Determination of phenolic content and in vitro antioxidant potential of ethanol extract of seven sources of Ayurvedic drug ‘Pittapapda’

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‘Pittapapda’ is a well known crude drug used in Indian system of traditional medicine for diarrhoea, liver disorders, leprosy, skin diseases, etc. This study presents antioxidant activity of ethanol extracts of seven botanically different sources of ‘Pittapapda’, viz. Glossocardia bosvallia (L.f.) DC., Rostellularia procumbens (L.) Nees, Rungia repens (L.) Nees, Naregamia alata Wight & Arn., Fumaria vaillantii Loisel., Mollugo pentaphylla L. and Polycarpaea corymbosa (L.) Lam. by means of various assays including 1,1-diphenyl 2-picryl hydrazyl test by TLC and spectrophotometry, nitric oxide, hydrogen peroxide, trolox equivalent antioxidant capacity (TEAC), radical scavenging potential using photo-chemiluminescence and lipid peroxidation. The trend of phenol content was as: G. bosvallia > F. vaillantii > N. alata > P. corymbosa > M. pentaphylla > R. procumbens > R. repens. Out of seven species, ethanol extracts of G. bosvallia, F. vaillantii and N. alata having high percentage of phenol content showed potent in vitro antioxidant activity followed by moderate activity of M. pentaphylla and P. corymbosa. The above study supports use of G. bosvallia, F. vaillantii and N. alata to treat liver disorder in traditional medicine as their ethanol extract exhibits potent inhibition of free radicals than other four species.

Keywords: Pittapapda species, Ethanol extracts, Antioxidant, TLC-DPPH, Photo-chemiluminescence.

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Introduction

‘Pittapapda’ is a well-known crude drug used in Indian system of traditional medicine for diverse pharmacological activities like anthelmintic, antipsoriatic, hypoglycaemic, anti diarrhoeal and hepatoprotective. It is a major constituent of Ayurvedic preparations like ‘Parpatadi-kwath’, ‘Parpatadi-arishta’, ‘Parpatadi-arka,’ etc. During crude drug market survey it was observed that whole plant of seven botanically different species, viz. Glossocardia bosvallia (L. f.) DC. (Asteraceae), Rostellularia procumbens (L.) Nees syn. Justicia procumbens L. (Acanthaceae), Rungia repens (L.) Nees (Acanthaceae), Naregamia alata Wight & Arn. (Meliaceae), Fumaria vaillantii Loisel. syn. F. indica (Haussk.) Pugsley (Fumariaceae), Mollugo pentaphylla L. (Molluginaceae), Polycarpaea corymbosa Lam. (Caryophyllaceae) are being used as sources of ‘Pittapapda’ in various geographical areas of India.

The literature survey indicated that, though these species are well known medicinal herbs and used to treat liver disorder, no previous work is available with respect to their comparative antioxidant potential. Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism. Reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (OH$^-$) are closely involved in human diseases such as Alzheimer’s disease, aging, cancer, inflammation, rheumatoid arthritis, liver diseases and atherosclerosis. There has been an increased interest in identifying antioxidant phytochemical because these molecules can inhibit the propagation of free radical reactions to protect the human body from diseases and retard lipid oxidative rancidity in food.

Hence, it was proposed to evaluate comparative antioxidant efficacy and potency of above species by means of different assays including 1,1-diphenyl 2-picryl hydrazyl (DPPH) test by TLC and spectrophotometry, nitric oxide, hydrogen peroxide, superoxide radicals, trolox equivalent antioxidant capacity (TEAC), photo-chemiluminescence along with lipid peroxidation. Total phenolic content in each ethanol extract also determined. Hence, in present study, a battery of techniques was used to demonstrate antioxidant activity.
**Material and Methods**


**Chemicals**

1,1-diphenyl 2-picryl hydrazyl (DPPH), Griess reagent, 2, 2’-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), gallic acid, butylated hydroxytoluene were obtained from Sigma Chemical Co. (St. Louis, U.S.A.). Ferrous sulphate (FeSO₄), trichloroacetic acid (TCA), thiobarbituric acid (TBA), acetic acid, sodium nitroprusside, ammonium molybdate, sodium phosphate, hydrogen peroxide, dimethyl sulfoxide (DMSO), butylated hydroxytoluene (BHT) were obtained from Sd. Fine Chemicals (Mumbai, India). Other analytical grade reagents were also purchased from Sd. Fine Chemicals (Mumbai, India).

