ISSN : 0970-2539
e-ISSN : 0976-3880

The Journal of Plant Science Research

VOLUME 37 NUMBER 1, 2021

Prints Publications Pvt Ltd
New Delhi

SOCIETY FOR THE PROMOTION OF PLANT SCIENCE RESEARCH

The Journal of Plant Science Research

2021 Volume 37 Number 1

CONTENTS

Continued on inside back cover

Content contd...

Society for the Promotion of Plant Science Research

Submitting Articles to the Journal of Plant Science Research

Contribution/manuscripts should be sent to Prof. C P Malik, Editor-in-chief, E-46, Sector-41, Gautam Budh Nagar, Noida, 201301 (UP) or through e-mail on cpm_malik@yahoo.com. Manuscript may also be sent to Dr. (Mrs.) Sanjeev Thind, Dr. Navita Ghai, Department of Botany, Punjab Agriculture University, Ludhiana-141004, India.

SUBSCRIPTIONS :

Annual Subscription Price	
₹ 2500.00 (National)	US$ 250.00 (International)

Online version free with print subscription

All remittance must be paid in favour of **Prints Publications Pvt Ltd** payable at **New Delhi.**

Periodicity : This journal is published Bi-annually.

Electronic Version : This Journal can be accessed electronically at http://www.printspublications.com

DOI No. : 10.32381/JPSR

This journal is Indexed in
NAAS, Proquest (USA), EBSCO Publishing (USA), CABI, Genamics (Journal Seek)

Published by :

Prints Publications Pvt Ltd
Viraj Tower 2, 4259/3,
Ansari Road, Darya Ganj,
New Delhi-110 002. India

Phone : +91-11-45355555
E-mail : **contact@printspublications.com**
Website : **www.printspublications.com**

The Journal of Plant Science Research

Introduction

The Journal of Plant Science Research is a reputed peer reviewed International Journal which is published bi-annually. This Journal disseminates knowledge in all related fields of Plant Scince Research such as Plant Physiology, Agriculture, Bio-Chemistry and Botany. It imparts the latest advances in the field of biotechnology and genetic engineering which is proved beneficial for the upcoming Geneticists, Plant Physiologists, Botanists, Biochemists and Biotechnologists. It regularly supplies latest information on researchers, education, public and projects on Plant Science at national and international level

Manuscript Submission

The Society for the Promotion of Plant Science Research invites review papers, full papers and short communications for publication in its official publication "The Journal of Plant Science Research". Submitted papers will be accepted only if found suitable by the referee.

All Contributions and editorial correspondence should be addressed to

Prof. C.P. Malik,
E-46, Sector- 41, Gautam Budha Nagar,
Noida – 201301 (U.P.) India.
Papers for publication must be sent by e-mail on
cpm_malik@yahoo.com

Ethical Statement

The cover letter should include a written statement from the author(s) that:

1. The manuscript is an original research work and has not been published elsewhere including open access at the internet.
2. The data used in the research has not been manipulated, fabricated, or in any other way misrepresented to support the conclusions.
3. No part of the text of the manuscript has been plagiarised.
4. The manuscript is not under consideration for publication elsewhere.
5. The manuscript will not be submitted elsewhere for review while it is still under consideration for publication in the The Journal of Plant Science Research.

The cover letter should also include an ethical statement disclosing any conflict of interest that may directly or indirectly impart bias to the research work. Conflict of interest most commonly arises from the source of funding, and therefore, the name(s) of funding agency must be mentioned in the cover letter. In case of no conflict of interest, please include the statement that "the authors declare that they have no conflict of interest".

