Int J Pharm Bio Sci 2015 Jan; 6(1): (P) 723 - 732

**Research Article** **Natural chemistry**

**International Journal of Pharma and Bio Sciences** **ISSN 0975-6299**

# EVALUATION OF ANTIOXIDANT ACTIVITY OF HOMONOIA RIPARIA LOUR., KIRGANELIA RETICULATA (POIR) BAILL., PHYLLANTHUS FRATERNUS WEBSTER AND PEDILANTHUS TITHYMALOIDES (LINN.) POIT. AND ITS CORRELATION WITH THE TOTAL PHENOLIC AND FLAVONOID CONTENTS.

**UJWALA C. BAPAT* AND DEEPALI R. MHAPSEKAR**

*Department of Botany, St. Xavier's College, Mahapalika Marg, Mumbai 400 001, Maharashtra State, India*

## ABSTRACT

Plants of Euphorbiaceae are used to cure various diseases in traditional systems of medicine. For present study, *Homonoia riparia, Kirganelia reticulata, Phyllanthus fraternus* and *Pedilanthus tithymaloides* were selected on the basis of their availability and antimicrobial activity. Plant phenolics and flavonoids are known to possess antioxidant activity. Hence, it was decided to evaluate the antioxidant activity of these extracts and correlate it with the total phenolic and flavonoid contents of the same. The antioxidant activity and total phenol and flavonoid contents were determined by the standard methods. The results showed that the highest antioxidant activity and the total phenolic and flavonoid contents were observed in DMSO extract of *P. fraternus*. There was a positive correlation between the total antioxidant capacity and total phenolic and flavonoid contents in all the extracts. The $IC_{50}$ values obtained indicated maximum antioxidant potential in the extract of male flowers of *H. riparia*.

**KEYWORDS:** **Antioxidant activity, *Homonoia riparia, Kirganelia reticulata*, *Phyllanthus fraternus*, *Pedilanthus tithymaloides*, phenols**

**UJWALA C. BAPAT**
**Department of Botany, St. Xavier's College, Mahapalika Marg, Mumbai 400 001, Maharashtra State, India**

***Corresponding author**

# INTRODUCTION

The plants of Euphorbiaceae are reported to be used in the treatment of skin diseases, nervine, urinary and venereal disorders, abdominal troubles, asthma, cancer, rheumatism, dysentery, diarrhea, jaundice, etc [1]. The decoction of roots of *Homonoia riparia* Lour. is given in piles, stones in bladder, chest pain, gonorrhoea and syphilis while, the pounded leaves and fruits are applied as poultice for skin diseases[2]. *Kirganelia reticulata* (Poir) Baill. is used in small pox, syphilis, asthma, diarrhoea and bleeding from gums[3]. *Phyllanthus fraternus* Webster is employed for treatment of biliary and urinary conditions including gall bladder and kidney stones, hepatitis, colds, flu, tuberculosis, viral infections, liver diseases, anaemia, bacterial infections such as cystitis and prostatitis, diabetes, malaria, dysentery, fever, pain, tumours, vaginitis, gonorrhoea and dyspepsia[4]. *Pedilanthus tithymaloides* (Linn.) Poit. has been reported to possess febrifuge, anticancer and anti-inflammatory properties and it is also applied to wounds for rapid healing[5,6]. The antimicrobial (antibacterial and antifungal) activity of the extracts of *H. riparia*, *K. reticulata*, *P. fraternus* and *P. tithymaloides* was carried out against the Gram +ve and Gram –ve bacteria and against *Trichophyton mentagrophytes* and *T. rubrum* causing dermatophytosis in humans. The aqueous extract of leaves of *H. riparia*, DMSO extracts of flowers of *H. riparia* and *P. tithymaloides*[7] and leaves of *K. reticulata*[7] and *P. fraternus*[8] exhibited the antimicrobial activity in earlier studies. Selection of the plants was done on the basis of their traditional medicinal uses, their availability in the wild and their antimicrobial activity. Preliminary phytochemical tests indicated the presence of phenolics and flavonoids. Several studies have shown that medicinal plants contain a wide variety of natural antioxidants such as phenolics and flavonoids[9]. Recent studies have shown that the antioxidant effect of plant products is mainly due to phenolic compounds such as flavonoids, phenolic acids and tannins[10,11]. Antioxidant activity of the extracts of *K. reticulata*, *P. fraternus* and *P. tithymaloides* was reported[12,13,14].Total phenolic and flavonoid contents of these extracts were estimated in separate experiments by other group of researchers[15,16,17,18]. However, the correlation between the antioxidant activity and the total phenolic content was shown only in the extracts of dried leaves of *K. reticulata* and *P. fraternus*[19,20]. Hence, present study was undertaken to evaluate the antioxidant activity of the extracts of leaves and flowers of *H. riparia*, leaves of *K. reticulata* and *P. fraternus* and flowers of *P. tithymaloides* and correlate it with the total phenolic and flavonoid contents of the same.