**Extraction procedure**

All samples were shade dried, coarsely powdered and stored in an airtight container at 25°C ± 4°C. Powdered material (75 g) was extracted with ethanol using ASE 100 accelerated solvent extractor (Dionex, Vienna, Austria). Extraction was performed at of 100 bar and temperature 60°C for 20 min in five replicate cycles. Yield of ethanol extract of *G. bosvallia* (GBP), *N. alata* (NAP), *F. vaillantii* (FIP), *M. pentaphylla* (MP), *P. corymbosa* (PCP), *R. procumbens* (RPP) and *R. repens* (RUP) were 8.67, 7.34, 9.24, 9.56, 6.77, 5.88 and 7.33%, respectively.

**Radical-scavenging effect of extracts in DPPH radicals**

DPPH radical-scavenging ability of extracts was assessed qualitatively and quantitatively by TLC autographic and spectrophotometric method, respectively. Ethanol extract of seven samples were subjected to the Thin Layer Chromatography (TLC) study. Extract samples were applied on a pre-coated silica gel F₂₅₄ (Merck, Darmstadt, Germany) TLC plates with band width 6 mm, by employing Linomat IV sample applicator (Camag, Switzerland). The plate loaded with samples was developed up to 8.0 cm in a Camag Twin trough chamber previously saturated with mobile phase toluene: ethyl acetate: formic acid (5:5:0.3 v/v/v). Then dried plate was sprayed with 0.5 mM DPPH solution in methanol, dried in darkness at ambient temperature for exactly 90 s and heated for 30 s at 60°C⁹. Gallic was used as a positive control. The area of yellow bands against purple background determined radical scavenging activity.

DPPH radical-scavenging ability was assessed according to method of Jung *et al*¹⁰. Briefly, to a methanolic solution of DPPH (60 mM, 2 mL), 50 µl of each test extract at different concentrations (5-50 µg/ml) dissolved in methanol was added. Absorbance measurements commenced immediately at 515 nm. The decrease in absorbance was determined after 70 min when the absorbance stabilized. The absorbance of the DPPH radical without extracts and the control was measured. Ascorbic acid was used as reference antioxidant. The percent inhibition of the DPPH radical in the samples was calculated according to the formula given below:

\[
\% \text{ Inhibition} = \left( \frac{A_{C(0)} - A_{A(t)}}{A_{C(0)}} \right) \times 100
\]

where \( A_{C(0)} \) is the absorbance of the control at \( t=0 \) min and \( A_{A(t)} \) is the absorbance in presence of antioxidant at \( t=70 \) min.

**Nitric Oxide Scavenging Activity**

Nitric oxide scavenging activity was measured according to method of Marcocci *et al*¹¹. Sodium nitroprusside (5 mM) in phosphate buffered saline was mixed with each test extract of different concentrations (5-50 µg/mL) and incubated at 25°C for 30 min and then 1.5 mL of the incubated solution was removed and diluted with 1.5 mL of Griess’ reagent. The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent coupling with naphthylethylene diamine was measured at 546 nm along with control. Ascorbic acid was used as reference antioxidant. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance values of control and test samples.

**Hydrogen Peroxide (\( \text{H}_2\text{O}_2 \)) Decomposition**

Decomposition of \( \text{H}_2\text{O}_2 \) was determined by method of Sinha¹². The assay mixture contained 4 mL of \( \text{H}_2\text{O}_2 \) solution (80 mM) and 5 mL of phosphate buffer...
(pH 7.4). One milliliter of each extract dissolved in water (25 µg/mL) was rapidly mixed with the reaction mixture by a gentle swirling motion at room temperature. 1 mL portion of the reaction mixture was then blown into 2 mL of dichromate/acetic acid reagent at 60 s intervals. The decomposition of H$_2$O$_2$ was determined based on the standard plot for H$_2$O$_2$ and the monomolecular velocity constant $K$ was determined by using the formula:

$$K = \frac{1}{t} \log_{10} \frac{S_0}{S}$$

where, $S_0$ is the initial concentration and $S$ is the final concentration of H$_2$O$_2$.

