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Research Article

SCREENING OF ANTIOXIDANT ACTIVITY AND PHYTOCHEMICALS STRENGTH OF SOME HERBAL PLANTS

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ABSTRACT

Objective: The aim of present study was to assess the total antioxidant activity and phytochemical constituents (i.e. total phenols, flavonoids, carotenoids, non protein thiols and vitamin C) in ethanolic crude extracts of *Oxalis corniculata*, *Phyllanthus fraternus* and *Trichosanthes cucumerina* plants.

Methods: The total antioxidant activity was assayed by DPPH free radicals scavenging method. The phytochemicals in the ethanolic extracts of plants were determined quantitatively using standard methods.

Results: DPPH radicals scavenging activity was evaluated on concentration dependent and observed maximum in *Phyllanthus fraternus* with IC_{50} values 202.22 ±1.89 µg/ml in comparison to *Oxalis corniculata*. Antioxidant activity in the plant parts of *Trichosanthes cucumerina* was found to be highest in the fruits than its leaves, stems and roots where least activity was perceived in roots at all concentrations of extracts. The phytochemicals screening showed that *Phyallanthus fraternus* had greater strength with 19.67 ± 0.68 mg flavonoids, 4.63 ± 0.14 mg carotenoids, 1.39 ± 0.16 mg ascorbic acid and 33.50 ± 1.14 µmole non protein thiols each in 100mg residual weight (RW). *Trichosanthes cucumerina* fruits displayed maximum phytochemicals over leaves, stems, roots and contained 2.43 ± 0.14 mg phenols, 16.33±0.78 mg flavonoids, 2.48±0.12 mg carotenoids, 0.89±0.13 mg ascorbic acid and 29.34 ±1.22 µmole non protein thiols each per 100mg RW. All values were found significantly different at $\alpha = 0.05$, $p < 0.0001$.

Conclusion: The study concluded that ethanolic extracts of *Phyallanthus fraternus* have better antioxidative potential than *Oxalis corniculata* and *Trichosanthes cucumerina* fruits however all of three are good sources of natural antioxidants.

Keywords: *Oxalis corniculata, Phyallanthus fraternus, Trichosanthes cucumerina*, ethanolic extracts, antioxidant activity, DPPH and phytochemicals.

INTRODUCTION

An Oxidative stress is caused by free radicals, which form stable electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation. It is responsible for many of today's diseases that results from an imbalance between formation and neutralization of pro-oxidants [1]. In defence against this oxidative stress, body have their own system including various enzymes, proteins and vitamins, which are known as Anti-oxidants. As the age increases, the level of anti-oxidants declines in body which requires external source of anti-oxidants to defend [2]. Currently there has been an increased interest globally to identify antioxidant compounds that are pharmacologically potent and have low or no side effects for use in preventive medicine and food industry [3]. Plants are good sources of natural antioxidants such as polyphenolic compounds, vitamins and other secondary metabolites for the human diet, containing many different antioxidant components which provide protection against harmful free radicals and have been strongly associated with reduced risk of chronic diseases, in addition to other health benefits [4]. The antioxidant activity of these natural phyto-products is due to their redox properties, which allow them to act as reducing agents, hydrogen donators, singlet oxygen quenchers, and metal chelators [5, 6]. Although many plants resources have been studied but a large number of herbal plants (wild and domestic types) still need proper documentation for use as therapeutically applications. *Oxalis corniculata* a weed of Oxalidaceae family (commonly known as creeping wood sorel or teen patiya weed) rich with oxalic acid in their foliage having various pharmacological effects as antiscorbutic in the treatment of scurvy, antimicrobial, antifungal, wound healing, anti implantation, abortifacient, cardiorelaxant and nematocidal activities [7]. Another weed plant *Phyllanthus fraternus* Webster of Euphorbiaceae family (commonly called; gulf leaf- flower, chanca piedra, quebra pedra, stone braker, bhoomi amlaki etc.) [8], is employed for analgesic, carminative, digestive, laxative, stomachic, tonic, and vermifuge [9, 10]. *Trichosanthes cucumerina* a vegetable of Cucurbitaceae family commonly called as snake gourd, viper gourd, snake tomato or chichinga. Its fruit is usually consumed as a vegetable by human beings. It has a prominent place in various pharmacological activities like antidiabetic, hepatoprotective, anticytotoxic, anti inflammatory, larvicidal effects [11]. The aim of the present study is to establish the antioxidant activity of ethanolic extract of *Oxalis corniculata*, *Phyallanthus fraternus* and *Trichosanthes cucumerina* plants and to evaluate their phytochemicals strength to validate antioxidative activities.