# MATERIALS AND METHODS

### *Collection and authentication of plant material*

The plant materials were collected from Sanjay Gandhi National Park, Borivili, Vasai and Byculla, Mumbai and authenticated by studying the morphological characters and by comparing with the Blatter Herbarium specimens. The plants were washed under running water, shade dried, powdered and sieved (mesh size 1mm).

### *Preparation of extracts*

To prepare 20 % extracts, 2 g of dry plant powders were soaked overnight in 10 ml of distilled water / DMSO. In case of *P. fraternus*, the extract of fresh leaves was prepared by crushing the leaves in DMSO. The extracts were then filtered through Whatman No. 1 filter paper and the filtrates were used after diluting with appropriate amount of ethanol to obtain the required concentrations[21]. The extracts were prepared in triplicates.

### *Assays for Antioxidant activity*

#### *1. Determination of total antioxidant capacity*

The method described by Prieto *et al* (1999) was used to determine the total antioxidant capacity of the extracts[22]. The antioxidant capacity was expressed as ascorbic acid equivalent (AAE).

#### *2. Reducing power assay*

The method proposed by Oyaizu *et al* (1986) was used for the reducing power assay[22]. The

reducing power of the extracts was expressed in terms of absorbance and compared with that of ascorbic acid standard.

***3. DPPH radical scavenging activity***

The DPPH radical scavenging activity was carried out as per the method adopted by Brand-Williams *et al* (1995)[23]. The range of concentrations of extracts used for DPPH radical scavenging activity were as follows:

*H. riparia* - leaves -10-50 µg of residue
*H. riparia* - flowers- 4-20 µg of residue
*K. reticulata* - leaves- 5-25 µg of residue
*P. fraternus* - 6-30 µg of residue
*P. tithymaloides* - flowers 5-25 µg of residue

(The linear relationship between the DPPH radical scavenging activity and the concentration of extract was observed at these concentrations). The activity was calculated using following equation: DPPH scavenging activity (%) = $[(A_o-A_1)/A_o] \times 100$ where, $A_o$ was the absorbance of the blank (i.e. only DPPH solution, no sample) and $A_1$ was the absorbance in presence of the test compound / ascorbic acid standard.

***Estimation of total phenolics and flavonoids***

The total phenolic content of the extracts was estimated by modified Folin-Ciocalteau method which was proposed by Eberhardt *et al* (2000)[24] while, the total flavonoid content was estimated by Aluminium chloride method which was described by Zhishen *et al* (1999)[24]. The total phenolic content was expressed as mg of gallic acid equivalents per mg of residue and the flavonoid content as mg of rutin equivalents per mg of residue.

***Statistical analysis***

Linear regression analysis was used to calculate $IC_{50}$ values for DPPH radical scavenging assay. The correlation analysis was carried out to determine the relationship between the total antioxidant capacity and the total phenolic and flavonoid contents.

