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Phytoconstituents and *In-vitro* Antioxidant Activity of Methanol crude extract of *Putranjiva roxburghii* Wall. Leaf

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Abstract

The present article describes the phytoconstituents and antioxidant capacity of the methanol crude drug extract of *Putranjiva roxburghii* Wall. leaves. Antioxidant, quantitative, and qualitative techniques were used to evaluate crude drug extract in methanol. Both the qualitative screening and the quantitative analysis were conducted using standard methods. Qualitative screening revealed the presence of alkaloids, flavonoids, glycosides, phenols, saponins, phytosterols/ terpenoids, tannins, coumarins, lipids, and fixed oil. The antioxidant test was performed using three methods: the DPPH radical scavenging test, the hydrogen peroxide test, and the ferric reducing antioxidant power test. The DPPH free radical scavenging test used 2, 2-diphenyl-1-picrylhydrazyl, the hydrogen peroxide test used H_2O_2, and the FRAP test used potassium ferricyanide and $FeCl_3$. The quantitative study results show that phenols are more concentrated in the leaves *of Putranjiva roxburghii*, followed by flavonoids and alkaloids. Among the three antioxidant assay techniques, the methanol crude extract has the greatest capacity to neutralise free radicals in the DPPH assay. This is followed by the ferric reducing power and hydrogen peroxide assay. *Putranjiva roxburghii* leaves demonstrated significant antioxidant activity.

Key words : DPPH Assay, FRAP Assay, Phytoconstituents, *Putranjiva roxburghii* leaf, Phenols, Antioxidant.

Plants contain phytoconstituents with therapeutic and pain-relieving properties. Plants are utilised as medications and have real utility. Plants, which contain a variety of secondary metabolites with antibacterial and antioxidant properties, such as alkaloids, flavonoids, terpenoids, steroids, phenols, tannins, and other phytochemicals, have been used for medical purposes for as long as humans have existed[12]. Atherosclerosis, arthritis, ischemia, damage to the central nervous system, gastritis, cancer, and more than 100 disorders

in humans are caused by free radicals, chemical species with one or more unpaired electrons that make them extremely unstable and damage other molecules by removing electrons from them to achieve stability. *Putranjiva roxburghii* is said to be native to Assam and the tropical region of the Eastern Himalaya, Indochina, Nepal, Thailand, Bangladesh, and Myanmar, but it is found throughout the Indian Subcontinent and Sri Lanka as an ornamental and roadside avenue tree[3]. Antioxidants assist in preventing oxidative stress, which is brought on by free radical damage[4]. *Putranjiva roxburghii* Wall. (Syn. *Drypetes roxburghii*) is a moderately large, evergreen tree that can reach a height of 12 to 18 meters. Roxburgh described the tree's name, "Pootranjeeva," which is a Sanskrit term, with "Putra" meaning a "Son" and "Jeeva" meaning "Life." It has dark grey bark with horizontal lenticels and hanging branches. Simple, dark green, elliptic, oblong, and distantly serrated leaves are placed in an alternating pattern.

The medicinal plant *Putranjiva roxburghii* is an ancient Indian herb used in the treatment of infertility in women and uterus related problems. Its leaves, fruits and seeds are traditionally used for medicinal purposes. The plant has been used in the treatment of various diseases such as infertility, antimicrobial, burning sensation, elephantiasis and azoospermia, etc. Hence, the present study aims to analyse the phytoconstituents and antioxidant activity of the leaves of *Putranjiva roxburghii* Wall. through successive extraction of leaf crude extract in methanol.

Plant Collection :

The *Putranjiva roxburghii* Wall. Fresh plant material was collected from the Vijayapura district, Karnataka, India, in December 2024.

Processing of plant material[6] *:*

The freshly harvested leaf material was thoroughly cleaned with running tap water and then distilled water. To prevent any chemical changes that would break down the bioactive components, it was shade-dried after washing. A mixer grinder was used to powder the dried material. An airtight glass bottle was used to contain the powder substance. Methanol was used as a solvent to extract the crude drug from the powder.

