

EXTRACTION, PHYTOCHEMICAL SCREENING AND ANTIOXIDANT POTENTIAL OF ETHANOLIC EXTRACT OF *DRYPETES ROXBURGHII*

Abstract

Humans are affected by free radicals in over a hundred different ways. Synthetic antioxidants have been linked to harmful health consequences, prompting substantial investigation into naturally occurring antioxidants, particularly those found in plants. The goal of this study was to determine the phytochemical components and in vitro antioxidant activity of *Drypetes roxburghii* (*D. roxburghii*, Euphorbiaceae) leaves collected in the Bhopal district of Madhya Pradesh. The 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) test was used to measure antioxidant activity. The presence of cardiac glycosides, flavonoids, phenol, proteins, carbohydrate, and saponins in the ethanolic extracts of *D. roxburghii* leaves was discovered using phytochemical screening. The ethanolic and aqueous extracts of *D. roxburghii* leaves yielded 1.9 and 2.6 percent w/w, respectively. The ethanolic extract of *D. roxburghii* leaves contained 1.48 and 0.70 mg/100 mg of total phenolic and flavonoids, respectively. When comparing the DPPH scavenging activity of leaf ethanolic extract to Ascorbic acid (standard), the IC₅₀ value was 87.80. It suggests that the plant has the ability to scavenge free radicals, and it might lead to new discoveries in the continuing hunt for natural antioxidants in medicinal plants that can be utilised to treat illnesses caused by free radical reactions.

Keywords: *Drypetes roxburghii*, Qualitative, Quantitative phytochemical, Antioxidant activity.

Introduction

Plants have long been used as therapeutic ingredients, and humans continue to rely on them for healthcare [1]. Plant-based remedies are still used by over 80% of the population in poor and underdeveloped nations to treat their problems [2]. Compounds generated from nature have played an important role in the development of novel chemical entities. The process of discovering drugs from nature is multi-disciplinary and incorporates numerous fields such as ethnobotany, phytochemistry, biology, and different chemical separation methods, as well as combinatorial synthesis approaches. Approximately 87 percent of medications are considered to be obtained directly or indirectly from nature. In nature, there are around 420 000 plant species [3]. Antioxidants fight oxidation and help to preserve lipids from radical peroxidation. The human body is equipped with a sophisticated antioxidant defence system[4]. The capacity of an antioxidant to trap free radicals is its most important feature. Free radicals and oxygen species with high reactivity can be found in biological systems from

a variety of sources. These free radicals can cause degenerative illness by oxidising nucleic acids, proteins, lipids, or DNA. Antioxidant substances such as phenolic acids, polyphenols, and flavonoids scavenge free radicals including peroxide, hydroperoxide, and lipid peroxy, inhibiting the oxidative pathways that contribute to degenerative illnesses. A number of clinical investigations show that the antioxidants found in fruits, vegetables, tea, and red wine are the major reason for these foods' reported efficacy in lowering the prevalence of chronic illnesses like heart disease. The plant *D. roxburghii* Wall is a member of the Euphorbiaceae family. *Putranjiva roxburghii* Wall is its synonym. It grows wild or farmed in practically every corner of India, up to a height of 750 metres. It is a dioecious, evergreen tree with grey bark that grows to a height of up to 18 metres and a girth of up to 2 metres. Long, elliptic oblong to ovate-lanceolate leaves with uneven sides at the base, dark green in colour and gleaming. Saponins, mannitol, arachidic acid, linoleic acid, palmitic acid, glucoputranjivin, putranjivoside, putranoside, -sitosterol, carboxylic acid, putric acid, putranjivic acid, glucosides, fatty oil, alkaloids, and gallo-tannins are among the phytochemicals found in *D. roxburghii*. For colds and fevers, the leaves and stones are decocted; they are also used for rheumatism, elephantiasis, burning sensations, azoospermia, constipation, and infertility. It is also used as an antidiabetic [6, 7]. The aim of this work was to determine the quality (types), quantity (amount) of bioactive compounds and *in vitro* antioxidant activity of leaves of *D. roxburghii* in Bhopal region of Madhya Pradesh.