MATERIALS AND METHODS

Plant materials

Oxalis corniculata, *Phyallanthus fraternus* and *Trichosanthes cucumerina* plants were collected from Allahabad district, identified and authenticated by an Agronomist, Department of Agronomy, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, India.

Preparation of extracts

The whole plant of *Oxalis corniculata*, *Phyllanthus fraternus* and various parts (like fruits, leaves, stems and roots) of *Trichosanthes cucumerina* were collected, washed, dried under shade and powdered into fine particles. The 50g powder of each was macerated in 600ml of 95% ethanol at room temperature for 48 hours with occasional shaking at 8 hours. It was then filtered by Whatmann filter paper (size no.1) and the filtrate was evaporated on rotary evaporator to concentrate at 40ºC. There, 6.56 g light green residue (13.12% w/w) of *O. corniculata*, 6.50 g dark green residue (13% w/w) of *P. fraternus*, 10.26 g reddish residue (20.52% w/w) of *T. cucumerina*'s fruits, 3.99 g dark green residue (7.98% w/w) of *T. cucumerina*'s leaves, 3.50 g light green residue (7.0% w/w) of *T. cucumerina*'s stems and 4.0 g brownish residue (8% w/w) of *T. cucumerina*'s roots, were obtained respectively. These were kept in air tight bottle in a refrigerator until used [12, 13].

DPPH radicals scavenging activity assay

The free radical scavenging activity of ethanolic crude extracts of all plants were determined by DPPH method [14, 15]. DPPH solution (0.004% w/v) was prepared in 95% methanol. The crude extracts were mixed with 95% methanol to prepare solution of known concentration as 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml respectively in five test tubes. Freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes and after 10 minutes, the absorbance was taken at 517nm wavelength. Ascorbic acid was used as a reference standard. 95% methanol was used as blank. The percentage scavenging of the DPPH free radicals was measured using the following equation-

$$(A_{control}\text{-}A_{Test})/A_{control} \text{ X } 100$$

Where, A is absorbance.

The IC_{50} value for each sample was calculated from % Inhibition vs. Conc. graph. IC_{50} value is defined as the concentration of the test sample causing 50% inhibition of the initial DPPH free radical activity.

Phytochemicals analysis

Total flavonoids determination

Total flavonoids content was assayed by Chang *et al*. method [16]. Each crude extract (0.5mL of 1:10g/mL-1) in methanol was separately mixed with 1.5mL of methanol, 0.1mL of 10% aluminium chloride, 0.1mL of 1M potassium acetate and 2.8mL of distilled water. It remained at room temperature for 30 minutes. Absorbance of the reaction mixture was measured at 415nm wavelength using a single beam Systronics UV/Visible spectrophotometer. The calibration curve was prepared by standard quercetin solutions at concentrations 12.5 to 100g/ml in methanol.

Total carotenoids determination

Total carotenoids content in each crude extract was estimated by Mahadevan and Sridhar method [17]. 50 mg of each crude residue was dissolved in 3 ml absolute ethanol. Then 0.3 ml of 60% aqueous KOH was added and kept overnight at room temperature. After that they were washed with 5% ice-cold saline water to remove alkali and saline washings were extracted with ether (3:15 v/v). The ether extract from both were mixed together followed by washing with cold water till alkali free. The alkali free ether extracts were evaporated to crude residue form. These residues were dissolved in minimum volume of ethanol and absorbance was measured at λmax 450 nm by using ethanol as blank. The calibration curve was prepared by pure β carotene solution (1mg/ml).