General Guidelines

- The contributions should be submitted through proper channel. It is presumed that the papers simultaneously submitted have not been previously and will not be simultaneously submitted and published in any other journal.
- On receipt of the paper an acknowledgement giving registration number of the paper will be sent to the corresponding author. This number should always be quoted.
- Review paper should not mention headings such as Material and Methods and Results and Discussion.
- The review papers should critically summarize the existing state of knowledge pointing out gaps in research and suggest possible line of future research.
- Review papers are generally invited but good reviews are considered for publication.
- Full paper should be based on original research reporting some new findings.
- Manuscripts of full paper should be prepared under the following headings: Title, Abstract (without heading), Key Words (not more than 5), Introduction, Material and Methods, Results and Discussion and References (all headings flush left).
- Short communication should include reports on experiments in progress which author wants to share with the readers urgently and it should have no headings/subheadings. For format please consult the latest issue of the journal. First line of the paragraph is not to be indented.
- The manuscript must be typed double-spaced on one side of good grade paper. An electronic copy of the paper done in Word Perfect 6.00 or MS Word should be sent in a CD.
- Title must be short and specific. The by line should comprise name(s) of the author(s) and complete address of the institute.
- Title pages should include a running title of not more than 7 words.
- Abstract must be self-explanatory and should include the reason(s) for carrying out the investigations objectives, methodology, important observations and a conclusion.
- Introduction should be brief and limited to the statement of the problem and aim of the experiment.
- Material and Methods should be clear and concise.
- Results and Discussions should be written concisely in a logical order.

Reference Style Guidelines

- References should be cited in text as Vijayaraghvan & Shukla (1977) or (Vijayaraghvan & Shukla 1977) depending upon the structure of the sentence.
- Indicate more than two authors as Truernit et al (1999) or (Truernit et al 1999), as the case be.
- If there is more than one publication by the same author(s) in the same year the letter 'a' or 'b' should be added after the year both in the text and list of references.
- The World List of Scientific Periodicals should be followed for abbreviations of the title of the journal.
- The general pattern of the references is given below :

Journal

Takahashi M 1993 Am J Bot 80 192 . .
Vijayaraghvan M R & Shukla A K 1977 Ann Bot 41 923 . .
Truernit E Stadler R Baier K & Santer N 1999 Plant J 17 191

Book

Stanley R G & Linskens H F 1974 Pollen Biology Biochemistry and Management Springer Verlag Berlin .

Edited Book.

Malik CP Kaur B Wadhwani C & Verma A 2007 In Current Topics in Biotechnology (eds Malik CP Kaur B & Wadhwani C) p 101 MD Publications Pvt Ltd New Delhi.

Thesis

Kaur A 2002 M Sc Thesis Punjab Agricultural University Ludhiana .

Table Guidelines

- Tables should be numbered e.g., Table 1, consecutively and titled. <>br• Sources of data need to be given below each table unless otherwise mentioned in the text.
- Each table should contain a short caption explaining the content of the table.
- All tables column should have an explanatory heading

Figure and Artwork Guidelines

- Illustrations should be very clear for photographic reproduction. Line drawings and photographs should be separately grouped. Photographs should be of high quality not less than 600 dpi. A full page figure should not be larger than 6.70 x 9.00 cm..

Accompanying Material

The manuscripts should be accompanied by:

- A note about the author(s) not exceeding 50 words.

Copyright Transfer

Once the manuscript is accepted for publication, the corresponding author will receive an E-mail informing about the acceptance of the article. The publication of an article in the "The Journal of Plant Science Research" means that the author(s) transfer the Copyright of the article to the Journal. The responsibility of statements, whether of fact or opinion, rests entirely with the author(s) thereof.

Announcements about scientific, events life meetings, conferences, workshops, trainings, awards, competitions and information on personal achievements, honors, awards, etc. and obituaries will be published free of cost.

Jour Pl Sci Res **37 (1)** 203-207 2021

Quantification of major bioactive compounds from *Diospyros chloroxylon Roxb.*

Nageswara Rao Naik B[1], Jyothi D[2] and Vishnuvardhan Z[3]

[1] *Department of Environmental sciences, Acharya Nagarjuna University, Guntur, Andhra Pradesh.*

[2] *Department of Botany SRR & CVR Govt. College Vijayawada Andhra Pradesh.*

[3] *Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, Andhra Pradesh.*

Corresponding author e-mail: bnnaikenviro@gmail.com

Diospyros **is an important genus of Ebenaceae family with more than 500 species. These have drawn the attention of investigators by the presence of valuable phytochemicals in them. They have proved their utility in the amelioration of various ailments. The present study constitutes the quantification of major bioactive compounds viz., alkaloids, flavonoids, phenolics, steroids and terpenoids from** ***Diospyros chloroxylon*** **an endemic species. The pharmacological potential of any plant is being determined based on its chemical constituents. The significant quantities of bioactive compounds quantified in** ***Diospyros chloroxylon*** **rates it an useful species having antioxidative and antibacterial potential.**

Keywords: Phytoconstitutents, Phenolic compounds, Flavonoids, Steroids, Antimicrobials.