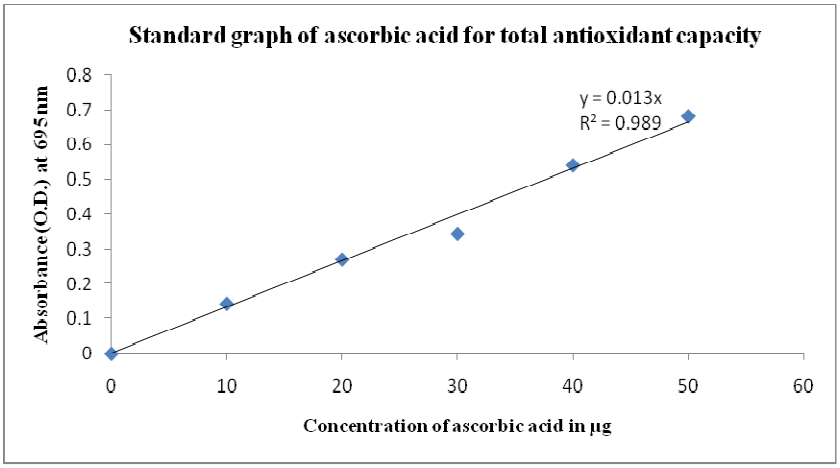
## RESULTS AND DISCUSSION

***Assays for Antioxidant activity***

***1. Determination of total antioxidant capacity***

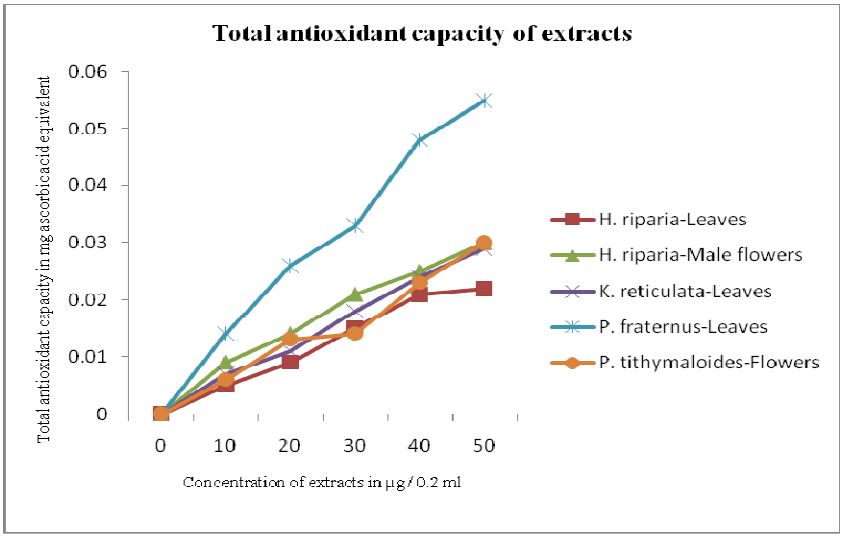
The total antioxidant capacity was determined by phosphomolybdenum method, which is based on the reduction of Mo (+6) to Mo (+5) by the sample analyte and the subsequent formation of green phosphate/ Mo (+5) complex with a maximum absorption at 695 nm[25]. The standard graph of ascorbic acid for total antioxidant capacity is given below (Graph 1):

**Graph 1**
***Standard graph of ascorbic acid for total antioxidant capacity***




It can be seen that there is a strong +ve correlation between the concentration of ascorbic acid and the absorbance. The total antioxidant capacity of the extracts is shown in Graph 2.

**Graph 2**
***Total antioxidant capacity of extracts***

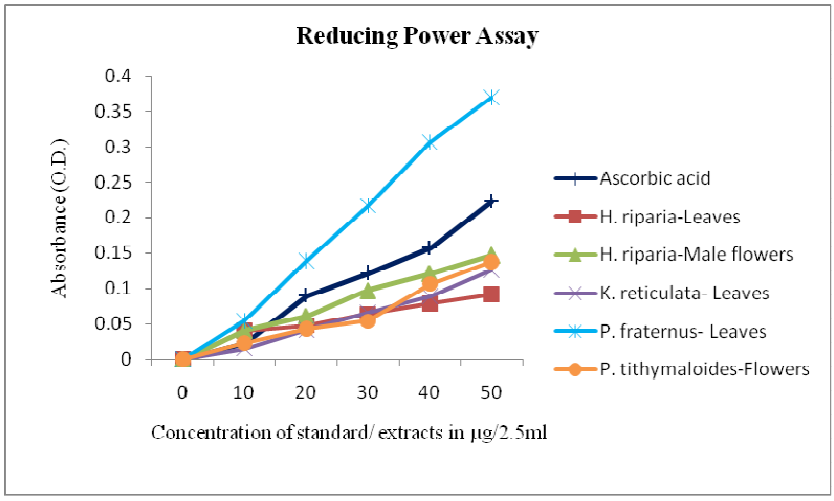



Graph 2 indicates that as the concentration of extracts increases, there is an increase in the total antioxidant capacity. The lowest antioxidant capacity was recorded for the aqueous extract of dry leaves of *H. riparia* (0.022±0.008 mg AAE/ 50 µg of residue). The antioxidant capacity of DMSO extracts of leaves of *K. reticulata* and flowers of *H. riparia* and *P. tithymaloides* was 0.029±0.002, 0.030±0.001 and 0.030±0.011 mg AAE/ 50 µg of residue respectively. The highest antioxidant capacity was observed for the DMSO extract of fresh leaves of *P. fraternus* (0.055±0.024 mg AAE/ 50 µg of residue). Koffuor *et al* (2011) had reported increase in the total antioxidant capacity of the ethanolic extract of dried leaves of *P. fraternus* with increasing concentration[20]. They observed antioxidant activity of 0.070 µg ascorbic acid equivalent per 10 mg dried leaves which was very poor as compared to that of 0.055 µg ascorbic acid equivalent per 50 µg fresh leaves in the present study. This suggests that there was a loss of antioxidant activity of the leaves of *P. fraternus* during drying.