Total phenols determination

Total phenols in crude extracts were determined by Bray and Thorpe method [18]. 5 mg of each crude extracts were dissolved in 3 ml of (1:1) solution of methanol and distilled water. 0.5 ml of FCR solution (1:10 diluted) and 3 minute after 2 ml of 20% Na_2CO_3 solution were added. Slightly heated the mixture in boiling water bath for exactly one minute, cooled and measured the absorbance at 650 nm wavelength against the reagent blank. The calibration curve was prepared by Gallic acid equivalent at conc. 1mg/ml (1:10 diluted).

Ascorbic acid determination

Harris method [19] with 2, 6-dichloro phenol indophenols dye was used for ascorbic acid assay in ethanolic extracts. 50 mg of each crude extracts were dissolved in 15 ml of 4% oxalic acid solution and titrated against dye (V_2). Stock solution of pure ascorbic acid in 4% oxalic acid (as 1mg / ml) diluted 10 times. 5 ml of this solution dissolved in 10 ml of 4% oxalic acid and titrated against dye (V_1). The amount of ascorbic acid (mg/100mg) was calculated by using formula -

$$EV \text{ X } V_1 \text{ X } 100 \text{ / } V_2 \text{ X } W$$

Where EV is ascorbic acid equivalent of dye and W is weight of sample (in mg).

Determination of non protein thiol

Non-protein thiol (NPT) was assayed by Ellman method [20]. 5 mg of each ethanolic crude extract was dissolved in 1 ml ice-cold 5% (w/v) sulfosalicylic acid solution. After centrifugation at 10,000 rpm and 4°C for 30 min, the supernatants were collected and immediately assayed. 300µl of this supernatant was mixed with 1.2 ml of 0.1M PBS (pH 7.6). After taking of initial absorbance at 412 nm, 25µM DTNB solution (6mM DTNB dissolved in 5mM EDTA, 0.1M PBS, pH 7.6) was added, and further increase in absorbance was recorded. The calibration curve was prepared by using reduced glutathione as standard (3 to 12µg/ml) and results were expressed in µmol/ 100 mg of extract.

Statistical analysis

All data were analysed by one sample t-test using graph pad prism software (version 5.03) for windows (Graph Pad Software, San Diego, USA).

RESULTS

Total antioxidant activity

Total antioxidant activity of ethanolic extracts of plants were evaluated by DPPH free radicals scavenging method and results presented in Table -1 shows the increase in percentage of DPPH radicals scavenging activity as increase in the concentrations of ethanolic extracts of Oxalis corniculata, Phyllanthus fraternus and Trichosanthus cucumerina (fruits, leaves, stems, and roots) and recorded maximum at 100µg/ml plant extract. However P. fraternus showed significantly ($\alpha = 0.05$, $p < 0.0001$) higher DPPH radicals scavenging activity among all the plants studied with its lowest IC_{50} (202.22 ± 1.89µg/ml) and displayed 38 % & 18.5% more activity than O. corniculata and T. cucumerina fruits respectively. T. cucumerina fruits and leaves also exhibited comparatively better antioxidant activity than stems and roots extracts but found much in its fruits. Further O. corniculata extract (IC_{50} = 352.34 ± 4.58 89µg/ml) had higher antioxidant activity than stem and root extracts of T. cucumerina.

Table 1: Percentage of DPPH radicals scavenging activity and IC_{50} values of plants ethanolic crude extracts.

Plants Extracts	Percentage of DPPH radicals scavenging activity at					IC_{50} value (in µg/ml)	t value for IC_{50} at df=3
	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml		
OC	4.63 ± 0.15	6.32 ± 0.11	7.11 ± 0.21	9.56 ± 0.42	11.86 ± 0.15	352.34* ± 4.58	23.1
PF	9.30 ± 0.29	11.42 ± 0.19	15.18 ± 0.14	17.61 ± 0.18	19.18 ± 0.23	202.22* ± 1.89	95.4
TCF	7.31 ± 0.14	9.31 ± 0.15	11.47 ± 0.15	13.79 ± 0.15	15.62 ± 0.19	260.30* ± 2.23	93.7
TCL	6.59 ± 0.15	8.72 ± 0.18	10.15 ± 0.19	11.93 ± 0.16	12.66 ± 0.21	278.29* ± 2.98	107
TCS	2.84 ± 0.16	4.40 ± 0.12	6.71 ± 0.44	7.24 ± 0.16	8.97 ± 0.15	462.44* ± 7.42	104
TCR	1.28 ± 0.19	2.88 ± 0.36	3.22 ± 0.34	4.66 ± 0.40	5.14 ± 0.29	920.38* ± 4.31	84.6
Asc as standard	21.16 ± 0.47	36.11 ± 0.50	52.31 ± 0.42	67.30 ± 0.39	88.22 ± 1.12	58.67** ± 3.18	330