Materials and methods

Plant material

Fresh *D. roxburghii* leaves were taken from Vindhya Herbals' Botanical Garden in Bhopal. The leaves were properly cleansed with regular tap water and then sterile distil water. The leaves were then dried at room temperature in a shady environment. Using a grinding machine, the leaves were crushed into powder. The powder was kept at 4°C in an airtight storage bottle.

Reagents for chemistry

Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Ltd. (Mumbai, India), and SRL Pvt. Ltd. (Mumbai, India) provided all of the chemicals utilised in this work (Mumbai, India).

The substances employed in this experiment were all analytical grade.

Extraction by maceration method

By maceration, 175 gramme of powdered *D. roxburghii* leaves were extracted using various solvents such as chloroform, ethyl acetate, ethanol, and aqueous. The extract was evaporated at temperatures higher than their boiling points. Finally, the dried extracts' percent yield was calculated. After that, the extracts were reduced in a rotary evaporator before being kept in airtight containers at 4°C for later use.

Evaluation of organoleptic properties

Color, odour, taste, texture, and other organoleptic characteristics of dried extracts of *Drypetes roxburghii* were assessed. The sample's organoleptic characteristics were assessed using the approach outlined by Siddiqui and Hakim [8].

Qualitative phytochemical screening

Crude extracts were screened to identify the occurrence of primary and secondary metabolites, viz. carbohydrates, alkaloids, glycosides, polyphenols, flavonoids, tannins, saponins, terpenoids, proteins and fixed oils, using standard screening test and phytochemical procedures [9, 10].

Estimation of total phenolic content

The modified Folin-Ciocalteu technique (Parkhe and Bharti, 2019) [11] was used to determine the total phenolic content of the extract. In methanol, 10 mg Gallic acid was dissolved in 10 ml methanol, and different aliquots of 5- 25g/ml were produced. We dissolved 10 mg of dried extracts in 10 ml methanol and filtered it. For the phenol determination, two millilitres (1 mg/ml) of this solution were utilised. 1 ml Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) sodium carbonate were combined with 2 ml of each extract or standard. The mixture was vortexed for 15 seconds before being let to sit for 15 minutes to develop colour. A spectrophotometer was used to measure the absorbance at 765 nm.

Estimation of total flavonoids content

The total flavonoids content was determined using the aluminium chloride technique [10]. In 10 ml methanol, 10 mg quercetin was dissolved, and different aliquots of 5- 25g/ml were made in methanol. We dissolved 10 mg of dried extracts in 10 ml methanol and filtered it. The flavonoid concentration was determined using three millilitres (1 mg/ml) of this solution. 1 ml of a 2 percent AlCl_3 methanolic solution was added to 3 ml of extract or standard and allowed to remain at room temperature for 15 minutes before measuring absorbance at 420 nm.

Antioxidant activity

DPPH method

Total free radical scavenging capacity of the ethanolic extract obtained from *Drypetes roxburghii* was estimated according to the previously reported method with slight modification (Parkhe and Bharti, 2019) [13]. Solution of DPPH (6 mg in 100ml methanol) was prepared and stored in dark place. Different concentration of standard and test (10- 100 µg/ml) was prepared. 1.5 ml of DPPH and 1.5 ml of each standard and test was taken in separate test tube; absorbance of this solution was taken immediately at 517nm. 1.5 ml of DPPH and 1.5 ml of the methanol was taken as control absorbance at 517nm. The percentage inhibition of free radical DPPH was calculated from the following equation:

$$\% \text{ inhibition} = [(\text{absorbance of control} - \text{absorbance of sample}) / \text{absorbance of control}] \times 100\%.$$