Preparation of Plant crude extract[6] *:*

The Soxhlet extractor's glass chamber was filled with 50 g of plant leaf powder that had been weighed and placed in a thimble. A 500 ml round-bottom flask was filled with 300–500 ml of methanol. The Soxhlet appliance was then successfully operated. Next, a round-bottom flask holding the solvent was set on top of the Soxhlet extractor. The process was repeated numerous times until the solvent from the siphon tube that fell into the distillation flask turned colourless, indicating that no phytoconstituents remained to be dissolved. To collect the plant crude extract, the solvent from the distillation flask was gathered, filtered, and allowed to evaporate at room temperature.

Phytochemical Screening of Putranjiva roxburghii Leaf :

The qualitative analysis was performed according to[1,5,6]

1. ***Test for Carbohydrates :***
Benedict's Test : To the 1ml of crude extract, 0.5ml of Benedict's reagent was added and heated on a water bath for 5 minutes. The formation of the characteristic brown colour indicates the presence of carbohydrates.

2. ***Test for Proteins :***
Biuret Test : 2ml of crude extract was treated with Biuret reagent, and the formation of blue to violet or purple colouration indicated the presence of proteins and amino acids.

Ninhydrin Test: To 2ml of crude extract, a few drops of ninhydrin reagent were added. Formation of blue colour indicated the presence of proteins.

Xanthoprotetic Test: 2ml of crude extract was treated with a few drops of concentrated HNO_3. The formation of yellow colour indicated the presence of proteins.

3. ***Test for Alkaloids :***
Mayer's Test : 2ml of crude extract was treated with a few drops of Mayer's reagent, and 1ml of HCl was added. Formation of a yellow precipitate indicated the presence of alkaloids.

Wagner's Test : 2ml of crude extract was treated with a few drops of Wagner's reagent and 1ml of dilute hydrochloric acid. Formation of yellow or reddish-brown precipitate indicated the presence of alkaloids.

Dragendroff's Test : 1 mL Dragendroff's reagent and 1ml HCl were added to the plant crude extract. Formation of orange or reddish-brown precipitate indicated the presence of alkaloids.

Hager's Test : 2ml of crude extract was treated with a few drops of Hager's reagent (saturated solution of picric acid). The formation of a yellow-coloured precipitate signified a positive result.

4. ***Test for Flavonoids :***
Lead acetate Test : 1ml of crude extract was treated with 1ml 10% lead acetate solution. Formation of white curdy precipitate indicated the presence of flavonoids.

Alkaline Test : 2-3ml of crude extract was treated with a few drops of NaOH solution. Formation of intense yellow colour, which turned colourless on addition of a few drops of dilute HCl, indicates the presence of flavonoids.

Ammonium Test : 1ml of crude extract was shaken with 1% dilute ammonia solution. The layers were allowed to separate. A yellow colour observed in the ammonia layer indicated the presence of flavonoids.

Shinoda Test : a few magnesium turnings and 5 drops of concentrated hydrochloric acid were added dropwise to 1ml of the test solution. A crimson red colour appeared after a few minutes, confirming the presence of flavonoids.

Pew's Test : 2-3ml of crude extract was treated with zinc powder in a test tube, followed by drop-wise addition of Conc.

HCl. Formation of purple, red or cherry colour indicates the presence of flavonoids.

5. *Test for Phenols :*

Potassium dichromate Test: 2ml of crude extract was treated with 5% potassium dichromate solution. The positive result of phenol was confirmed by the formation of a brown precipitate.

Ellagic Acid Test : 2ml of crude extract was treated with a few drops of 5% glacial acetic acid (v/v) and sodium nitrate (w/v). The formation of muddy yellow or drive brown, or Niger brown colour indicated the presence of phenols.

6. *Test for Glycosides :*

Keller Killian Test (Cardiac glycosides test): 2ml crude extract was treated with 1ml glacial acetic acid, a few drops of 5% $FeCl_3$ and 0.5ml Conc. H_2SO_4. A brown ring of the interface indicated the presence of cardiac glycosides.

7. *Test for Saponins :*

Foam Test : 2ml crude extract was diluted with 2-3ml of distilled water and shaken for 5 minutes. The foamy-like appearance indicates the presence of saponins.

