HPTLC study to determine the antioxidant activity of dried leaves of *Portulaca oleracea* L.

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**ABSTRACT**

This present study involves the assessment of the anti-oxidant activity study of the sample which was obtained from the methanolic extracts of dried leaves of *Portulaca oleracea* L. (common name Purslane). Purslane is a rich source of Vitamin A, Vitamin-C and some other B-complex vitamins like riboflavin, niacin, pyridoxine and carotenoids which are known powerful natural anti-oxidants. Anti-oxidants are compounds that inhibit oxidation. This methanolic extract of leaves was evaluated for the determination of its anti-oxidant efficiency by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) by using Silica TLC plates on Camag High-Performance Thin Layer Chromatography (HPTLC) system using visionCATS software. Densitograms and chromatographs obtained show the presence of anti-oxidant activity. It is a rapid, inexpensive and straightforward method to measure anti-oxidant properties of substances after separation by HPTLC. It involves the use of the free radical, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate anti-oxidant activity. When Anti-oxidants substances react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor (e.g., a free radical scavenging anti-oxidant) and is reduced to the DPPHH. As a consequence, the absorbance's decreased from the DPPH.

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**INTRODUCTION**

*Portulaca oleracea* L. (Purslane) has wide cosmopolitan distribution around the globe as it can be seen in fields, gardens, vineyards, lawns, driveways, dunes and river banks. It is better known as wild plants but also an edible vegetable rich in many beneficial nutrients for human consumption ([Alam et al., 2014](https://doi.org/10.26452/ijrps.v12i1.4174)). It contains more omega-3 fatty acids and alpha-linolenic acid (both are good anti-oxidants) in than any other leafy vegetable plant. ([Chowdhary, 2013](https://doi.org/10.26452/ijrps.v12i1.4174)), and is a nutritious food rich in omega-3 fatty acids and anti-oxidants ([Simopoulos et al., 1992](https://doi.org/10.26452/ijrps.v12i1.4174)). It possesses many anti-oxidant properties due to the excellent value contents of vitamins, minerals, essential fatty acids and other compounds and having rich medicinal properties ([Rahimi, 2018](https://doi.org/10.26452/ijrps.v12i1.4174)), possess potent pharmacological activities as anti-oxidant ([Naeem and Khan, 2013](https://doi.org/10.26452/ijrps.v12i1.4174)).
Purslane is a nutritious vegetable used for human consumption (Dkhil et al., 2011) and having anti-oxidative property. Phytochemical investigations revealed that this plant has a wide range of secondary metabolites including alkaloids, terpenoids, flavonoids and organic acids (Iranshahy et al., 2017). Anti-oxidants are the compounds that inhibit oxidation and oxidation is a chemical reaction that can produce free radicals (Sicari et al., 2018), thereby leading to chain reactions that may damage the cells of organisms. Many anti-oxidants like thiols or ascorbic acid (vitamin C) terminate these chain reactions.
Kesri Nandan Sharma and Nitu Bhatnagar, Int. J. Res. Pharm. Sci., 2021, 12(1), 254-261

Figure 7: Derivatized HPTLC Plate image in white light

Figure 8: Derivatized HPTLC Plate image at 366 nm

reactions. To balance the oxidative stress, plants and animals maintain complex systems of overlapping anti-oxidants, such as glutathione and enzymes (e.g., catalase and superoxide dismutase), produced internally, or the dietary anti-oxidants vitamin C & vitamin E (Hefnawy and Ali, 2015).

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a free radical which produces a violet solution dissolved in Methanol (Erkan, 2012). It is stable at room temperature and ambient environmental conditions. Anti-oxidants present in the extract reacts with the DPPH free radicals and breaks the chain reaction and further formation of free radicals (Sanja et al., 2009) this causes a colour change in DPPH to yellow. The formula of DPPH is C18H12N5O6 and its Molecular Weight is 394.32 g/mol. Structure of DPPH (Figure 1). High-Performance Thin Layer Chromatography (HPTLC) is a powerful and versatile chromatographic technique for the separation and analysis of natural products as compared to other techniques such as HPLC, spectrophotometry, titrimetric. HPTLC system of Camag brand consists of following components such as Plate Developing Chambers (Twin trough), TLC Applicator (Linomat 5), TLC plate heater, Derivatier Sprayer, Detection component (Scanner 4), Evaluation is done by software (visionCATS) and documentation by TLC Visualizer.

MATERIALS AND METHODS

The whole plant of Portulaca oleracea along with leaves on which study is performed was authenticated by Raw Materials Herbarium & Museum, Delhi (RHMD), National Institute of Science Communication & Information Resources (CSIR-NISCAIR) New Delhi and requisite certificates are obtained.

DPPH reagent (2,2-Diphenyl-1-picrylhydrazyl), Ethanol, Camag HP-TLC System comprising vision CATS- Software, Linomat 5, Twin Trough Chamber, Visualizer, Scanner4, Dipping Chamber, Centrifuge machine, Ultrasonic bath, Silica gel TLC plates, Ethyl Acetate, Methyl ethyl ketone, Formic Acid, Distilled Water

Reagent Preparation

Exactly 7.89 mg of DPPH reagent is weighed by analytical balance and diluted to 100 ml by 99.5 % ethanol. The concentration of this solution is 0.2 mM. It is kept in an amber-coloured bottle which is then wrapped with aluminium foil as DPPH activity is light sensitive.

