



<https://ijrps.com>

ISSN: 0975-7538

Research Article

## Quantitative estimation of tannins, phenols and antioxidant activity of methanolic extract of *Imperata cylindrica*

Padma R, Parvathy N.G, Renjith V, Kalpana P. Rahate\*

Amrita School of Pharmacy, AIMS Health Sciences Campus, Kochi- 682041, Kerala, India

### ABSTRACT

The aim of present study was to evaluate the antioxidant activity of the Methanolic root extract of *Imperata cylindrica* (MEIC) by using various in-vitro models of antioxidant activity. The extract was comprised to phytochemical screening and quantitative determination of tannins and total phenols by spectrophotometric methods. Scavenging of nitric oxide by MEIC was estimated by using Gries's reagent. Reducing the ability of MEIC was studied by using  $\text{FeCl}_3$  reagent. Ascorbic acid was used as standard in all the three methods. The extract showed the  $\text{IC}_{50}$  value for nitric oxide scavenging method as  $400.15 \pm 1.934 \mu\text{g/ml}$  as compared to the standard ascorbic acid with  $\text{IC}_{50}$  value  $269.75 \pm 0.852 \mu\text{g/ml}$ . The extract was found to have been strong reducing power and the activity was comparable to the standard ascorbic acid. The hydrogen peroxide scavenging capacity of MEIC was reported as  $\text{IC}_{50}$  value of  $185.6 \pm 1.551 \mu\text{g/ml}$  as compared to the  $\text{IC}_{50}$  value of the standard ascorbic acid  $128.5 \pm 0.683 \mu\text{g/ml}$ . MEIC showed significant antioxidant activity in all the three antioxidant models comparable to the standard. The phytochemical screening of the MEIC revealed the presence of carbohydrates, glycosides, triterpenoids, phenolic compounds/tannins, flavonoids, proteins and volatile oils. The tannin content of MEIC was found to be  $12.53 \pm 0.56 \text{mg}$  tannic acid equivalent  $\text{g}^{-1}$  extracted powder. The total phenolic content was found to be  $7.09 \pm 0.14 \text{mg}$  gallic acid equivalent  $\text{g}^{-1}$  extracted powder. The antioxidant potential of the methanolic extracts of *Imperata cylindrica* may be due to the presence of tannins and phenolic compounds.

**Keywords:** *Imperata cylindrica*; antioxidant activity; Nitric oxide; reducing power; hydrogen peroxide; tannins.

### INTRODUCTION

Reactive oxygen species (ROS), such as superoxide ( $\text{O}_2^-$ ), hydroxyl (OH) and peroxy (OOH, ROO) radicals are generated during oxidative stress. Accumulation of these radicals results into the oxidative damage and thus there is need for antioxidants. These reactive oxygen species play an important role related to the degenerative processes of various serious diseases such as ageing, cancer, coronary heart disease, atherosclerosis, cataract, inflammation and neurodegenerative disorders like Alzheimer's disease. (Smith MA *et al.*, 1996; Diaz MN *et al.*, 1997, Aruoma OI *et al.*, 1998). The reactive oxygen species are also known to activate matrix metalloproteinase (e.g. collagenase) causing increased destruction of tissues, e.g. collagenase damage seen in various arthritic reactions. (Cotran RS *et al.*, 1994).

Antioxidants can prevent or slow the oxidative damage to our body. They act as "free radical scavengers" and hence prevent and repair damage caused due to these free radicals. They are present in plant sources such as

vitamins, minerals, flavanoids, phenolics and carotenoids, etc. Synthetic and natural antioxidants are added as additives for prevention of food deterioration. The use of synthetic antioxidants is being restricted because of their carcinogenicity. (Diaz MN *et al.*, 1997). In recent years, much attention has been devoted to natural antioxidants and their association with health benefits. (Ali SS *et al.*, 2008).