8. *Test for Phytosterols/ Terpenoids :*

Liebermann–Burchard's Test : 2ml of crude extract was dissolved in 2ml of acetic acid anhydride, heated to boiling, cooled and then 1ml of Conc. H_2SO_4 was added along the side of the test tube. A brown ring formation at the junction confirmed the test for the presence of phytosterols.

9. *Test for Triterpenoids :*

Salkowski Test : Dry crude extract (2mg) was shaken with chloroform(1ml) and a few drops of Conc. H_2SO_4 were added along the side of the test tube. A red-brown colour formed at the interface indicated the presence of triterpenoids.

10. *Test for Tannins :*

Ferric Chloride Test: a few drops of 5% (w/v) $FeCl_3$ solution were added to the 2ml crude extract. Formation of bluish black colour indicated the presence of hydrolysable tannins.

Braymer's Test : 2ml of crude extract was treated with 2ml H_2O and followed with 2-3 drops of $FeCl_3$(5%). Green precipitate proved the presence of tannins.

11. *Test for Fats and fixed oils :*

2ml of crude extract was treated with 1ml of 1% $CuSO_4$ solution and a few drops of 10% NaOH solution. Blue colouration indicated the presence of fixed oils.

12. *Test for Coumarins :*

2ml of crude extract was treated with 3ml of 10% NaOH solution. Yellow colouration indicates the presence of coumarins.

Total Phenols content :

The Folin-Ciocalteu method was used to quantitatively measure phenols[1,16]. Catechol standard: To create a working standard, 100 milligrams of catechol are dissolved in 100 millilitres of distilled water in a volumetric flask and then diluted ten times.

Aliquots (0.1 and 0.5ml) are combined with distilled water to make a final volume of 3 ml. 0.5 ml of Folin-Ciocalteu's reagent (FCR) and 2 ml of 20% (w/v) Na_2CO_3 were added to these tubes and thoroughly mixed after three minutes. After a minute of incubation in a boiling water bath, all of these were cooled. In a spectrophotometer, the absorbance of the blue colour (caused by the molybdenum complex) is measured at 650 nm in relation to the blank reagent. 1% catechol is used as the standard when plotting a standard curve.

$$\text{Phenol in mg/100ml} = \frac{\text{Graphical value} \quad X \quad \text{Volume of total content}}{\text{Wt. of the plant material} \quad X \quad \text{Volume taken for reading}} \text{ X } 100$$

Total Flavonoids content :

The vanillin reagent technique was used to measure flavonoids[12] Vanillin Reagent: One gram of crystalline vanillin is dissolved in 100ml of 70% Con. H_2SO_4.

After diluting 0.1 and 0.2 ml of extract with 2 ml of distilled water in a test tube, 4 ml of Vanillin reagent was immediately added. After 15 minutes, the brick red colour was measured at 599 nm using a spectrophotometer against reagent black. The standard curve is plotted using different concentrations of phloroglucinol as the standard.

The flavonoid present in the sample was calculated with the help of a standard curve.

$$\text{Flavonoids in mg/100ml} = \frac{\text{Graphical value} \quad X \quad \text{Volume of total content}}{\text{Wt of the plant material} \quad X \quad \text{Volume taken for reading}} \text{ X } 100$$

Total Alkaloids content :

The Horneborn method was used to quantitatively measure the alkaloids[1,16] 20% acetic acid in ethanol (100ml), Concentrated NH_4OH

1g powder sample was treated with 40 ml of 20% acetic acid and kept for 4 hrs. The solution was filtered, and the volume was reduced to one-fourth in a water bath. To this sample, concentrated ammonium hydroxide was added dropwise for a precipitation reaction. The whole solution was allowed to settle, and the precipitate was collected by filtration and weighed. The percentage of total alkaloid content was calculated as,

$$\text{Percentage of total alkaloids} = \frac{\text{Weight of residue}}{\text{Weight of the sample taken}} \text{ X } 100$$

In Vitro Antioxidant Activity :

DPPH radical scavenging assay :

The DPPH technique was used to conduct the Free Radical Scavenging Assay (FRSA)[11,14]. 100 mL of analytical-grade methanol was used to prepare the DPPH (0.004%) methanol solution.