### *2. Reducing power assay*

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity[26]. Antioxidants which have reduction potential, react with potassium ferricyanide ($Fe^{3+}$) to form potassium ferrocyanide ($Fe^{2+}$), which then reacts with ferric chloride to form $Fe^{2+}$-Ferrozine complex that has an absorption maximum at 700 nm[27]. The reducing power of the extracts when compared with that of ascorbic acid standard showed following graph.

**Graph 3**
***Reducing power assay***

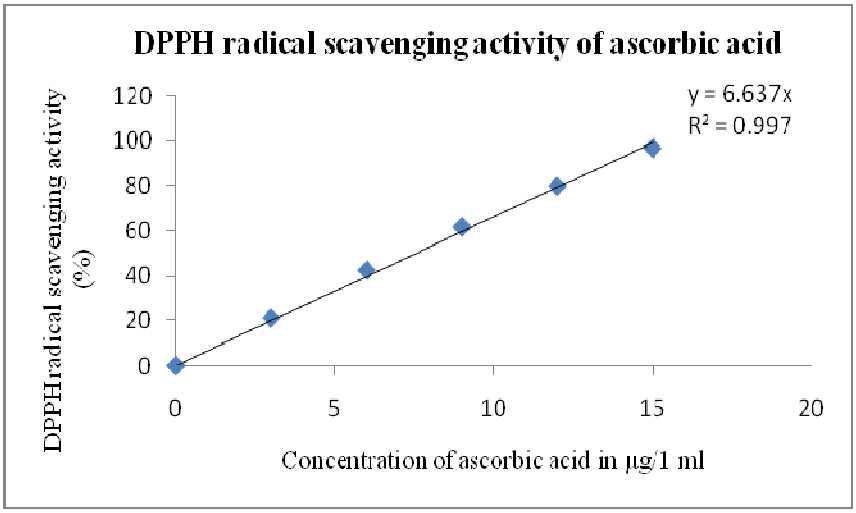



Graph 3 shows that as the concentration of standard / extracts increases, there is an increase in the absorbance indicating the increase in the reducing power. The highest reducing power was observed with the DMSO extract of fresh leaves of *P. fraternus* (0.370±0.014 Abs for 50 µg) which is higher than that of standard ascorbic acid. Koffuor *et al* (2011) had also observed a concentration dependant reducing activity for the dried leaves of *P. fraternus*[20]. The lowest reducing power was observed with the aqueous extract of dry leaves of *H. riparia* (0.092±0.03 Abs for 50 µg). The reducing power of extracts of dry flowers of *H. riparia* and *P. tithymaloides* and that of leaves of *K. reticulata* for 50 µg was 0.147±0.02, 0.126±0.01 and 0.138±0.06 Abs respectively. However, Abreu *et al* (2008) reported comparatively less reducing power ($IC_{50}$ value of 950.0±5.1 µg/ml) in the 70 % ethanolic extract of powdered stem and leaves of *P. tithymaloides*[18].

***3. DPPH radical scavenging activity***

DPPH (2, 2-diphenyl-1-picryl hydrazyl) is the most commonly used stable free radical, to test the potential of compounds as free radical scavengers of hydrogen donors and to investigate the antioxidant activity of plant extracts[28]. Antioxidant molecules when incubated, react with DPPH and convert it into 2, 2 -diphenyl-1-picryl hydrazine, which is a measure of the scavenging potential of plant extracts and can be measured at 520 nm[29]. DPPH radical scavenging activity of ascorbic acid (standard) is given below (Graph 4)

**Graph 4**
***DPPH radical scavenging activity of ascorbic acid***




A strong +ve correlation between the concentration of ascorbic acid and the DPPH radical scavenging activity is observed in the above graph. DPPH radical scavenging activity of the extracts showed following results (Table 1).