Researchers have shown that crude extracts of fruits, herbs, vegetables, cereals and other plant materials rich in phenolics are of great interest in food industry as they retard oxidative degradation of lipids and thereby improve the quality of nutritional value of food. (Cotran RS *et al.*, 1994). Hence, the herbs that have been reported to possess antioxidant properties are being used for antioxidant formulations. Tannins, phenols, polyphenols including flavanoids have been reported to exhibit a wide range of activities and prevent the attack of free radicals on the human body. (Narasimhan S *et al.*, 2006).

The plant *Imperata cylindrica* usually known as *Cogon-grass* is found throughout tropical and subtropical regions on every continent except Antarctica. *Imperata cylindrica* is also known as Thatch grass (English) and *Darbh* (Hindi). (Anonymous, Ayurvedic Pharmacopoeia of India, Part 1, 2006). It has been used traditionally as antibacterial, astringent, diuretic, sialagogue, emol-

\* Corresponding Author

Email: rahatekalpana@gmail.com

Contact: +91-9895872328 Fax: +91-4842857925

Received on: 07-05-2012

Revised on: 04-01-2013

Accepted on: 09-01-2013

lient, febrifuge, styptic, restorative and antiageing property. (Anonymous, The Ayurvedic Formulary of India, Part II., 2000). It is an important drug of Tripanchmool and used in urinary calculi, retention of urine, diabetes, cardiac disorders, gout, rheumatism, piles, common cough and cold, anemia. (Anonymous, The Useful Plants of India, 2000). No detailed studies have been carried out to investigate the antioxidant potential of the plant. The free radical species like nitric oxide, hydrogen peroxide were investigated and the effect produced was compared with that of the standard drug ascorbic acid.

## MATERIALS AND METHODS

### Plant material

The plant *Imperata cylindrica* was collected from the local regions of Ernakulam and was botanically identified and confirmed. The roots were separated and washed thoroughly with sufficient water. It was dried under shade for two weeks and powdered in an electrical blender. The powder was sieved through a sieve no 40.

### Preparation of the extract

250 g of powdered root of *Imperata cylindrica* was defatted with petroleum ether. The marc was successfully extracted with each of chloroform, dichloromethane, acetone, methanol by the cold maceration process for one week. The aqueous extract was prepared by boiling the powder with successive volumes of water and combined. The macerated pulp from each solvent was filtered and the filtrate was dried at reduced temperatures.

### Preliminary Phytochemical studies

Phytochemical screening of the root extracts of *Imperata cylindrica* (MEIC) was carried out to identify the

secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, steroids, saponins and cardiac glycosides according to standard phytochemical methods. (Harborne JB *et al.*, 1998; Trease GE *et al.*, 1989)

### Estimation of tannins

Content of tannins in MEIC was determined by Folin-Denis method. (Polshettiwar SA *et al.*, 2007). Colorimetric estimation of tannins is based on the measurement of blue colour formed by the reduction of phosphotungstomolybdic acid by tannin like compound in alkaline medium. 1.0 ml of extract and standard solution of tannic acid (100 – 800 µg/ml) was made up to 7.5 ml with distilled water. Then 0.5 ml Folin denis reagent and 1 ml Na<sub>2</sub>CO<sub>3</sub> solution were added. The volume was made up to 10 ml with distilled water and absorbance was measured at 700 nm. The total tannic acid content was expressed as mg of tannic acid equivalent per gram of extract.

### Determination of total phenolic composition

The total content of phenols in MEIC was determined by modified Folin-Ciocalteu colorimetric method using gallic acid as a standard. (Lubica *et al.*, 2007). To 0.5 ml of the sample (three replicates) of plant extract solution (1 mg/ml) & standard solution of gallic acid (10 – 90 µg/ml) in DMSO, 400 µl of Folin-Ciocalteu reagent was added. After 3 min. The solution was diluted to 10 ml with 7.5 % Na<sub>2</sub>CO<sub>3</sub> solution and incubated at 25°C for 2 hrs. It was then centrifuged at 5000 rpm for 5 min and the absorbance was measured at 760 nm. The total phenolic content was expressed as mg of gallic acid equivalent per gram of extract.