**Superoxide Scavenging Activity**

Superoxide scavenging activity was carried out by using alkaline DMSO method$^{13}$. Solid potassium superoxide was allowed to stand in contact with dry DMSO for at least 24 h and the solution was filtered immediately before use. Filtrate (200 mL) was added to 2.8 mL of an aqueous solution containing nitroblue tetrazolium (56 mM), EDTA (10 mM) and potassium phosphate buffer (10 mM, pH 7.4). Each test extracts (1 mL) of different concentrations (5-50 µg/mL) in water was added to the reaction mixture and the absorbance was recorded at 560 nm against a control in which pure DMSO has been added instead of alkaline DMSO.

**Evaluation of Trolox Equivalent Antioxidant Capacity (TEAC)**

Total antioxidant activity of the extract was measured using the TEAC assay$^{14}$ with minor modifications. The TEAC value is based on the ability of the antioxidant to scavenge the blue-green 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS$^+$) radical cation relative to the ABTS$^+$ scavenging ability of the water soluble vitamin E analogue, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox). ABTS$^+$ radical cation was generated by the interaction of ABTS$^+$ (100 µM), H$_2$O$_2$ (50 µM) and horse raddish peroxidase enzyme (4.4 unit/mL). To measure antioxidants capacity, 0.25 mL of each extract (25 µg/mL) was mixed with an equal volume of ABTS$^+$, H$_2$O$_2$, peroxidase and deionized water. The reaction mixture was stand for 10 min at room temperature. The decrease in absorption at 734 nm after the addition of the reactant was used to calculate the TEAC value. The TEAC value is expressed as the milimolar concentration of Trolox solution having an antioxidant equivalent to a 1000 ppm solution of the sample under investigation. The higher the TEAC value of the sample, the stronger the antioxidant ability.

**Photo-chemiluminescence Assay**

Method of Photochemiluminescence (PCL) was used for determination of integral antioxidative capacity (AC) of methanol and water-soluble substances in extracts. Photochem® apparatus (Analitik jena AG, Germany) was used to determine the antioxidant capacity of the extracts against superoxide anion radicals generated from luminol where plays a double role of photosensitizer as well as the radical detecting agent. The activity was measured using standard kit ACW and ACL (Analitik jena AG, Germany). Each extract was measured at 10 µg/mL concentrations. A standard curve was plotted and the results were calculated for methanol soluble substance in trolox and for water soluble substance in ascorbic acid equivalents (n mol/g).

**Lipid Peroxidation Inhibition - Liver Homogenate**

Adult albino mice (6-8 weeks old) of either sex breed in the animal house of Agharkar Research Institute, Pune were used for the preparation of liver homogenate. The approval for this work using animals was taken from the Institutional Animal Ethical Committee 101/1999/CPCSEA of Agharkar Research Institute, Pune. The mice were kept in the departmental animal house at 26±2°C and relative humidity 44-55% light and dark cycles of 10 and 14 h, respectively for one week before the experiment. Animals were provided with rodent diet (Amruth, India) and water ad libitum. Randomly selected mice were fasted overnight and were sacrificed by cervical dislocation, dissected and abdominal cavity was perfused with 0.9% saline. Whole liver was taken out and visible clots were removed and weighed amount of liver was processed to get 10% homogenate in cold phosphate buffered saline, pH 7.4 using glass teflon homogenizer and filtered to get a clear homogenate.

**Assay of Lipid Peroxidation**

Degree of lipid peroxidation was assayed by estimating the thiobarbituric acid-reactive substances$^{15}$ (TBARS) with minor modifications$^{16}$. Each test extract (25 µg/mL in water) was added to the liver homogenate. Lipid peroxidation was initiated by adding 100 µL of 15 mM ferrous sulphate solution to 3 mL of liver homogenate (final concentration was 0.5 mM). After 30 min, 100 µL of this reaction
mixture was taken in a tube containing 1.5 mL of 10% TCA. After 10 min tubes were centrifuged and supernatant was separated and mixed with 1.5 ml of 0.67% TBA in 50% acetic acid. The mixture was heated in a hot water bath at 85°C for 30 min to complete the reaction. The intensity of pink coloured complex formed was measured at 535 nm. The values of TBARS were calculated from a standard curve of absorption against concentration of tetraethoxy propane and expressed as nmol/mg of protein. The percentage inhibition of lipid peroxidation was calculated by comparing the results of the test with those of control not treated with the extracts.