Sample preparation

One gram of fine powder made from dried leaves of Portulaca oleracea (Purslane) is precisely weighed and dissolved in 10 ml of Methanol and then kept in Ultrasonic bath for 15 minutes after its supernatant is taken with the help of a pipette. After that, it is centrifuged at 3000 rpm for 10 minutes in a centrifuge machine, and the final extract is taken for the analysis.

PROCEDURE

The study is performed in a dark environment only as the DPPH activity is light sensitive, DPPH reagent is dissolved in ethanol and dissolved. After application of the sample on stationary phase HPTLC plates silica gel 60 F 254 of size 10 cm x10 cm the and standard are run in the mobile phase which contains Ethyl Acetate: Methyl ethyl ketone: Formic Acid: Water in a ratio of 5:3:1:1 volume/volume.
Figure 9: *Chromatograph A1, A2, A3, A4 and A5 obtained at 254 nm
Figure 10: Chromatograph B1, B2, B3, B4 and B5 obtained at 366 nm
### Table 1: Evaluation result HPTLC Chromatograph are obtained at 254 nm

|   | Volume | Peak | Max | Area  |
|---|--------|------|-----|-------|
|   |        |      | Height | %    |        | A   | %   |
| A1| 2.0 µl | 1    | 0.124 | 0.0326 | 22.66 | 0.00103 | 11.81 |
| A1| 2.0 µl | 2    | 0.876 | 0.1112 | 77.34 | 0.00767 | 88.19 |
| A2| 4.0 µl | 1    | 0.039 | 0.0201 | 7.50  | 0.00036 | 2.21  |
| A2| 4.0 µl | 2    | 0.124 | 0.0503 | 18.77 | 0.00153 | 9.45  |
| A2| 4.0 µl | 3    | 0.173 | 0.0291 | 10.85 | 0.00125 | 7.70  |
| A2| 4.0 µl | 4    | 0.381 | 0.0153 | 5.71  | 0.00062 | 3.82  |
| A2| 4.0 µl | 5    | 0.745 | 0.0460 | 17.15 | 0.00277 | 17.07 |
| A2| 4.0 µl | 6    | 0.887 | 0.1073 | 4.02  | 0.00968 | 5.75  |
| A3| 6.0 µl | 1    | 0.037 | 0.0222 | 6.76  | 0.00045 | 3.21  |
| A3| 6.0 µl | 2    | 0.126 | 0.0699 | 21.28 | 0.00213 | 15.11 |
| A3| 6.0 µl | 3    | 0.174 | 0.0417 | 12.70 | 0.00173 | 12.26 |
| A3| 6.0 µl | 4    | 0.385 | 0.0229 | 6.98  | 0.00093 | 6.59  |
| A3| 6.0 µl | 5    | 0.750 | 0.0526 | 16.02 | 0.00321 | 22.78 |
| A3| 6.0 µl | 6    | 0.935 | 0.1190 | 36.24 | 0.00564 | 40.05 |
| A4| 8.0 µl | 1    | 0.039 | 0.0272 | 7.13  | 0.00053 | 3.18  |
| A4| 8.0 µl | 2    | 0.126 | 0.0832 | 21.83 | 0.00274 | 16.52 |
| A4| 8.0 µl | 3    | 0.177 | 0.0526 | 13.81 | 0.00217 | 13.06 |
| A4| 8.0 µl | 4    | 0.384 | 0.0284 | 7.45  | 0.00118 | 7.09  |
| A4| 8.0 µl | 5    | 0.753 | 0.0565 | 14.82 | 0.00318 | 19.17 |
| A4| 8.0 µl | 6    | 0.939 | 0.1332 | 34.95 | 0.00680 | 40.97 |
| A5| 10.0 µl| 1    | 0.039 | 0.0324 | 7.38  | 0.00070 | 3.50  |
| A5| 10.0 µl| 2    | 0.129 | 0.0937 | 21.33 | 0.00320 | 16.08 |
| A5| 10.0 µl| 3    | 0.181 | 0.0610 | 13.89 | 0.00242 | 12.15 |
| A5| 10.0 µl| 4    | 0.836 | 0.3062 | 38.26 | 0.01653 | 44.23 |
| A5| 10.0 µl| 5    | 0.948 | 0.1549 | 35.28 | 0.00847 | 42.61 |