### Antioxidant activity by Nitric oxide scavenging method

The effect of MEIC on nitric oxide radical scavenging activity was measured by using ascorbic acid as stan-

**Table 1: Percent extractive value**

Extract	% Extractive value (w/w)
Chloroform	1.06 %
Dichloromethane	2.41 %
Acetone	2.45 %
Water	12.44 %
Methanol	9.16 %

**Table 2: Phytochemical and antioxidative components of the methanol extract of *Desmostachya bipinnata***

Parameters studied	Methanolic extract of <i>Imperata cylindrica</i>
Phytoconstituents	Carbohydrates, glycosides, triterpenoids, phenolic compounds/tannins, flavonoids, proteins and volatile oils
Total tannins content	12.53 ± 0.56*
Total phenol content	7.09 ± 0.14b**

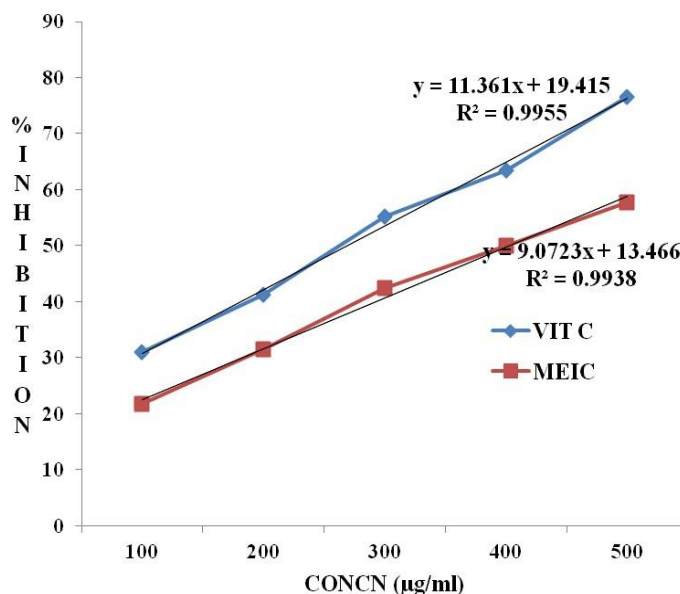
\* mg of Tannic acid equivalent per gram of extract

\*\* mg of Gallic acid equivalent per gram of extract

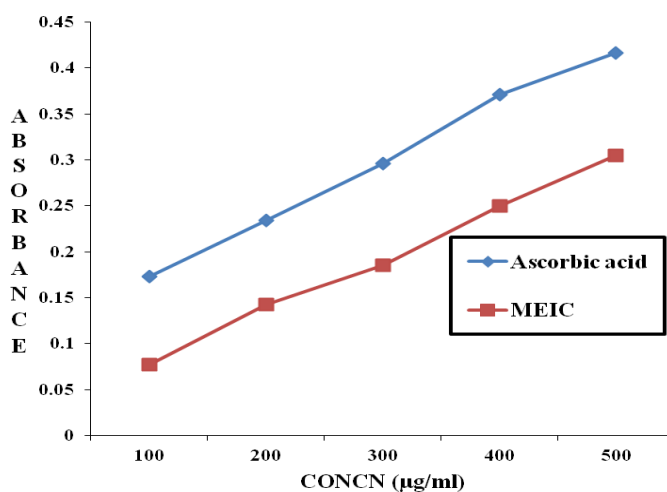
Values represent in the results are mean±SD of three replicates

**Table 3: Scavenging of reactive oxygen species (IC50 values) of methanolic extract of *Imperata cylindrica***

	Nitric oxide µg/ml	Hydrogen peroxide µg/ml
<b>MEIC</b>	400.15 ± 1.934 (R <sup>2</sup> 0.993)	185.6 ± 1.551 (R <sup>2</sup> 0.969)
<b>STANDARD (Ascorbic acid)</b>	269.75 ± 0.852 (R <sup>2</sup> 0.995)	128.5 ± 0.683 (R <sup>2</sup> 0.995)



**Figure 1: Nitric oxide anion scavenging activity of methanolic root extract of *Imperata cylindrica***



**Figure 2: Reducing power of methanolic root extract of *Imperata cylindrica***

dard. (Sreejayan N et al., 1997 and Govindarajan R et al., 2003). Aqueous sodium nitroprusside generates nitric oxide at physiological pH, which interacts with oxygen to produce nitrite ions that can be estimated using Gries's reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions.