Values are mean ±SD of triplicates; * statistically significant at $\alpha = 0.05$ and $p < 0.0001$, ** statistically significant at $\alpha = 0.05$ and $p = 0.0002$

Note: OC= *Oxalis corniculata*, PF= *Phyllanthus fraternus*, TCF= *Trichosanthes cucumerina fruit*, TCL= *Trichosanthes cucumerina* leaves, TCS= *Trichosanthes cucumerina* stem, TCR= *Trichosanthes cucumerina* root, Asc= ascorbic acid.

Phytochemical constituents

T. cucumerina fruit was observed to be comparatively richer source of total phenols (**Fig. 1**) than the other plants and plant parts studied. The fruit extract of *T. cucumerina* examined nearly 440.0%, 129.24% and 53.79% higher phenols than its roots, stems and leaf respectively whereas 28.4% more phenols observed than the *Oxalis corniculata.* Even *Phyllanthus fraternus*

whole plant extracts which displayed a good source of total phenol (2.14±0.12 mg per 100 mg Residual Weight) had shown bit lesser than *T. cucumerina* fruits (2.43 ±0.14 mg per 100 mg RW).

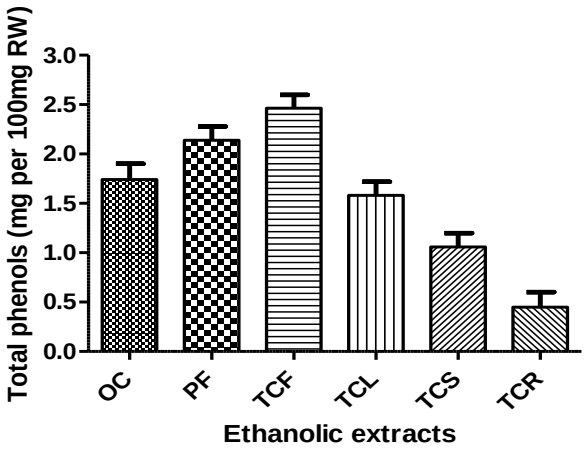

Fig. 1: Total phenols content in plants ethanolic extracts.

Total flavonoids content was observed to be significantly higher in *P. fraternus* extracts and possessed 19.67±0.68 mg per 100 mg RW that was noted around 17.99%, 20.45% higher flavanoids than *O. corniculata* which fruits of *T. cucumerina* respectively (**Fig. 2**). Fruits of *T. cucumerina* contained nearly same amount of total flavonoids as recorded in *O. corniculata* (16.67±0.58 mg per 100mg RW) extracts. Fruits of *T. cucumerina* had retained much more flavonoids than its other parts and observed to the 58.08%, 44.13% and 18.67% higher than its roots, shoots and leaves respectively.

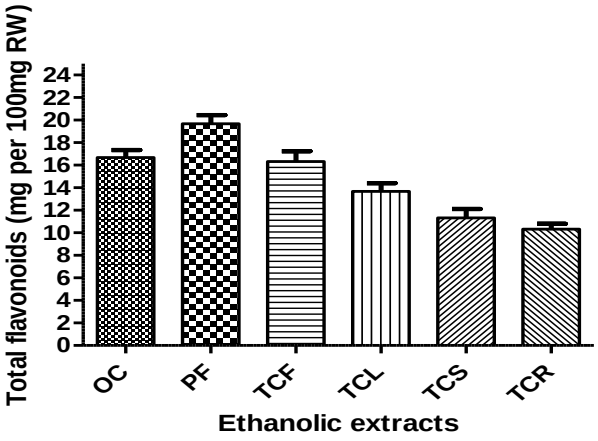

Fig. 2: Total flavonoids content in plants ethanolic extracts.