Results and discussions

To achieve the real yield of extraction, the crude extracts produced after each consecutive maceration extraction step were concentrated on a water bath by fully evaporating the solvents. Table 1 shows the yield of extracts collected from the plants' leaves using chloroform, ethyl acetate, ethanol, and water as solvents. Table 2 shows the findings of a qualitative phytochemical study of *D. roxburghii* leaf crude powder. *D. roxburghii* ethanolic and aqueous extracts included cardiac glycosides, flavonoids, phenol, proteins, carbohydrate, and saponins. The total phenolic content of a sample is measured in mg gallic acid equivalents per 100 mg dry weight of the sample. The extracts' total flavonoids content was calculated as a percentage of quercetin equivalent per 100 mg dry weight of sample. TPC of *D. roxburghii* ethanolic and aqueous extracts revealed concentration values of 1.48 and 1.08, respectively. Table 3 shows that the total flavonoid concentration of *D. roxburghii* ethanolic and aqueous extracts was 0.70 and 0.32, respectively. The DPPH test has been widely utilised to determine the in vitro general antioxidant activity of pure chemicals and plant extracts [14, 15]. The reduction in DPPH radical absorbance with increasing extract concentration, as seen by the fast discoloration of the purple DPPH, suggests that the *D. roxburghii* hydro alcoholic extracts have antioxidant activity due to its proton donating capacity. The extracts were shown to have dose-dependent action, implying that DPPH scavenging activity increased according to the rise in extract concentration. Table 4 also included the IC₅₀ values for scavenging DPPH radicals for the AA and extract. When compared to AA, the extract's IC₅₀ value for DPPH radical activity was determined to be 87.80.

Conclusion

Despite continuous scientific research on this species, this is the first attempt to investigate the phytochemical contents as well as the antioxidant activities of *D. roxburghii* leaves

ethanolic extracts that has been identified despite a comprehensive literature review to date. The primary method to identifying new secondary metabolites as unaltered form, semi-synthetic, or pharmacological templates is to understand the phytochemical elements of the plant. This research shows that hydroalcohol extracts have the capacity to scavenge free radicals. As a result, distinct active secondary metabolites are likely to be present in these extracts. Furthermore, the action of this plant ingredient may aid in scientifically elucidating the reason for this plant species' ethnomedical usage. Based on our findings, further studies are necessary to elucidate the mechanism lying with these effects of the plant extracts and could be open a new window in the search for new bioactive drug lead components of this plant extracts.

Table 1: Organoleptic evaluation and extractive values of *D. roxburghii*

Sr. No	Extracts	Colour	Taste/odor	Texture	% Yield (W/W)
1.	Chloroform	Dark green	Characteristics	Sticky	1.8%
2.	Ethyl acetate	Dark green	Characteristics	Sticky	1.3%
3.	Ethanolic	Dark green	Characteristics	Sticky	1.9%
4.	Aqueous	Dark brown	Characteristics	Sticky	2.6%

Table 2: Result of phytochemical screening of *D. roxburghii*

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Ethanolic extract	Aqueous extract
1.	Alkaloids Hager's Test:	-Ve	-Ve	-Ve	-Ve
2.	Cardiac Glycosides Keller-Killani test:	-Ve	+Ve	+Ve	+Ve
3.	Flavonoids Lead acetate Test: Alkaline test:	-Ve +Ve	+Ve -Ve	+Ve +Ve	+Ve +Ve
4.	Diterpenes Copper acetate Test:	+Ve	+Ve	-Ve	-Ve
5.	Phenol Ferric Chloride Test:	-Ve	-Ve	+Ve	+Ve
6.	Proteins Xanthoproteic Test:	+Ve	-Ve	+Ve	+Ve

7.	Carbohydrate Fehling's Test:	+Ve	-Ve	+Ve	+Ve
8.	Saponins Froth Test:	-Ve	+Ve	+Ve	+Ve
9.	Tannins Gelatin test:	-Ve	-Ve	-Ve	-Ve