**Table 1**
***Determination of DPPH radical scavenging activity of extracts of H. riparia, K. reticulata, P. fraternus and P. tithymaloides.***

| Extract | Concentration in µg/ ml | DPPH radical scavenging activity** (%) | $IC_{50}$*** µg/ml |
|---|---|---|---|
| *H. riparia* Leaves | 10 | 25.94±2.61 | 28.89 |
| | 20 | 40.06±5.46 | |
| | 30 | 55.98±3.66 | |
| | 40 | 69.46±5.27 | |
| | 50 | **80.14±6.35** | |
| *H. riparia* Male flowers | 4 | 21.58±2.54 | 13.24 |
| | 8 | 35.41±0.66 | |
| | 12 | 45.91±0.83 | |
| | 16 | 62.72±1.29 | |
| | 20 | **69.98±1.53** | |
| *K. reticulata* Leaves | 5 | 15.48±0.45 | 18.79 |
| | 10 | 27.16±1.82 | |
| | 15 | 41.27±4.44 | |
| | 20 | 49.58±5.99 | |
| | 25 | **67.99±13.11** | |
| *P. fraternus* Leaves | 6 | 18.31±2.48 | 19.72 |
| | 12 | 28.00±3.36 | |
| | 18 | 51.91±0.95 | |
| | 24 | 57.41±4.99 | |
| | 30 | **75.46±2.07** | |
| *P. tithymaloides* Flowers | 5 | 21.16±7.29 | 15.87 |
| | 10 | 40.22±2.69 | |
| | 15 | 48.31±4.18 | |
| | 20 | 58.96±2.19 | |
| | 25 | **76.87±1.5** | |

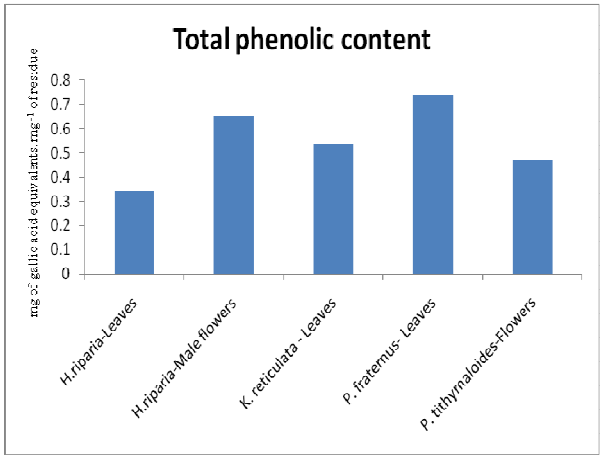
***** values with ± std deviation***
******IC50 value for ascorbic acid is 7.53 µg/ ml***

The table shows that as the concentration of extract increases, there is an increase in the DPPH radical scavenging activity. The highest $IC_{50}$ value was observed for the extract of dry leaves of *H. riparia* (28.89 µg/ ml) indicating lowest DPPH radical scavenging activity. The lowest $IC_{50}$ value was observed for extract of dry flowers of *H. riparia* (13.24 µg/ ml) indicating the highest DPPH radical scavenging activity. In present study, the $IC_{50}$ value of 18.79 µg/ ml was obtained for the extract of dry leaves of *K. reticulata* whereas, Eldeen *et al* (2011) obtained 10.8 µg/ ml for the same[19]. The DPPH radical scavenging activity with the $IC_{50}$ value of 19.72 µg/ ml was observed with the extract of fresh leaves of *P. fraternus*. However, Koffuor *et al* (2011) observed a very high $IC_{50}$ value (11.40 mg/ml) with the methanolic extract of dry leaves indicating the loss of DPPH scavenging activity during drying process[20]. $IC_{50}$ value of 15.87 µg/ ml was observed for the extract of dry flowers of *P. tithymaloides*. Abreu *et al* (2008) reported the DPPH scavenging activity with $IC_{50}$ value of 310 µg/ ml for 70 % ethanolic extract of powdered stem and leaves of *P. tithymaloides*[18]. These observations indicate that the flowers of *P. tithymaloides* have higher DPPH radical scavenging activity. The $IC_{50}$ values of all the extracts were greater as compared to that of ascorbic acid (7.53 µg/ ml) indicating that the ascorbic acid had the highest DPPH radical scavenging activity.

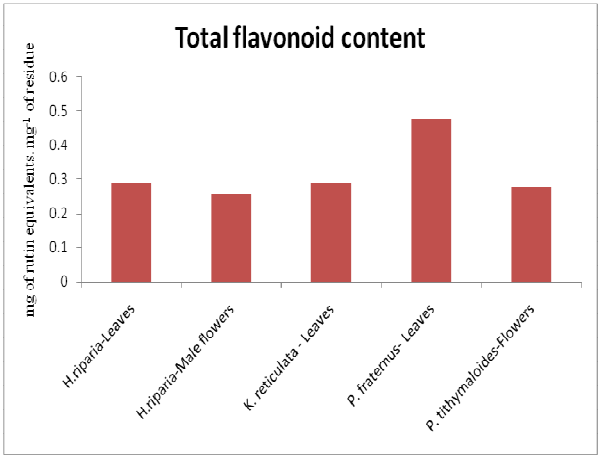
***Estimation of total phenolics and flavonoids***

