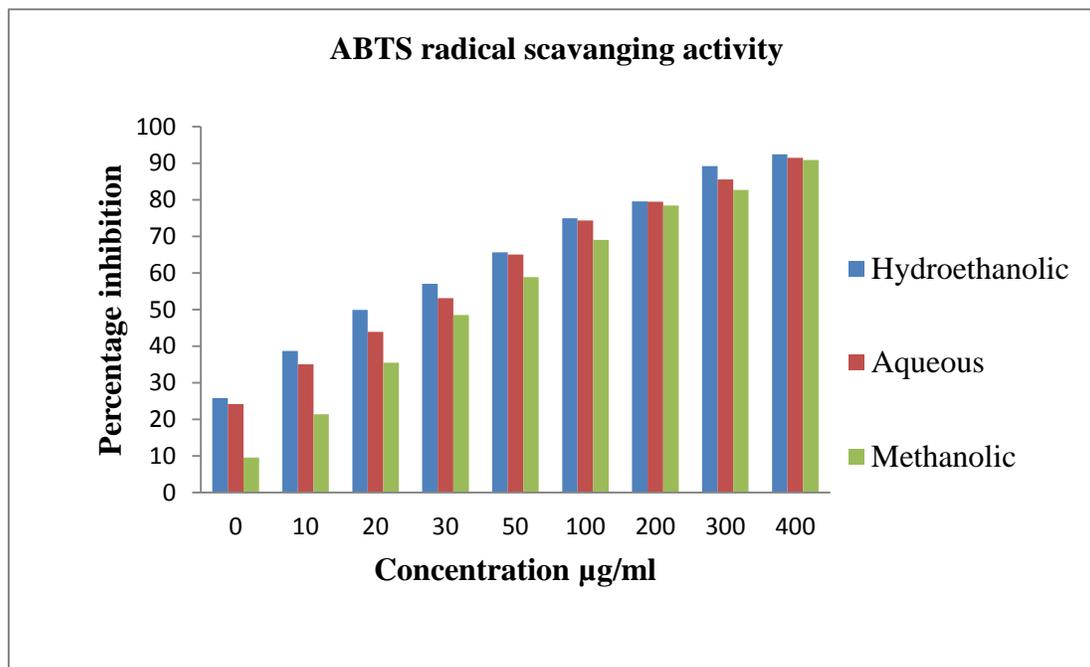
Concentration	HEETF	AQETF	METF	Standard
10 µg/ml	25.87%	28.58%	9.59%	31.85%
20 µg/ml	38.67%	35.08%	21.73%	41.68%
30 µg/ml	49.92%	43.92%	35.46%	50.69%
50 µg/ml	57.05%	53.12%	48.51%	58.06%
100 µg/ml	65.71%	65.02%	58.85%	67.56%
200 µg/ml	74.94%	74.39%	69.08%	75.83%
300 µg/ml	76.93%	78.46%	79.48%	83.94%
400 µg/ml	89.19%	82.74%	85.56%	90.09%
500 µg/ml	92.40%	91.48%	90.85%	95.98%
IC <sub>50</sub> µg/ml	3.35 µg/ml	3.58 µg/ml	4.86 µg/ml	3 µg/ml



**Figure 4:** Standard line equation curve of ascorbic acid antioxidant using ABTS free radical scavenging activity



**Figure: 5** *In vitro* concentration dependent percentage inhibition of DPPH radical by different extracts.



**Figure: 6** *In vitro* concentration dependent percentage inhibitions of ABTS radical by different extracts

#### Discussion & Conclusion

The antioxidant properties of the different solvent extracts of *T. foliolosum* were significantly corroborated by the phytochemical constituents of the extracts. Phenolic compounds are known as powerful chain breaking antioxidants and they are very important plant constituents because of their scavenging ability, which is due to their hydroxyl groups (Shahidi and Wanasundara, 1992).<sup>18</sup> Total phenolic assays by using Folin-Ciocalteu reagent is a simple convenient and reducible method. It is employed routinely in studying phenolic antioxidants.

Flavonoids are a group of polyphenolic compounds, which exhibit several biological effects such as antiinflammatory, antihepatotoxic, antiulcer, antiallergic, antiviral and anticancer activities (Umamaheswari and Chatterjee, 2008).<sup>19</sup> They are capable of effectively scavenging the reactive O<sub>2</sub> species because of their phenolic hydroxyl groups and so they are potent antioxidants also (Cao *et al.*, 1997).<sup>20</sup> In view of their wide pharmacological and biological actions, they have a greater therapeutic potential. The presence of high phenolic and flavonoid content in the different solvent extracts of *T. foliolosum* has contributed directly to the antioxidant activity by neutralizing the free radicals. Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infection, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases (Jamuna *et al.*, 2012).<sup>21</sup> They are also involved in autoimmune disorder like rheumatoid arthritis, etc. Our results demonstrated that the different

solvent extracts of *T. foliolosum* possess free radical scavenging activity with different *in vitro* models like DPPH, ABTS radical scavenging activity, total phenolic content and total flavonoid content assays.

In the present study whole plant of *T. foliolosum* has been subjected to phytochemical characterization, antioxidant activity with a view to assess its pharmacological potentials. The presence of phytochemicals such as alkaloids, flavanoids, phenols, quinones, saponins, and glycosides has been confirmed in the different extracts of the selected plant.

DPPH radical scavenging activity of, hydroethanol, methanol, and aqueous extracts of the whole plant of *T. foliolosum* were scavenging effect increases with the increase in the concentration of the extracts and also the standard. Among the solvents tested, hydroethanol extract exhibited the highest DPPH radical scavenging activity.

By reducing the ABTS and potassium persulfate, this assay generates a blue/green ABTS chromophore. All the extracts of *T. foliolosum* neutralized ABTS radical in a concentration dependent way (10- 500 µg/ml). The free radical scavenging activity of different extracts on ABTS radicals were in the following order- HEETF > AQETF > METF > The IC<sub>50</sub> value of the standard was found to be 3µg/ml.

The naturally occurring substance phenol flavonoids can be responsible for the antioxidant activity in plants. Further research on isolation and identification of active compounds and its efficacy needs to be done that is responsible for its antioxidant property.

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