Varying concentrations of MEIC sample/standard solution were mixed with 0.5 ml phosphate buffer solution (pH 7.4) and 2.0 ml sodium nitroprusside solution. The mixture was incubated at 25°C for 2.5 hours. To 0.5 ml of the reaction mixture, 1.0 ml of sulphanilic acid was

added and incubated at room temperature for 5 minutes for complete diazotization. Finally, 1.0 ml of 5% N-Naphthyl ethylene diamine dihydrochloride was added, mixed and incubated at room temperature for 5 minutes to form pink colored chromophore. The absorbance was then measured at 546 nm against the corresponding blank solution. Decreased absorbance of the reaction mixture indicated increased scavenging activity. The percentage inhibition was calculated by using the formula,

$$\% \text{ Inhibition} = \frac{A(\text{control}) - A(\text{sample})}{A(\text{control})} \times 100$$

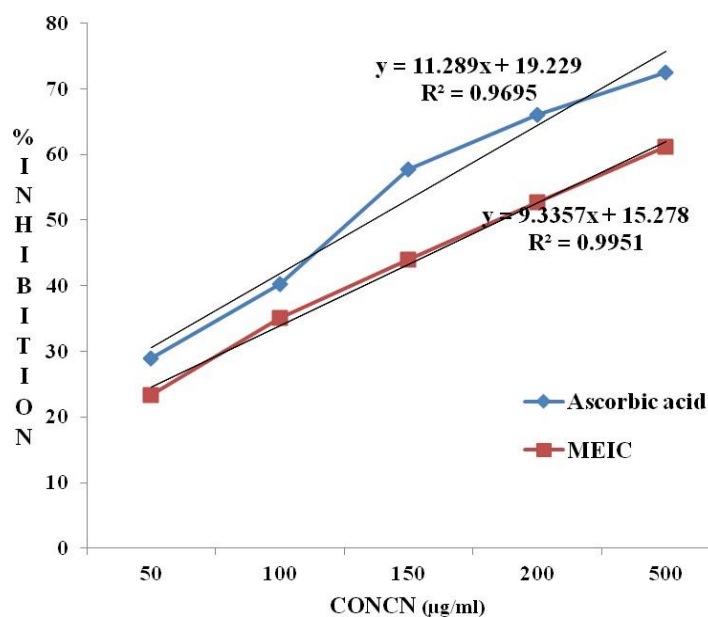


Figure 3: Hydrogen peroxide scavenging activity of methanolic root extract of *Imperata cylindrica*

#### Reducing power determination

The capacity of MEIC to bring about reduction of  $\text{FeCl}_3$  was evaluated. (Oyaizu M, 1986 and Rakesh et al., 2009). Varying concentrations of MEIC were mixed with 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of  $\text{K}_3\text{Fe}(\text{CN})_6$  (1% w/v) solution. The resulting mixture was incubated for 20 min at  $40^\circ\text{C}$  followed by the addition of 1.5 ml of trichloroacetic acid (10% w/v). The mixture was centrifuged at 3000 rpm for 10 min. 2.5 ml of the supernatant liquid was mixed with 2.5 ml distilled water and 0.5 ml of  $\text{FeCl}_3$  (0.1%, w/v). The absorbance was measured at 700 nm against the blank sample. Vitamin C was used as positive control.