The total phenolic and flavonoid contents of the extracts are shown in Graphs 5 and 6. The highest total phenolic content was observed in the extract of fresh leaves of *P. fraternus* (0.743±0.249 mg of gallic acid equivalents per mg of residue). Koffuor *et al* (2011) reported the phenolic content of 0.3551 mg tannic acid equivalent/ 2.5 mg residue of dried leaves of *P. fraternus*[20]. The total phenolic content of 0.343±0.153 mg of gallic acid equivalents per mg of residue was observed in the aqueous extract of dry leaves of *H. riparia*, whereas that in the DMSO extract of its dry flowers, it was 0.654±0.021 mg of gallic acid equivalents per mg of residue. The total phenolic content in the DMSO extract of dry leaves of *K. reticulata* was 0.537±0.081 mg of gallic acid equivalents per mg of residue. Eldeen *et al* (2011)[19] reported 127.8 mg of gallic acid equivalent/ g residue and Sankannavar *et al* (2012)[30] obtained 22.5 mg of gallic acid equivalent/ g residue obtained from the extract of dried leaves of *K. reticulata*. The total phenolic content of the DMSO extract of dry flowers of *P. tithymaloides* obtained was 0.472±0.117 mg of gallic acid equivalents per mg of residue (Graph 5). Abreu *et al* (2008) reported the content of total phenolics to be 76.0 ± 4.8 mg of gallic acid equivalent/ g residue obtained from 70% ethanolic extract of powdered stem and leaves of *P. tithymaloides*[18]. Thus, these findings showed that the phenolic content is more in the flowers of *P. tithymaloides* than the stem and leaves.

**Graph 5**
***Total phenolic content of the extracts (Mg of gallic acid equivalents mg$^{-1}$ of residue)***




**Graph 6**
***Total flavonoid content of the extracts (Mg of rutin equivalents mg$^{-1}$ of residue)***




The highest total flavonoid content was observed in the DMSO extract of fresh leaves of *P. fraternus* (0.478±0.250 mg of rutin equivalents per mg of residue). Das *et al* (2011) had observed 75.5 mg quercetin equivalent/ g sample of total flavonoids in the methanolic extract of dry *P. fraternus*[31]. The lowest content was found in the DMSO extract of dry male flowers of *H. riparia* (0.256±0.084 mg of rutin equivalents per mg of residue). The aqueous extract of dry leaves of *H. riparia* and DMSO extract of dry leaves of *K. reticulata* showed presence of 0.289±0.069 mg of rutin equivalents per mg of residue. Loganayaki *et al* (2010) obtained 46.7 g rutin equivalent / 100 g of fresh fruits of *K. reticulata*[32]. The total flavonoid content in DMSO extract of dry flowers of *P. tithymaloides* was 0.278±0.069 mg of rutin equivalents per mg of residue (Graph 6). Abreu *et al* (2008) had reported only 9.8±0.4 mg of rutin equivalent/ g residue obtained from the extract of powdered stem and leaves[18] indicating the higher content of flavonoids in the flowers. The total antioxidant capacity was correlated with the total phenolic and flavonoid contents. The results are mentioned in Table 2.

**Table 2**
***Correlation between the total antioxidant capacity and the total phenolic and flavonoid contents***

| Plant | $R^2$ value ( phenolic content) | $R^2$ value (flavonoid content) |
|---|---|---|
| *H. riparia* - Leaves | 0.931 | 0.845 |
| *H. riparia* - Male flowers | 0.819 | 0.715 |
| *K. reticulata* - Leaves | 0.887 | 0.853 |
| *P. fraternus* - Leaves | 0.958 | 0.968 |
| *P. tithymaloides* - Flowers | 0.914 | 0.916 |

There was a strong positive correlation between the total antioxidant capacity and total phenolic content of the leaves of *H. riparia* and *P. fraternus* and flowers of *P. tithymaloides*, whereas a positive correlation in case of male flowers of *H. riparia* and leaves of *K. reticulata*. Eldeen *et al* (2011) reported a strong correlation between the total phenolic content and the DPPH radical scavenging activity of extract of dried leaves of *K. reticulata*[19]. There was a strong positive correlation between the total antioxidant capacity and total flavonoid content of the leaves of *P. fraternus* and flowers of *P. tithymaloides* whereas, a positive correlation in case of leaves and male flowers of *H. riparia* and leaves of *K. reticulata*.