2,2-diphenyl-1-picrylhydrazyl (DPPH), which changes colour from deep purple to yellow, was used to assess the potential of the methanol crude extract of *Putranjiva roxburghii* leaf to scavenge free radicals. After adding a freshly made 1mL of 0.004% DPPH to various concentrations of methanol crude extract (20, 40, 60, 80, and 100 µg/mL), the test tubes were left in the dark for approximately 20 minutes at room temperature. A spectrophotometer was used to measure the mixture's absorbance at 517 nm. As a control, 1 ml of DPPH solution and 3 ml of methanol were added. The standard is ascorbic acid.

The following formula was used to calculate the DPPH radical scavenging activity.

Percentage of inhibition = (Ac – As)/Ac X 100
Where Ac is the absorbance of the control and
As is the absorbance of the test sample

Hydrogen peroxide radical scavenging assay:

The Hydrogen peroxide radical scavenging assay was carried out according to published findings[2,14]. The various concentration of methanol crude extract was prepared, such as 20, 40, 60, 80 and 100µg/ml in phosphate buffer saline (PBS) mixed with 0.6ml of 4mM H_2O_2 solution prepared in PBS and incubated for 10 mins. The absorbance was measured at 230nm. The standard is ascorbic acid.

Percentage of inhibition = (Ac – As)/Ac X 100
Where Ac is the absorbance of the control and
As is the absorbance of the test sample

Ferric reducing antioxidant power assay :

The Ferric reducing antioxidant power assay was carried out according to published findings[14] To the various concentrations (20, 40, 60, 80, and 100µg/ml) of methanol crude extract, 2.5ml of 0.2M phosphate buffer (pH 6.6) and 2.5ml 1% Potassium ferricyanide were added. After adding, shake it vigorously and keep it in incubation at 50°C for 20 mins. Then add 2.5ml 10% Trichloroacetic acid and centrifuge at 1500rpm for 10 minutes. 2.5 ml of supernatant solution was diluted with an equal volume of distilled water and add 0.5ml of $FeCl_3$ (0.1%)[14]. The absorbance was measured at 700nm by a UV-Visible Spectrophotometer. Phosphate buffer as blank and Ascorbic acid as standard.

Percentage of inhibition = (Ac – As)/Ac X 100
Where Ac is the absorbance of the control and
As is the absorbance of the test sample

Phytochemical Analysis :

The methanol crude extract of *Putranjiva roxburghii* Wall. The leaf was subjected to qualitative and quantitative analyses and evaluated for *in vitro* antioxidant activity. The qualitative analysis (Table-1) showed the presence of many pharmacologically important phytoconstituents, including Alkaloids, Flavonoids, Glycosides, Phenols, Carbohydrates, Saponins, Triterpenoids, Phytosterols/Terpenoids, Tannins, Proteins, fat, and fixed oil.

In quantitative analysis (Table-2), the phenol content was found to be (156.08±0.09 mg/100g), the flavonoid content was found to be (0.095±0.40 mg/100g), and the alkaloid content was found to be (0.05±0.02 mg/100g). The phenol content was found to be maximum in the *Putranjiva roxburghii* leaf (156.08±0.09 mg/100g), followed by flavonoids (0.095±0.40 mg/100g) and alkaloids (0.05±0.02 mg/100g).

Table-1. Qualitative analysis of the methanol crude extract of Putranjiva roxburghii Leaf

Sl. No	Phytochemical Test	Methanol
1	Alkaloids	+++
2	Flavonoids	++
3	Glycosides	+
4	Phenols	+
5	Carbohydrates	-
6	Saponins	+
7	Triterpenoids	-
8	Phytosterols/Terpenoids	+
9	Tannins	+
10	Proteins	-
11	Coumarins	+
12	Fats and Fixed Oil	+

Note: Presence (+), Absence (-)

Table-2. Total Phytoconstituents of the methanol crude extract of *Putranjiva roxburghii* leaf

Sl.No	Phytoconstituents	mg/100g
1	Phenols	156.08±0.09
2	Flavonoids	0.095±0.40
3.	Alkaloids	0.05±0.02

The Data expressed as Mean± Standard Deviation using Microsoft Excel.