**Total phenolic content**

Total phenolic content in each extract was determined with Folin-Ciocalteu reagent using pyrocatechol as a reference standard. 2 mL of 2 % Na₂CO₃ was added to 0.1 mL extract and mixed thoroughly. After 5 min of incubation, 0.1 mL of 50 % Folin-Ciocalteu reagent was added and allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm. Concentration of total phenol content was determined using the formula:

\[ \text{Absorbance} = 0.001 \times \text{pyrocatechol (µg)} + 0.0033 \]

**Statistical Analysis**

Experimental results were expressed as means ± SD of six replications for each data point. The results of lipid peroxidation experiments were analyzed by using Student’s t-test to calculate significance of the results. The \( P \) values < 0.05 were considered as significant and values for \( P < 0.001 \) as very significant.

**Results and Discussion**

In present investigation, the antioxidant activity was studied using different *in vitro* models of seven sources of ‘Pittapapda’ used in Indian system of traditional medicine. The DPPH radical scavenging assay is commonly employed to evaluate the ability of antioxidants to scavenge free radicals. In the present study, this ability was screened qualitatively by means of TLC; the plate was developed with DPPH reagent. Active extracts as well as positive control were observed as clear bands that contrast with purple background of plate (Plate 1). The highest activity was found in the extract of GBP, FIP and NAP followed by PCP whereas GBP showed 7 spots at \( R_f \) 0.04, 0.07, 0.18, 0.41, 0.46, 0.65, 0.77; FIP extract showed 4 spots at \( R_f \) 0.54, 0.59, 0.66, 0.72; NAP extract showed 4 spots at \( R_f \) 0.53, 0.60, 0.64, 0.71;

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Plate 1—TLC of extracts of ‘Pittapapda’ samples developed with DPPH reagent GBP PCP FIP NAP MPP RRP RUP STD *G. bosvallia* (GBP), *N. alata* (NAP), *F. vaillantii* (FIP), *M. pentaphylla* (MPP), *P. corymbosa* (PCP), *R. procumbens* (RRP) and *R. repens* (RUP).
PCP extract showed 3 spots at Rf 0.04, 0.086, 0.66; MPP extract showed 2 spots at Rf 0.46, 0.82. RRP and RUP showed one active spot at Rf 0.67 and 0.47, respectively. The standard gallic acid showed spot at Rf 0.26. Furthermore, the results obtained from spectrophotometric assay confirmed the observation made by means of TLC (Table 1). Of the seven ethanol extracts assayed, three showed highest activity. GBP, NAP and FIP had high antioxidant capacity which decreased the DPPH radical as compared to standard and IC50 values of each were 14.5±0.67, 11.4±0.89, 11.4±0.89 and 11.6±0.74 µg/mL, respectively. These values are comparable with standard ascorbic acid (4.5±0.09 µg/mL) and BHT (5.6±0.22 µg/mL). PCP and MPP showed moderate activities with IC50 values 21.5±0.56 and 29.3±0.85 µg/mL, respectively. RRP and RUP showed weak activities with IC50 values 38.6±0.23 and 43.3±0.67 µg/mL, respectively (Table 1).

Sodium nitroprusside (SNP) is known to decompose in aqueous solution at physiological pH (7.2) producing nitric oxide. SNP spontaneously releases nitric oxide (NO) in solution and the amount of NO released can be inferred by using the Griess reagent. This reagent reacts with nitrite, which is one of the two primary, stable and non-volatile breakdown products of NO and therefore, allows an indirect estimation of the amount of NO released in the solution18. GBP, NAP and FIP inhibited nitric oxide generation which was comparable to standard ascorbic acid (3.8 ± 0.21 µg/mL), BHT (3.2±0.34 µg/mL) and IC50 values were 15.6 ± 0.78, 11.1 ± 0.45 and 12.5 ± 1.67 µg/mL, respectively. PCP and MPP showed moderate activities with IC50 values 21.5 ± 0.56 and 29.3 ± 0.85 µg/mL, respectively. RRP and RUP showed weak activities with IC50 values 38.6±0.23 and 43.3±0.67 µg/mL, respectively (Table 1). H2O2 is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. In the body, H2O2 is rapidly decomposed into oxygen and water.