## CONCLUSION

Present study revealed that the aqueous extract of leaves of *H. riparia* and DMSO extracts of flowers of *H. riparia* and *P. tithymaloides* and leaves of *K. reticulata* and

*P. fraternus* possessed antioxidant properties. Among all the extracts tested, the highest antioxidant capacity and the reducing power activity were observed in the extract of fresh leaves of *P. fraternus*, whereas the highest DPPH radical scavenging activity was observed in the extract of dry flowers of *H. riparia*. There is a positive correlation between the total antioxidant capacity and the total phenolic and flavonoid contents.The antioxidant components such as phenolics and flavonoids found in the said plants may be isolated and identified in future to explore their potential as a source of new antioxidants.

## ACKNOWLEDGEMENT

The authors gratefully acknowledge University Grants Commission for the financial assistance provided through the grant of Major Research Project to carry out this work. The authors express their sincere thanks to Principal, St. Xavier's College for providing the facilities. Thanks to Dr. Rajani Athawale and Ms. Meenakshi Nehete, S.N.D.T. Women's University, Mumbai for their guidance.

### CONFLICT OF INTEREST

There is no conflict of interest among authors of this publication.

## REFERENCES

1. Kirtikar KR and Basu BD. Indian Medicinal Plants, 2nd Edn, Vol 3, International Book Distributors: Uttaranchal, 2196-2290, (2005).
2. National Institute of Science Communication, CSIR (IN). The Wealth of India - A Dictionary of Indian Raw Materials and Industrial Products, Raw Materials, Vol. V: H-K, CSIR: New Delhi, 114, (1959).
3. Shruthi SD, Ramachandra YL, Rai SP and Jha PK. Pharmacognostic evaluation of leaves of *Kirganelia reticulata* Baill.(Euphorbiaceae). The Asian and Australasian Journal of Plant Science and Biotechnology, 4 (1): 62-65, (2010).
4. Singh B, Dutt N, Kumar D, Singh S and Mahajan R. Taxonomy, ethnobotany and antimicrobial activity of *Croton bonplandianum, Euphorbia hirta* and *Phyllanthus fraternus*. Journal of Advances in Developmental Research, 2 (1): 21-29, (2011).
5. Mongkolvisut W and Sutthivaiyakit S. Antimalarial and antituberculous Poly-*O*-acylated jatrophane diterpenoids from *Pedilanthus tithymaloides*. J. Nat. Prod., 70: 1434–1438, (2007).
6. Sriwiroch W, Chungsamarnyart N, Chantakru S,Pongket P, Saengprapaitip K and Pongchairerk U. The effect of *Pedilanthus tithymaloides* (L.) Poit. crude extract on wound healing stimulation in mice. Kasetsart J. (Nat. Sci.), 44: 1121–1127, (2010).
7. Bapat UC and Mhapsekar DR. Study of antimicrobial activity and phytochemical evaluation of *Jatropha gossypifolia*, *Sapium sebiferum*, *Kirganelia reticulata*, *Phyllanthus fraternus* and *Pedilanthus tithymaloides*. International Journal of Pharmaceutical Sciences and Research, 5 (11): 4933-4941, (2014).
8. Bapat UC, Mhadnak TR and Mhapsekar DR. Antibacterial activity studies of extracts of *Phyllanthus fraternus* Webster, *Jatropha gossypifolia* Linn. and *Sapium sebiferum* (L.)Roxb. Xplore, 3 (1): 1-9, (2012).
9. Muruhan S, Selvaraj S and Viswanathan PK. *In vitro* antioxidant activities of *Solanum surattense* leaf extract. Asian Pacific Journal of Tropical Biomedicine, 3 (1): 28-34, (2013).
10. Merinal S and Viji SBG. *In vitro* antioxidant activity and total phenolic content of leaf extracts of *Limonia crenulata* (Roxb.). J. Nat. Prod. Plant Resour., 2 (1): 209-214, (2012).
11. Sharma S and Grewal RK. *In vitro* studies on the antioxidant activities of extracts from the flowers of *Gomphrena globosa*. International Journal of Pharma and BioSciences, 5 (3): 457-465, (2014).
12. Aswatha RHN, Shreedhara CS, Falguni PG and Sachin BZ. *In vitro* free radical scavenging potential of methanol extract of entire plant of *Phyllanthus reticulatus* Poir. Pharmacologyonline, 2: 440-451, (2008).
13. Singh SK and Prakash V. Screening of antioxidant activity and phytochemicals strength of some herbal plants. International Journal of Pharmacy and Pharmaceutical Sciences, 5 (3): 296-300, (2013).
14. Chougale AD, Bhosale PM, Jadhav UU and Padul MV. Antibacterial and antioxidant