### Table 2: Evaluation result HPTLC Chromatographs are obtained at 366 nm

|   | Volume | Peak | Max | Area  |
|---|--------|------|-----|-------|
|   |        |      | Height | %    |        | A   | %   |
| B1| 2.0 µl | 1    | 0.827 | 0.1072 | 28.53 | 0.00450 | 30.49 |
| B1| 2.0 µl | 2    | 0.933 | 0.2685 | 71.47 | 0.01025 | 69.51 |
| B2| 4.0 µl | 1    | 0.831 | 0.2239 | 35.97 | 0.01148 | 40.74 |
| B2| 4.0 µl | 2    | 0.940 | 0.3985 | 64.03 | 0.01670 | 59.26 |
| B3| 6.0 µl | 1    | 0.064 | 0.0175 | 2.19  | 0.00046 | 1.22  |
| B3| 6.0 µl | 2    | 0.260 | 0.0141 | 1.77  | 0.00039 | 1.04  |
| B3| 6.0 µl | 3    | 0.836 | 0.3062 | 38.26 | 0.01653 | 44.23 |
| B3| 6.0 µl | 4    | 0.948 | 0.4625 | 57.79 | 0.02000 | 53.50 |
| B4| 8.0 µl | 1    | 0.067 | 0.0191 | 2.15  | 0.00042 | 0.97  |
| B4| 8.0 µl | 2    | 0.840 | 0.3647 | 41.03 | 0.01978 | 46.29 |
| B4| 8.0 µl | 3    | 0.953 | 0.5050 | 56.81 | 0.02253 | 52.74 |
| B5| 10.0 µl| 1    | 0.067 | 0.0230 | 2.31  | 0.00056 | 1.15  |
| B5| 10.0 µl| 2    | 0.265 | 0.0199 | 1.99  | 0.00059 | 1.23  |
| B5| 10.0 µl| 3    | 0.844 | 0.4033 | 40.48 | 0.02209 | 45.89 |
| B5| 10.0 µl| 4    | 0.958 | 0.5501 | 55.22 | 0.02489 | 51.72 |
After the development of standard and sample, the plate is then derivatized with DPPH reagent. A working program is prepared in visionCATS software. A specific volume of sample 2μl, 4μl, 6μl, 8μl and 10μl is taken by using 100 μl syringe and applied through sample applicator. After application, the plate is air-dried for 10 minutes. For the development of chromatogram, a filter paper lined in the Twin Trough Chamber for uniform saturation chamber is used. Chamber saturation is done for 20 minutes (according to USP chapter 202). Then the applied plate is placed in a twin trough chamber layer facing towards the paper. The plate is a run-up to the marked position, i.e. 70 mm and then removed and air-dried for 5 minutes. The dip tank is used for derivatization with DPPH reagent. It is kept in the dark place for 10 min for development of proper intensity of colour. Then images are taken in visible light if anti-oxidant is present it will show the yellow colour spot.

### RESULTS AND DISCUSSION

Images of HPTLC plate after application of sample of methanolic extract applied in a volume of by microsyringe in 2μl, 4μl, 6μl, 8μl and 10μl concentrations is obtained under normal white light (Figure 2), under U.V. light at 254 nm (Figure 3) and when under U.V. light at 366 nm (Figure 4).

First Densitogram is obtained by keeping the developed HPTLC plate in TLC Scanner and vision CATS software with settings at wavelength 254 nm and other as under:

- Scanner type used as Single λ; Optimization - Resolution; Measurement mode –absorption;
- Detector mode – Automatic ; Scanning speed - 20 mm/s; Data resolution 100 μm/step ; Slit 6 x 0.45 mm, micro; lamp- Deuterium Lamp (Figure 5).

Second Densitogram is obtained by keeping the developed HPTLC plates in TLC Scanner and vision CATS software with settings at wavelength 366 nm and other as under; Scanner type used as Single λ ; Optimization - Resolution ; Filter – K 400 ; Measurement mode–Fluorescence; Detector mode – Auto-

| Rf Value | Colour | Intensity |
|----------|--------|-----------|
| 0.87     | Yellow | High      |
| 0.78     | Yellow | High      |
| 0.18     | Yellow | Low       |
matic; Scanning speed - 20 mm/s; Data resolution 25 μm/step; Slit 6 x 0.45 mm, micro; lamp- Mercury Lamp (Figure 6).

The developed plate is then derivatized by using freshly prepared DPPH reagents and images are observed under white light (Figure 7) and at 366 nm (Figure 8).

**Evaluation of results**

Area, Area % and Rf values of the peaks detected during densitometry analysis of extract are given in Table 1 for five chromatographs are obtained at 254 nm on applying 2 μl, 4 μl, 6 μl, 8 μl and 10 μl consequently. (Figure 9) and in Table 2 for the other five chromatographs are obtained at 366 nm on applying the same concentrations as above consequently (Figure 10). TLC visualize used for imaging and documentation in which yellow colour bands can be seen at 0.18, 0.78 and 0.87 Rf values (Figure 11), and detailed results can be seen as in Table 3.

**CONCLUSION**

This study concludes that when five tracks of different concentrations, i.e. 2 μl, 4 μl, 6 μl, 8 μl and 10 μl were applied and studied at 254 nm and 366 nm and again studied at after derivatization by DPPH reagent, it is found that the yellow colour band present at Rf 0.18, Rf 0.78 and Rf 0.87 shows the presence of anti-oxidant activity present in the methanolic extract of *Portulaca oleracea* dried leaves.

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**Conflict of Interest**

The authors declare that they have no conflict of interest for this study.

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