Superoxide anions damage bio-macromolecules directly or indirectly by forming H2O2, OH, peroxyl nitrite or singlet oxygen during pathophysiologic events. Alkaline DMSO, used as a superoxide generating system reacts with NBT to give coloured diformazan. It measures the ability to inhibit reduction of NBT by K+O2- added directly as a solution in dimethylsulphoxide. GBP, NAP and FIP showed higher scavenging activity than other test samples with IC50 values 11.8±0.33, 14.0±0.78 and 13.7 ± 0.67 µg/mL, respectively; these values were comparable with standard ascorbic acid (1.2 ± 0.07 µg/mL) and BHT (2.0 ± 0.11 µg/mL), respectively. IC50 values of other samples PCP, MPP, RRP and RUP were 23.4 ± 0.77, 29.7 ± 0.22, 49.0 ± 0.65 and 48.7 ± 0.34 µg/mL, respectively (Table 1). Peroxyl radicals or other oxidants like potassium persulphate oxidize ABTS to its radical cation, ABTST. The antioxidant capacities were determined by measuring decrease in the intensity

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**Table 1**—Percentage inhibition and IC50 values (µg/mL) of free radical scavenging capacity of extract of ‘Pittapapda’ samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>DPPH IC50 µg/mL</th>
<th>NO IC50 µg/mL</th>
<th>SOD IC50 µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>GBP</td>
<td>14.5±0.67</td>
<td>15.6±0.78</td>
<td>11.8±0.33</td>
</tr>
<tr>
<td>NAP</td>
<td>11.4±0.89</td>
<td>11.1±0.45</td>
<td>14.0±0.78</td>
</tr>
<tr>
<td>FIP</td>
<td>11.6±0.74</td>
<td>12.5±1.67</td>
<td>13.7±0.67</td>
</tr>
<tr>
<td>PCP</td>
<td>19.2±0.09</td>
<td>21.5±0.56</td>
<td>23.4±0.77</td>
</tr>
<tr>
<td>MPP</td>
<td>25.5±0.45</td>
<td>29.3±0.85</td>
<td>29.7±0.22</td>
</tr>
<tr>
<td>RRP</td>
<td>34.6±0.67</td>
<td>38.6±0.23</td>
<td>49.0±0.65</td>
</tr>
<tr>
<td>RUP</td>
<td>38.9±0.34</td>
<td>43.3±0.67</td>
<td>48.7±0.34</td>
</tr>
<tr>
<td>AA 100µM</td>
<td>4.5±0.09</td>
<td>3.8±0.21</td>
<td>1.2±0.07</td>
</tr>
<tr>
<td>BHT 100µM</td>
<td>5.6±0.22</td>
<td>3.2±0.34</td>
<td>2.0±0.11</td>
</tr>
</tbody>
</table>

Values are mean ± SD of three replicates

G. bosvallia (GBP), N. alata (NAP), F. vaillantii (FIP), M. pentaphylla (MPP), P. corymbosa (PCP), R. procumbens (RRP) and R. repens (RUP)

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Figure 1—Decomposition of hydrogen peroxide by ‘Pittapapda’ samples Decomposition of hydrogen peroxide by ‘Pittapapda’ samples at different time intervals with respect to rate constant K at 1 mg/mL concentration. Rate was calculated as mentioned in material and methods. Values are mean ±SD of six replicates.
of the blue colour as a result of reaction between the ABTS+ radical and the antioxidant compounds in the sample\textsuperscript{18}. The trend of TEAC values was FIP > GBP > NAP > PCP > MPP > RUP > RRP (Table 2).