Similar pattern was also observed in the retention of total carotenoids as total flavonoids content by the studied plants and plant parts (**Fig. 3**). *P. fraternus* plant extract displayed maximum content (4.63±0.14mg per 100 mg RW) of the total carotenoids then in order: *O. corniculata* (3.20±0.12mg per 100 mg RW), and fruits of *T. cucumerina* (2.48±0.12mg 100 mg RW) that was about respectively 44.68% and 86.69% lesser content. Least content of carotenoids was noted in the roots of *T. cucumerina* and was found about half of its fruits.

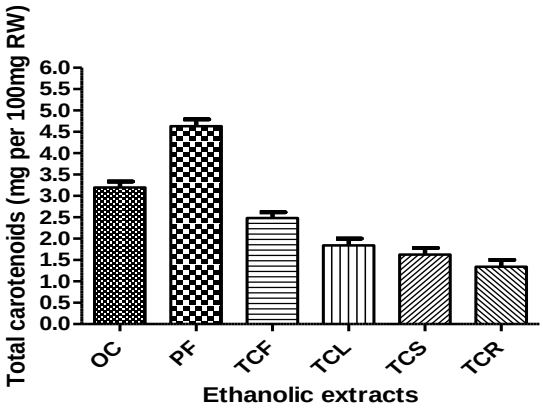

Fig. 3: Total carotenoids content in plants ethanolic extracts.

Thiols (-SH) play an important role in free electrons quenching and were found significantly different at $\alpha = 0.05$, $p < 0.0001$ in plants ethanolic extracts (**Fig. 4**). However, slight difference was observed in the extracts of *T. cucumerina* fruits, *P. fraternus* and *O. corniculata*. Stem and roots of *T. cucumerina* were perceived to be lowest in the thiols content. *P. fraternus* extract that displayed highest (33.50 ±1.14 µmole per 100 mg RW) non protein thiols was found to be double than the roots of *T. cucumerina* and observed 26.65%, 91.10%, higher than the leaves and stems of *T. cucumerina*.

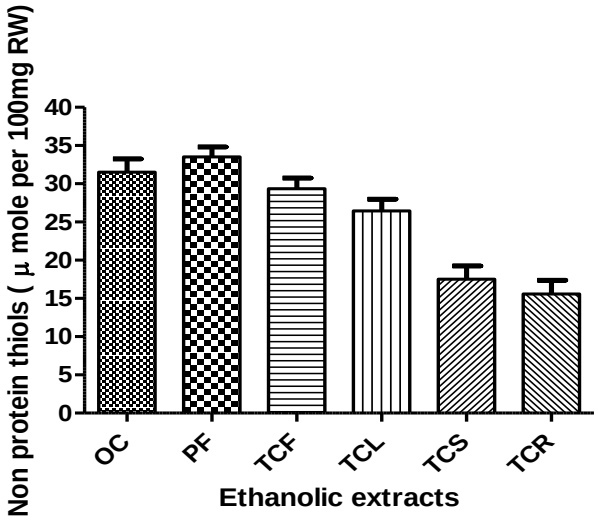

Fig. 4: Non protein thiols (-SH) content in plants ethanolic extracts.

P. fraternus was also observed richer source of ascorbic acid amongst the plants studied and possessed 1.39±0.16 mg per 100 mg RW ascorbic acid and displayed more than double of *O. corniculata* (**Fig. 5**). The fruits of *T. cucumerina* had also exhibited more than the *O. corniculata* and leaves, stems and roots of *T. cucumerina*. The fruit of *T. cucumerina* was reported to be 8 folds, 5 folds and more than 2 folds higher content than its root, stem and leaf respectively.

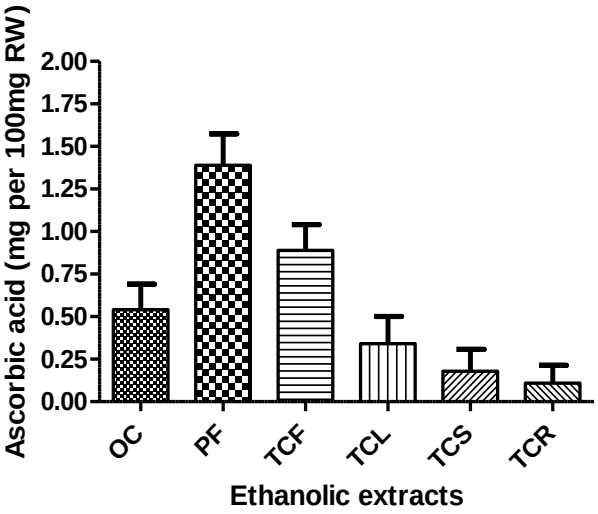

Fig. 5: Ascorbic acid content in plants ethanolic extracts.