In vitro antioxidant activity :

The *in vitro* antioxidant activity of *Putranjiva roxburghii* leaf was assessed for three different antioxidant assays, such as DPPH, Hydrogen peroxide, and $FeCl_3$ reducing power. The DPPH assay gives insight into the scavenging activity of the compounds against steady free radicals. DPPH shows high absorption in the visible range at 517nm. DPPH, upon reaction with an open radical, the single electrons get paired, which causes a change in colour from deep purple (violet) to light yellow, resulting in reduced absorption. The reduction in absorbance is the indicator of radical scavenging potential[14].

The Figs. 1, 2 & 3 revealed the antioxidant activity of different concentrations of methanol crude extract. The antioxidant capacity of the methanol crude extract was compared with that of ascorbic acid as a standard. In the present study, Fig. 1 shows the antioxidant capacity of the methanol crude extract of *Putranjiva roxburghii* leaves against the DPPH assay. The different concentrations of methanol crude extract exhibited DPPH free radical scavenging activities of 60.29, 66.55, 72.14, 80.90, and 87.92%. The methanol crude extract of *Putranjiva roxburghii* leaves seems to be equipotent to ascorbic acid, with a highest inhibition of 87.92% at 100µg/ml, whereas for ascorbic acid, it was 85.37% for the same concentration.

Fig. 2 shows the antioxidant capacity of the methanol crude extract of *Putranjiva roxburghii* leaves against the hydrogen peroxide assay. The different concentrations of methanol crude extract exhibited hydrogen peroxide free radical scavenging activities of 35.78, 51.37, 60.22, 59.35 and 73.69%. The standard ascorbic acid shows the highest inhibition of 93.33% at 100µg/ml, whereas the methanol crude extract shows 73.69% at 100µg/ml concentration.

Fig. 3 shows the antioxidant capacity of the methanol crude extract of *Putranjiva roxburghii* leaves against the ferric reducing antioxidant power assay. The different

concentrations of methanol crude extract exhibited FRAP free radical scavenging activities of 19.86, 40.27, 54.51, 60.56 and 69.83%. The methanol crude extract of *Putranjiva roxburgii* leaves appears to be equipotent to ascorbic acid, with the highest inhibition of 69.83% at 100 µg/ml, whereas the standard shows 58.31% at 100 µg/ml.

Among the three methods, the methanol crude extract shows high free radical scavenging activity in the 2, 2, -Diphenyl-1-picrylhydrazyl assay, followed by the Ferric reducing antioxidant power assay and Hydrogen peroxide assay.

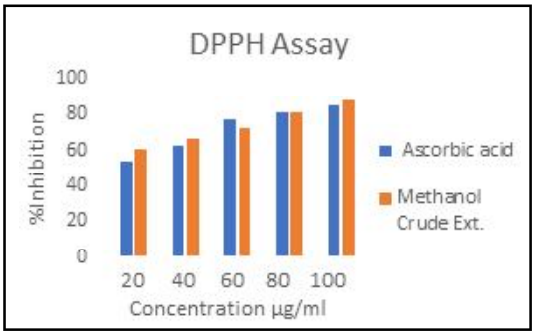

Fig. 1. DPPH Free Radical Scavenging Assay of Methanol Crude Extract of *Putranjiva roxburghii* leaf

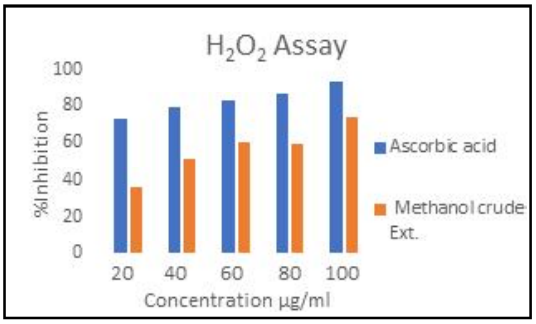

Fig. 2. Hydrogen Peroxide antioxidant assay of the methanol crude extract of *Putranjiva roxburghii* leaf