#### Hydrogen peroxide scavenging assay

The antioxidant activity of the MEIC and the standard were assessed based on their hydrogen peroxide scavenging ability. (Gulcin I et al., 2002 and J Javanmardi et al., 2003). To 0.5 ml of the standard ascorbic acid and the extract in phosphate buffer (pH 7.4) 0.6 ml of 4 mM  $\text{H}_2\text{O}_2$  solution in phosphate buffer solution (pH 7.4) was added. Absorbance of the solution was measured at 230 nm after 10 min against the blank solution containing phosphate buffer without hydrogen peroxide. Control was prepared by replacing the sample/standard with phosphate buffer. All samples were prepared and assayed in triplicate and averaged. The percentage inhibition was calculated by using the formula.

#### Statistical analysis

Statistical comparisons were made using a one-way ANOVA test.

### RESULTS AND DISCUSSION

The percent extractive value obtained is given in table 1. The yield of MEIC was found to be 9.16%.

#### Phytochemical analysis

The methanolic extract showed the presence of carbohydrates, glycosides, triterpenoids, phenolic compounds/tannins, flavonoids, proteins and volatile oils in the detectable amount (Table 2). The total phenolic content of the methanolic root extract was  $7.09 \pm 0.14$  mg gallic acid equivalent per gram of extract. The tannin content of the plant was  $12.53 \pm 0.56$  mg of tannic acid equivalent per gram of extract powder.

#### Antioxidant study

##### Nitric oxide Scavenging assay

The effect of MEIC on nitric oxide radical scavenging activity is shown in Figure 1. It exhibited potent nitric oxide radical scavenging activity comparable to that of the standard drug ascorbic acid. The  $\text{IC}_{50}$  value of the methanolic extracts ( $400.15 \pm 5.93$  µg/ml,  $R^2$  0.909) was found to be comparable with the  $\text{IC}_{50}$  value of ascorbic acid ( $269.75 \pm 2.55$  µg/ml,  $R^2$  0.947) (Table 3). Nitric oxide is an important bioregulatory molecule and plays a major role in maintaining blood pressure, signal transduction, platelet function but excess of it brings about cytotoxic effects like Alzheimer, AIDS, cancer. Sodium nitroprusside in aqueous solution at physiological pH generates nitric oxide that reacts with oxygen to produce nitrite ion. (Ialenti A et al., 1993). The scavengers of nitric oxide compete with oxygen and lead to reduced production of nitrite ion. (Govindarajan R et al., 2003).

##### Reducing power determination

Figure 2 shows the reducing power of the MEIC in comparison with ascorbic acid standard at 700 nm. The reducing capacity of the extract, another considerable indicator of antioxidant activity was also found to be substantial. Reducing power exhibited may be due to the presence of reductones those are electron donors and are capable of converting them into a more stable product and terminating the free radical reaction.

### Hydrogen peroxide scavenging assay

The effect of MEIC in scavenging hydrogen peroxide radicals is shown in figure 3. The extract was found to scavenge the hydrogen peroxide comparable to the standard (Table 3). Hydrogen peroxide is the most stable ROS and generated directly by divalent or monovalent reduction of O<sub>2</sub> by enzymes like xanthine oxidase, etc. When H<sub>2</sub> Q decomposed into H<sub>2</sub>O and O causes serious damage, including chromosomal aberrations. (Oyaizu M et al., 1986).

### CONCLUSION

Phytochemicals are potent antioxidants, metal chelators or free radical scavengers thus they own health promoting properties. (Cotelle N et al., 1996). The free radicals play a major role by bringing up number of disorders in human, including arthritis, gastritis, ageing, respiratory diseases, etc. (Gupta VK and Sharma BK, 2006). As the use of synthetic antioxidant possesses the serious threat like carcinogenicity researchers have turned towards to ponder the herbal plants with effective antioxidant property that is capable of protecting the cells against the damaging effects of free radicals. (Kumar V and Sharma SK, 2006). The methanolic extract of *Imperata cylindrica* showed significant antioxidant activity in all the three antioxidant models when compared to standards. The antioxidant potential of the plant may be due to the presence of tannins and phenolic compounds. The isolation of therapeutically active constituents from the extract will probably give better antioxidant activity than even standard at lower concentrations. More research in this plant can pave the way to newer phytochemicals. In conclusion, this plant is a promising resource for future as a natural source of antioxidant.