DISCUSSION

Antioxidant is one of the most essential ingredients of today's therapy since they reduce *in vivo* oxidative damages. Plants are the good resources for natural antioxidants [1]. Therefore, the great interest has been recently focused on searching of plants having natural antioxidants and good medicinal value. DPPH radicals scavenging method is widely used to investigate the total antioxidant activities in plants [21]. In present study *Phyllanthus fraternus* ethanolic extract showed maximum antioxidant activity with IC_{50} value 202.22±1.89 µg/ml. Even as ethanolic extract of *Oxalis corniculata* and *Trichosanthes cucumerina* fruits also displayed better antioxidant activity than others (**Table 1**) and may be employed together for enhancing antioxidant power. In earlier studies the ethanolic extract of *Phyllanthus fraternus* showed IC_{50} values 0.062 mg mL^{-1} [12], while ethylacetate fraction of *Oxalis corniculata* has maximum DPPH scavenging activity with the IC_{50} value of 4.04±0.08 µg/ml, among different solvent (n- BuOH, MeOH and aqueous) extracts [22]. *Trichosanthes cucumerina* fruits also exhibited ferric-reducing antioxidant power [26], which supports our results. So this study explored the comparative as well as individual antioxidative activity in selected plants extracts.

Phenolic substances and flavonoids are associated with antioxidant activity and play important role in stabilizing lipid peroxidation [15], by adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [23]. Present study entailed *Phyllanthus fraternus* extract has higher phytochemicals

strength than other, while ethanolic extracts: *Oxalis corniculata* and *Trichosanthes cucumerina* fruits also exhibited almost same phytochemicals strength (**Fig. 1, 2, 3, 4 & 5**) as studied in *Momordica charantia* fruit [25]. In previous studies phytochemicals screening of methanolic and aqeous exracts of these plants were done and found that extract of *Oxalis corniculata* possessed total phenol (25.62±0.10 mg), total flavonoids content (150.88±12.61mg) per gram of dry weight [24] while aqueous extract of *Phyllanthus fraternus* [8] and *Trichosanthes cucumerina* fruits [26] revealed the presence of alkaloids, flavonoids, tannins, glycosides, saponin, carbohydrates, resins and phenols. Thus present study reports a comparative phytochemicals strength which may enhance antioxidative power in vivo system.

CONCLUSION

Present study concluded that ethonolic extract of *P. fraternus*, *O. corniculat*a, fruits and leaves part of *T. cucumerina* possessed significant antioxidant activity and photochemical potency. Further investigation is required to find active components of these extracts and isolation of components responsible for activity.