+Ve = Positive, -Ve= Negative

Table 3: Results of total phenol and flavonoids content

S. No.	Extracts	Total phenol content	Total flavonoids content
		mg/100mg	
1.	Chloroform	-	0.59
2.	Ethyl acetate	-	0.60
3.	Ethanollic	1.48	0.70
4.	Aqueous	1.08	0.32

Table 4: % Inhibition of ascorbic acid and ethanolic extract using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
1	10	41.5	8.6
2	20	47.7	16.9
3	40	52.9	29.0
4	60	67.4	43.9
5	80	75.8	48.4
6	100	89.6	49.8
IC 50		27.82	87.80

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

NOTE:

The study highlights the efficacy of "Herbals" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable

References

1. Uddin MS, Asaduzzaman M, Mamun AA, Iqbal MA, Wahid F. Neuroprotective activity of *Asparagus racemosus* Linn against ethanolinduced cognitive impairment and oxidative stress in rats brain: Auspicious for controlling the risk of Alzheimer's disease. *J Alzheimers Dis Parkinsonism* 2016; 6(4): 1–2.
2. Hasan MF, Iqbal MA, Uddin MS. Antibacterial and antifungal activity of *Litsea monopetala* leaves on selected pathogenic strains. *Eur J Med Plants* 2016; 12(4): 1–8.
3. Vuorela P, Leinonen M, Saikku P, Tammela P, Rauha JP, Wennberg T, et al. Natural products in the process of finding new drug candidates. *Curr Med Chem* 2004; 11: 1375–1389.
4. Allesio HM. and Biasiz R..Antioxidant defense mechanism natural Antioxidant. *Res., Q.Exer.C.* 68; 1997: 292-94.
5. Miller HE., Rigelhof F., Marquart L., Prakash, A. and Kanter M. *Cereal Foods World.* 45(2); 2000: 59-63.
6. The Wealth Of India; A Dictionary of Indian Raw Materials and Industrial products – Raw Materials Series (Publications and Information Directorate, Council of Scientific & Industrial Research, New Delhi), 1976; Vol. VII: 325-326.
7. Sharma Ravindra, *Medicinal Plants of India, An Encyclopaedia.* Daya publishing house, New Delhi. 2003; 208.
8. Siddiqui S, Hakim MA. Format for the Pharmacopoeial Analytical Standards of Compound Formulation, Workshop on Standardization of Unani Drugs. New Delhi: Central Council for Research in Unani Medicine (CCRUM); 1995. p. 24-5.
9. Parkhe G, Jain P, Jain DK. Total phenolic content, total flavonoid & anti-bacterial activity of hydroalcoholic acacia catechu (l.f.) Willd roots extracts for treatment of mouth ulcer. *Pharmacologyonline* 2018; 2: 227-233.
10. Trease, G.E and Evans, W.C. (1978). *Pharmacology*, 11th Edition. Bailliere Tindall Limited, London, pages 60-75.
11. Geeta Parkhe, Deepak Bharti. Phytochemical Investigation and Determination of Total Phenols and Flavonoid Concentration in Leaves Extract of *Vitex trifolia* Linn. *Journal of Drug Delivery & Therapeutics.* 2019; 9(4-A):705-707.
12. Meda, A., Lamien, C.E., Romito, M., Millogo, J. and Nacoulma, O.G. (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry*, 91: 571-577.

13. Geeta Parkhe, Deepak Bharti. In vitro antioxidant activity, total phenolic and flavonoid contents of hydroalcoholic extract of leaves of *Lagerstroemia Parviflora* Roxb. Journal of Drug Delivery & Therapeutics. 2019; 9(4-A):708-711.
14. Koleva II, van Beek T, Linssen JPH, de Groot A, Evstatieva LN . Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem. Anal . 2002; 13:8 -17. 28.
15. Gonçalves C, Dinis T, Batist MT. Antioxidant properties of proanthocyanidins of *Uncaria tomentosa* bark decoction: a mechanism for anti -inflammatory activity. Phytochemistry. 2005; 66:89 -98.