### REFERENCES

- Ali SS, et al 2008. Indian medicinal herbs as sources of Antioxidants. *Food Res Int*, 41, 1-15.
- Anonymous, Ayurvedic Pharmacopoeia of India, Part 1 Vol.V, Ministry of Health and Family Welfare, Department of Indian System of Medicine and Homeopathy, Govt. of India New Delhi 2006.
- Anonymous, The Ayurvedic Formulary of India, Part II, Ministry of Health and Family Welfare, Department of Indian System of Medicine and Homeopathy, Government of India New Delhi 2000.
- Anonymous, The Useful Plants of India. National Institute Of Science Communication, New Delhi-2000.
- Aruoma OI, 1998. Free radicals, oxidative stress and antioxidants in human health and disease. *J Am Oil Chem Soc*, 75, 199-212.
- Cotelle N et al., 1996. Antioxidant Properties of Hydroxyl flavones, *Free Rad Bio and Med*, 20(1):35-43.
- Kalpana P. Rahate et al., (2013) Int. J. Res. Pharm. Sci., 4(1), 73-77
- Cotran RS, Kumar V, Robbins SL. In: Robbins Pathological Basis of Disease. Philadelphia: W.B. Saunders Company, 1994. pp.73.
- Diaz MN et al., 1997. Antioxidants and atherosclerotic heart disease. *N Engl J Med*, 337, 408-16.
- Govindarajan R et al., 2003. Studies on antioxidant activities of *Desmodium gangeticum* *Biopharm Bull*, 26(10), 1424-7.
- Gulcin I et al., 2002. Determination of antioxidant activity lichen *Certraria islandica* (L) Ach, *J Ethnopharmacol*, 79, 325-9.
- Gupta VK and Sharma BK, 2006. Plants as natural antioxidants. *Nat Prod Rad* 5(4), 326-334.
- Harborne JB, Methods of Extraction and isolation. In: Phytochemical methods, 3<sup>rd</sup> ed. Chapman and Hall, London 1998, pp. 60-66.
- Ialenti A et al., 1993. Modulation of adjuvant arthritis by endogenous nitric oxide. *Br J Pharmacol*, 110, 701-3.
- Javanmardi J et al., 2003. Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. *J Food Chem*, 547-50.
- Kumar V, Sharma SK, 2006. Antioxidant Studies on Some Plants:a review:Hamdard Medicus (Pakistan)XLIX, 4:25-36.
- Lubica L, Jan M, Irena M, Daniel G, 2007. Antioxidant Activity and Total Phenols in Different Extracts of *Staphylea* species, *Molecules*, 12, 98-102.
- Narasimhan S, Shobana R, Sathya TN 2006. Antioxidants – Natural rejuvenators that heal , detoxify and provide nourishment, In:Rakesh KS, Rajesh A (eds), Herbal Drugs :A Twenty First Century Prospective. New Delhi. JP brothers Medical Publishers, 548-57.
- Oyaizu M, 1986. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn J Nutri*, 44, 307-15.
- Polshettiwar SA, Ganjiwale RO, 2007. Spectrophotometric estimation of Total tannins in some ayurvedic eye drops. *Ind J Pharm Sci*. 69(4), 574-6.
- Rakesh SU et al., 2009. In-vitro antioxidant activity of the peel of unripe fruit of *Trichosanthes anguina* Linn. *Adv in Pharmacol and Toxicol*, 10(2), 25-34.
- Smith MA et al., 1996. Oxidative damage in Alzheimer's. *Nature*, 382, 120-21.
- Sreejayan N, Rao MNA, 1997. Nitric oxide Scavenging by Curcuminoids, *J Pharmacy and Pharmacol*, 49(1), 105-7.
- Trease GE, Evans WC, Textbook of Pharmacognosy, 12<sup>th</sup> ed. Balliere, Tindall, London, UK, 1989:57-59, 343-83.