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REFERENCES

1. Rajan S, Mahalakshmi S, Deepa VM, Sathya K, Shajitha S, Thirunalasundari T. Antioxidant potentials of *Punica granatum* fruit rind extracts. Int J Pharm Pharm Sci 2011; 3(3): 82-88.
2. Vadalia DN, Pethe AM, Chakraborthy GS. Antioxidant Activity of Polyherbal Formulation. J Pharma Res 2010; 3(8): 1756-1758.
3. Singh R, Jain SC, Jain R. Antioxidant Activity of Some Medicinally Important Arid Zone Plants. Asian J Exp Sci 2009; 23(1): 215-221.
4. Krishnaiah D, Sarbatly R, Bon A. Phytochemical antioxidants for health and medicine – A move towards nature. Biotechnology and Molecular Biology Review 2007; 1 (4): 097-104.
5. Morel I, Lescoat G, Cillard P, Cillard J. Role of flavonoids and iron chelation in antioxidant action. Methods Enzymol 1994; 234: 437- 443.
6. Rice-Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds. Trends Plant Sci 1997; 2: 152-159.
7. Kathiriya AK, Das K, Joshipura M, Mandal N. Review on *Oxalis corniculata* Linn. -The Plant of Indian subtropics. Herbal Tech Industry 2010, p 09-11.
8. Matur BM, Matthew T, Ifeanyi CIC. Analysis of the phytochemical and *in vivo* antimalaria properties of *Phyllanthus fraternus* webster extract. New York Science Journal 2009; 2(5): 12-19.
9. Oudhia P. *Phyllanthus fraternus* G.L.Webster. In: Schmelzer, G.H. & Gurib-Fakim, A. (Editors). Prota 11(1): Medicinal plants/Plantes médicinales 1. [CD-Rom]. PROTA, Wageningen, Netherlands; 2008.
10. Khatoon S, Raia V, Rawata AKS, Mehrotra S. Comparative pharmacognostic studies of three Phyllanthus species. J Ethnopharmacol 2006; 104(1-2): 79-86.
11. Sandhya S, Vinod KR, Chandra Sekhar J, Aradhana R, Nath VS. An updated review on *Tricosanthes cucumerina*. Int J Pharmaceutical Sci Rev Res 2010; 1(2, Article 011): 56-60.
12. Koffuor GA, Amoateng P. Antioxidant and Anticoagulant Properties of *Phyllanthus fraternu*s GL Webster (Family: Euphorbiaceae). J Pharmacol Toxicol 2011; 6: 624-636.
13. Devendra NK, Malashetty VB, Seetharam YN, Suresh P, Patil SB. Effect of ethanol extract of whole plant of *Trichosanthes cucumerina* var. *Cucumerina* L. on gonadotropins, ovarian follicular kinetics and estrous cycle for screening of antifertility activity in albino rats. Int J Morphol 2009; 27(1): 173-182.
14. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958; 181: 1199-1200.
15. Yen GC, Duh PD. Scavenging Effect of Methanolic Extracts of Peanut Hulls on Free Radical and Active Oxygen Species. J Agric Food Chem 1994; 42: 629-632.
16. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoids content in propolis by two complementary colorimetric methods. J. Food drug analysis 2002; 10: 178-82.
17. Mahadevan A, Sridhar. Secondary metabolites. In: Methods in Physiological Plant Pathology. 3rd ed. Sivakami Publications, 1986, Chennai, p 9-11.
18. Bray HG, Thorpe WV. Analysis of phenolic compounds of interest in metabolism. Method Biochem Anal 1954; 1: 27-52.
19. Harris LJ. Assessment of the level of nutrition: tests for vitamin-C on groups of poorly fed and well-fed school-children. The Lancet 1935; 236(6105): 259-263.
20. Ellman GL. Tissue sulphydril groups. Arch Biochem Biophys 1995; 82: 70-77.
21. Yeh-Lin Lu, Yuh-Hwa Liu, Jong-Ho Chyuan, Kur-Ta Cheng, Wen-Li Liang, Wen-Chi Hou. Antioxidant activities of different wild bitter gourd (*Momordica charantia* L. var. *abbreviata* Seringe) cultivars. Botanical Studies 2012; 53: 207-214.
22. Alam MB, Hossain MS, Chowdhary NS, Mazumdar MEH, Haque ME, Islam A. In *vitro* and in *vivo* Antioxidant and Toxicity Evaluation of different fraction of *Oxalis corniculata* L. J Pharmacol Toxicol 2011; 6(4): 337-348.
23. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. J Agri Food Chem 2001; 49: 5165-5170.
24. Sakat SS, Juvekar AR, Gambhire MN. *In vitro* Antioxidant and Antiinflammatory Activity of Methanol Extract of *Oxalis corniculata* L. Int J Pharm Pharm Sci 2010; 2(1): 146-155.
25. Patel S, Patel T, Parmar K, Patel B, Patel P. Evaluation of antioxidant activity, phenol and flavonoid contents of *Momordica charantia* L. fruit. Adv Res Pharmaceuticals biol 2011; 1(2): 120-129.
26. Adebooye OC. Phyto-constituents and anti-oxidant activity of the pulp of snake tomato (*Trichosanthes cucumerina*). Afr J Trad Compl Alt Med 2008; 5 (2): 173-179.