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

Abstract

Free radicals contribute to more than one hundred disorders in humans. The synthetic antioxidants have been suspected to cause or prompt negative health effects leading to extensive research on naturally occurring antioxidants especially from plant sources. The aim of the present study was to evaluate qualitative and quantitative phytochemical constituents and *in vitro* antioxidant activities of leaves of *Drypetes roxburghii* (*D. roxburghii*, Euphorbiaceae) collected from Bhopal region of Madhya Pradesh. Antioxidant activity was carried out by using 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) assay. The phytochemical screening of leaves of *D. roxburghii* revealed the presence of cardiac glycosides, flavonoids, phenol, proteins, carbohydrate and saponins in the ethanolic extracts. The percentage yield of ethanolic and aqueous extract of the leaves of *D. roxburghii* was found to be 1.9, 2.6% w/w. The total phenolic and flavonoids content of *D. roxburghii* leaves of ethanolic extract was found to be 1.48 and 0.70mg/100mg respectively. The results of DPPH scavenging activity for leave ethanolic extract showed IC_{50} value 87.80, when compared to Ascorbic acid (standard) was 27.82. It indicates the plant has the potency of scavenging free radicals and it may provide leads in the ongoing search for natural antioxidants from various medicinal plants to be used in treating diseases related to free radical reactions.

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

Introduction

Use of plants as medicinal substances is as old as human civilization and mankind continues to rely on them for healthcare [1]. At present, around 80% population residing in the developing or underdeveloped countries still use plant-based medicines to combat their ailments [2]. Naturally-derived compounds have significantly contributed in the discovery of new chemical entities. The process of drug discovery from nature involves multi-disciplinary approach and is interconnected with many disciplines like ethnobotany, phytochemistry, biology and various chemical separation processes along with combinatorial synthetic techniques. It is currently estimated that around 87% of drugs are derived directly or indirectly from nature. Approximately, 420 000 plant species occur in nature [3]. Antioxidant means against oxidation and the work to protect lipid from peroxidation by radicals. The human body as an elaborate antioxidant defense system[4]. The main characteristic of an

antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like Phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydro peroxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers [5]. The plant *D. roxburghii* Wall, belong to the family Euphorbiaceae. Its synonym is *Putranjiva roxburghii* Wall. It is found wild or cultivated almost in all parts of India, ascending of 750m. It is a dioeciously, evergreen tree, attaining a height of up to 18m and a girth up to 2m having grey bark. Leaves long, elliptic oblong to ovate-lanceolate, unequal sides at the base, dark green in colour and shinning in appearance. Phytochemicals present in *D. roxburghii* include saponins, mannitol, arachidic acid, linoleic acid, palmitic acid, glucoputranjivin, putranjivoside, putranoside, β-sitosterol, carboxylic acid, putric acid, putranjivic acid, glucosides, fatty oil, alkaloids and gallo-tannins. Leaves and stones are given in decoction for cold and fever; they are also used in rheumatism, elephantiasis, burning sensation, azoospermia, constipation, sterility. 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 leaves of *D. roxburghii* were collected from Botanical garden of Vindhya Herbals, Bhopal. The leaves were washed thoroughly with normal tap water followed by sterile distill water. Then leaves were dried under shaded condition at room temperature. Leaves were crushed to powder using grinding machine. Powder was stored at $4^{o}C$ in tight air container bottle.

Chemical reagents

All the chemicals used in this study were obtained from 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).All the chemicals used in this study were of analytical grade.

Extraction by maceration method

175 gram of powdered leaves of *D. roxburghii* was extracted with different solvent like chloroform, ethyl acetate, ethanol and aqueous by maceration method. The extract was evaporated above their boiling points. Finally, measured the % yield of the dried extracts. The recovered extracts were then reduced in a rotary evaporator and finally stored in airtight containers at 4°C for further use.

Organoleptic evaluation

Organoleptic evaluation was done for dried extracts of *Drypetes roxburghii* by observing color, odor, taste, texture, etc. The organoleptic characters of the sample were evaluated based on the method described 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 total phenolic content of the extract was determined by the modified Folin-Ciocalteu method (Parkhe and Bharti, 2019) [11]. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol. 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Meda *et al.,* 2005) [12]. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured 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: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%.

Results and discussions

The crude extracts so obtained after each of the successive maceration extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The yield of extracts obtained from the leaves of the plants using chloroform, ethyl acetate, ethanol and water as solvents are depicted in the Table 1. The results of qualitative phytochemical analysis of the crude powder of leaf of *D. roxburghii* were shown in Table 2. Ethanolic and aqueous extracts of *D. roxburghii* showed the presence of cardiac glycosides, flavonoids, phenol, proteins, carbohydrate and saponins. The determination of the total phenolic content, expressed as mg gallic acid equivalents and per 100 mg dry weight of sample. The total flavonoids content of the extracts was expressed as percentage of quercetin equivalent per 100 mg dry weight of sample. TPC of ethanolic and aqueous extract of *D. roxburghii* showed the content values of 1.48 and 1.08 respectively. The total flavonoid content of *D. roxburghii* ethanolic and aqueous extract showed the content values of 0.70 and 0.32 respectively Table 3. The DPPH assay has been largely used as a quick, reliable and reproducible parameter to search for the in vitro general antioxidant activity of pure compounds as well as plant extracts [14, 15]. The decrease in absorbance by the DPPH radical with increase in concentration of the extract which manifested in the rapid discolouration of the purple DPPH, suggest that the hydro alcoholic extracts of *D. roxburghii* has antioxidant activity due to its proton donating ability. It was found that the extracts exhibited a dose-dependent activity which indicates that DPPH scavenging activity was increased proportionately to the increase in the extracts' concentration. Additionally, the IC_{50} values of scavenging DPPH radicals for the AA and extract were shown in Table 4. Comparing with AA, the IC_{50} value for DPPH radical activity of extract was found to be 87.80.

Conclusion

Despite ongoing scientific research on this species, this study constitutes the first attempt to determined the phytochemical compositions as well as the antioxidant, activities of *D. roxburghii* leaves ethanolic extracts that could be found despite the throughout literature survey so far as we know. The knowledge of phytochemical constituents of the plant is the basic approach to identify novel secondary metabolites as unmodified form, semi-synthetic or drug templates. This study delineates that hydroalcohol extracts could be potentials in free-radical scavenging activity. So, it can be assumed that different active secondary metabolites were present in these extracts. Furthermore, the activity of this plant constituent can help to elucidate the justification for the ethno medicinal use of this plant species scientifically. 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.	Ethanolic	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.