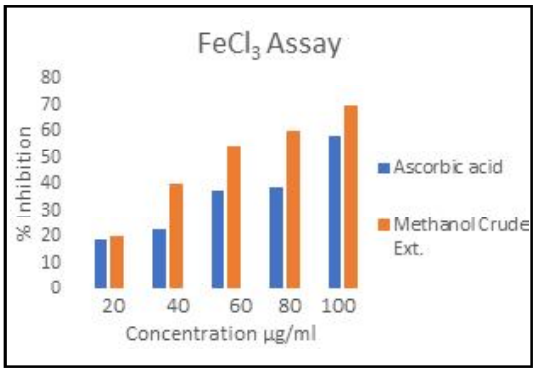

Fig. 3. Ferric reducing antioxidant power assay of the methanol crude extract of *Putranjiva roxburghii* leaf

References :

1. Ahmad, R., G. Parveen, N. A. Gauri and Neha Wal (2018). *International Journal of Fauna and Biological Studies 5*(6): 125-139.
2. Arya, N.N., K. Uniyal and K. Bhandari (2024). *Environment and Ecology 42*(2): 405-412, DOI: https;//doi.org/10.60151/envec/BFRU1901.ISSN 0970-0420
3. Beni, P., H. Konya, SK., M. Yashpal, and PK, Bechan La (2021). *Journal of Bioresources 8*(2): 25-35, ISSN: 2582-2276.
4. Chinmaya, A. S.J.S., N.C.V., T.R.P. Kekuda, A.N. Rajeshwara, S. Murthuza, and S.V. Praveen Kumar (2009). *Global Journal of Pharmacology 3*(1): 53-58, 2009ISSN 1992-0075.
5. Harborne J.B. (1998) Chapman and Hall. *49:* 302.
6. Hussain, M. A., M. Saquib, and M.F. Khan (2019). Springer Nature Singapore Pte Ltd. https://doi.org/10.1007/978-981-13-7205-6_8
7. Kavya H and Shrishail (2025). *Indian J.*

Applied & Pure Bio. 40(3): 1619-1627.

8. Kalemba, M. R. K., R. Makhuvele, and P. B. Njobeh (2024). *Heliyon 10*(2): e24435.
9. Kumar M. Prasath, K. Raja, K. Vasanth., A. Khushro, S. Sadhasivam, M. U. K. Sahibzada, M. R. A. Gawwad, D.A. Al Farraj, and M. S. Elshikh (2021). *Arabian journal of Chemistry 14:* 103345.
10. Mane, NB, NA Shaikh and SV Ingawale (2021). *Journal of Pharmacognosy and Phytochemistry 10*(1): 444-447.
11. Mauna, B.A. and T.P. Naik (2025). *Indian J. Applied and Pure Bio. 40*(3): 1694-1702.
12. Minj, E., S. J. britto, R.R. Marandi, I. Kindo and M. George (2015). *World Journal of Pharmacy and Pharmaceutical Sciences. 5*(1): 1157-66. ISSN 2278-4357
13. Mohanthy, SP., K. T. R., M. D. Mahesh, and Sonali Nayak (2023). *Journal of Ayurvedic and Integrated Medical Sciences. Vol. 8.*
14. Pandal, D.S., S.K. Pandya, N.K. Alruwaili, M. Gamah, R.K. Giri, and S.K Patro (2021). *Research journal of Pharmacy and Technology.* DOI:10.52711/0974-360X.2021.01076.
15. Penso, G., (1980). *J Ethnopharmacol* DOI: 10.1016/0378-8741(80)90013-6
16. Rathod, M. A., P. Mathad, S. Inamdar and N. Mahurkar (2025). *International Journal of Novel Research and Development.* 2456-4184.
17. Sarath P and R Sudha Bai (2019). *Journal of Pharmacognosy and Phytochemistry 8*(1): 1162-1166.
18. Shahwar D., M. A. Raza, A. Saeed, M. Riasat, F. L. Chattha, M. Javaid, S. Ullah and S. Ullah (2012). *African Journal of Biotechnology 11*(18): 4288-4295, DOI: 105897/AJB11.2564
19. Shetty, S. P., and R. R. Kale (2020) *International Journal of Research Culture Society* ISSN:2456-6683 Volume-4.