Photochem\textsuperscript{®} apparatus allowed precise method for the integral antioxidative capacity. Free radicals are generated in the instrument by means of photosensitizer and detected by their reaction with a chemiluminogenic substance. Luminol acts both as photosensitizer as well as the detecting reagent. In presence of radical scavengers in the extract intensity of PCL was attenuated as a function of concentration\textsuperscript{19}. In this way the antioxidative capacity of the extract could be quantified. The results revealed that extract of all samples had distinctly varied ACW and ACL values. GBP, FIP and NAP had higher antioxidant capacity (Table 2). Initiation of the lipid peroxidation by ferrous sulphate takes place either through ferryl-perferryl complex or through OH·radical by Fenton’s reaction\textsuperscript{16}. GBP, FIP and NAP very significantly ($P < 0.001$) inhibited lipid peroxidation while MPP and PCP showed significant ($P < 0.05$) inhibition of lipid peroxidation (Fig. 2). The inhibition could be caused by absence of ferryl-perferryl complex or by scavenging the OH radical or the superoxide radicals or by changing the $\text{Fe}^{3+}/\text{Fe}^{2+}$ or by chelating iron itself. Lipid hydroperoxide which is a product of reactive hydroxyl radical and polyunsaturated fatty acids can be decomposed to produce malondialdehyde (MDA). The low level of MDA showed highest inhibition of lipid peroxidation\textsuperscript{15}. GBP, FIP and NAP showed significantly low MDA levels which indicated the role of these extracts as an antioxidant. Standard ascorbic acid at 50 $\mu$M was found to have TBARS value of 0.153 nmol/mg protein and inhibited lipid peroxidation 95.45±0.34\% (Fig. 2).

The key role of phenolic compounds is the ability to scavenge free radicals and ROS such as singlet oxygen, superoxide free radical, and hydroxyl radicals. Phenolic compounds in the medicinal plants extracts are frequently responsible for the antioxidant status\textsuperscript{20}. Taking this into count total phenol content of extracts was assayed. The trend of phenol content was as: GBP (289 mg PCE/g) > FIP (272 mg PCE/g) > NAP (259 mg PCE/g) > PCP (202 mg PCE/g) > MPP (187 mg PCE/g) > RRP (141 mg PCE/g) > RUP (114 mg PCE/g). A significant correlation was shown by total phenolic content and free radical scavenging activities of all extracts. The results confirmed that greater antioxidant activity of GBP, FIP and NAP was probably due to their highest amount of phenolic compounds.

### Table 2—Free radical scavenging capacity of extract of ‘Pittapapda’ samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>TEAC (mM)</th>
<th>Photochemi luminescence ACW in ascorbic acid equivalent (nmol/g)</th>
<th>Photochemi luminescence ACL in trolox equivalent (nmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GBP</td>
<td>2.89±0.19</td>
<td>2.341</td>
<td>12.656</td>
</tr>
<tr>
<td>NAP</td>
<td>2.56±0.17</td>
<td>1.693</td>
<td>11.567</td>
</tr>
<tr>
<td>FIP</td>
<td>2.98±0.13</td>
<td>2.345</td>
<td>12.278</td>
</tr>
<tr>
<td>PCP</td>
<td>1.58±0.193</td>
<td>1.341</td>
<td>9.890</td>
</tr>
<tr>
<td>MPP</td>
<td>1.20±0.215</td>
<td>0.982</td>
<td>8.543</td>
</tr>
<tr>
<td>RRP</td>
<td>0.56±0.078</td>
<td>0.861</td>
<td>3.211</td>
</tr>
<tr>
<td>RUP</td>
<td>0.77±0.128</td>
<td>0.561</td>
<td>2.865</td>
</tr>
<tr>
<td>AA 100µM</td>
<td>3.89±0.23</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

TEAC- Trolox Equivalent Antioxidant Capacity, Values are mean ± SD of six replicates

$G. \text{bosvallia}$ (GBP), $N. \text{alata}$ (NAP), $F. \text{vaillantii}$ (FIP), $M. \text{pentaphylla}$ (MPP), $P. \text{corymbosa}$ (PCP), $R. \text{procumbens}$ (RPP) and $R. \text{repens}$ (RUP)

![Figure 2](image-url)
Conclusion

Out of seven sources of ‘Pittapapda’, the ethanol extract of *G. bosvallia*, *F. vaillantii* and *N. alata* showed potent *in vitro* antioxidant activity followed by moderate activity of the ethanol extract of *M. pentaphylla* and *P. corymbosa* as compared to the standard. The preliminary chemical examination of the ethanol extract of above three samples has shown higher content of phenolic compounds. The possible mechanism of antioxidant activity includes reducing ability, nitric oxide, hydrogen peroxide, trolox equivalent antioxidant capacity (TEAC) and free radical scavenging potential using Photo-chemiluminescence along with lipid peroxidation which may be due to the presence of higher phenolic phytoconstituents in the ethanol extracts.

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References