activity of plant latex. Journal of Pharmacy Research, 4 (2): 406-407, (2011).
15. Vaghasiya Y, Dave R and Chanda S. Phytochemical analysis of some medicinal plants from Western region of India. Research Journal of Medicinal Plant, 5 (5): 567-576, (2011).
16. Chanda S, Bhayani D and Desai D. Polyphenols and flavonoids of twelve Indian medicinal plants. The Bioscan, 8 (2): 595-601, (2013).
17. Abreu P, Matthew S, Gonza´lez T, Costa D, Segundo MA and Fernandes E. Anti-inflammatory and antioxidant activity of a medicinal tincture from *Pedilanthus tithymaloides*. Life Sciences, 78: 1578-1585, (2006).
18. Abreu PM, Matthew S, Gonza T, Vanickova L, Costa D, Gomes A, Segundo MA and Fernandes E. Isolation and identification of antioxidants from *Pedilanthus tithymaloides.* J Nat Med, 62: 67–70, (2008).
19. Eldeen IMS, Seowa E-M, Abdullaha R and Sulaimana SF. *In vitro* antibacterial, antioxidant, total phenolic contents and anti-HIV-1 reverse transcriptase activities of extracts of seven *Phyllanthus* sp. South African Journal of Botany, 77: 75-79, (2011).
20. Koffuor AG and Amoateng P. Antioxidant and anticoagulant properties of *Phyllanthus fraternus* GL Webster (Family: Euphorbiaceae). Journal of Pharmacology and Toxicology, 6 (7): 624-636, (2011).
21. Gupta MK, Lagarkha R, Sharma DK, Sharma PK, Singh R and Ansari HS. Antioxidant activity of the successive extracts of *Grewia asiatica* leaves. Asian Journal of Chemistry, 19 (5): 3417-3420, (2007).
22. Kumar ST, Shanmugam S, Palvannan T and Kumar BVM. Evaluation of antioxidant properties of *Canthium parviflorum* Lam. leaves. Natural Product Radiance, 7 (2): 122-126, (2008).
23. Motlhanka DMT, Habtemariam S and Houghton P. Free radical scavenging activity of crude extracts and 4'-O-methylepigallocatechin isolated from roots of *Cassine transvaalensis* Burtt-Davy from Botswana. African Journal of Biomedical Research, 11: 55-63, (2008).
24. Shanmugam S, Kumar ST and Selvam KP. Laboratory Handbook on biochemistry, PHI Learning Private Limited: New Delhi, 98-102, (2010).
25. Peer basha D, Ravishankar K, Kiranmayi GVN and Subbarao M. Antioxidant activity of different leaf extracts of *Ocimium sanctum*, *Mangifera indica* and *Hibiscus rosa sinensis*. Int. J. Pharm. Sci. Rev. Res., 25 (2): 296-299, (2014).
26. Pillai SS and Mini S. *In vitro* antioxidant activities of different solvent fractions from the ethanolic extract of *Hibiscus rosa sinensis* petals. International Journal of Pharmaceutical Sciences and Research, 5 (9): 3879-3885, (2014).
27. Jayanthi P and Lalitha P. Reducing power of the solvent extracts of *Eichhornia crassipes* (Mart.) Solms. International Journal of Pharmacy and Pharmaceutical Sciences, 3 (3): 126-128, (2011).
28. Behera DA, Bhatnagar S and Mahapatra AK. Cytotoxic and radical scavenging potential of Indian almond (*Terminalia catappa*) leaf extracts. British Biomedical Bulletin, 2 (1): 31-39, (2014).
29. Baby T, Saraswathi U, Revathi S and Malathi M. Screening of antioxidant and antityrosinase activities of a herbal formulation. International Journal of Innovative Drug Discovery, 4 (2): 93-98, (2014).
30. Sankannavar SH and Patil CG. *In vitro* studies on diversity of antibacterial activity in some species of *Phyllanthus* for human pathogenic bacteria. Asian J. Exp. Biol. Sci., 3 (3): 607-612, (2012).
31. Das R and Handique PJ. *In vitro* evaluation of phenol, flavonoid and antioxidant properties of methanolic extract of *Phyllanthus fraternus* Webster. Asian Journal of Science and Technology, 4: 59-64, (2011).
32. Loganayaki N and Manian S. *In vitro* antioxidant properties of indigenous underutilized fruits. Food Sci. Biotechnol, 19 (3): 725-734, (2010).