INTRODUCTION

Plants constitute an important source for secondary metabolites that are responsible for many biological activities (Sofowara 1996). *Diospyros* is the largest genus of Ebenaceae family with more than 500 species distributed across the world. The species of *Diospyros* genus are known for their use in traditional medicinal practices to cure a wide variety of ailments (Rathore *et al.,* 2012). Hence, there is an urgent need to analyse many species phytochemically to identify the major compounds of therapeutic significance. The advancement in chromatographic techniques has led to identification of multitude of bioactive ingredients from this genus (Katiyar *et al.,* 2012). The secondary metabolitic compounds of plants viz., flavonoids and phenolic compounds have been recognized with significant biological activities (Kim *et al.,* 2003). Terpenoids are found to be useful in the prevention and therapy of several diseases (Wagner & Elmadfa 2003). The flavonoids, terpenoids and steroids of many plants were reported to possess antibacterial activity (Feng zhu *et al.,* 2009).

Therefore, the quantification of alkaloids, flavonoids, phenolics, steroids and terpenoids of a plant will reflect on the correct assessment whether it has medicinal potential or not. Thus, the present research study reports the quantity of afore mentioned bioactive compounds in leaf, stem and bark extracts of *Diospyros chloroxylon* aqueous, methanolic and ethyl acetate leaf extracts.

MATERIALS AND METHODS

The stem, bark and leaf of *Diospyros chloroxylon* are collected from its natural habitat (i.e., Kondapalli Reserve forest) in Krishna district (Andhra Pradesh) India. The plant was identified by its Botanical name by the Botanical Survey of India, Deccan Regional Centre Hyderabad. The voucher specimen of the plant is BSI/DRC/2019-20/Tech./173. The herbarium specimens were deposited in the department of Environmental Sciences, Acharya Nagarjuna University, Guntur (A.P).

Preparation of plant extract

Leaves, stem and bark (500g) of *Diospyros chloroxylon* were used for extraction. The air dried leaves, stem and bark were made into a fine powder and taken in to a conical flask. Ethyl acetate methanol and water were used for extraction. The extraction was performed by soxhlation and the extraction was carried out until the extract becomes colourless. The polarity of Ethyl acetate, Methanol and Water is in

DOI:-https://doi.org/10.32381/JPSR.2021.37.01.25

the increasing order. The pooled extract was distilled under reduced pressure into syrup and evaporated in a porcelain basin over a water bath to give a sticky residue. This was kept in a desiccator for a few days to get the dry extract (Yield-500g).

The quantification of alkaloids, steroids, flavonoids, terpenoid and phenolic compounds present in different solvent extracts of *Diospyros chloroxylon* leaf, stem and bark was made with UV-visible spectro-photometry. A double beam UV visible spectrophotometer (Model: TECHOMP-2301) was used. Absorbance against blank at different wave lengths (470, 780, 510 & 750 nm) was recorded to work out the amount of above four groups of compounds in the solvent extracts. HITACHI UV, solutions 2.0 version software is also used.

The standard graphs (calibration curves) were drawn for alkaloids, steroids, flavonoids, terpenoid and phenolic compounds by using atropine, cycloartenol, quercetin and catechol as standards respectively. The total content of compound present in the extract was expressed in milligrams per gram of dry weight.

Total Phenolic Content

The total phenolic content of the extract was determined by the Folin–Ciocalteu method as explained by Shoib & Shaid (2015). Briefly, 1 µL of crude extract was made up to 3 mL with distilled water, mixed thoroughly with 0.5 mL of Folin–Ciocalteu reagent for 3 min, followed by the addition of 2 mL of 20% (w/v) sodium carbonate and mixture was allowed to stand for a further 60 min in the dark. The final volume was made up to 10 ml with distilled water and absorbance was measured at 650 nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent per g dry weight.

Total Flavonoid Content

The total flavonoid content of crude extract was determined by the aluminium chloride colorimetric method (Shoib & Shaid 2015). 1 mL of crude extract was mixed with 4 mL of distilled water and then 0.3 mL of 5% NaNO2 solution; 0.3 mL of 10% $AlCl_3$ solution was added after 5 min of incubation, and the mixture was allowed to stand for 6 min. Then, 2 mL of 1 mol/L NaOH solution was added, and the final volume of the mixture was brought to 10 mL with double-distilled water. The mixture was allowed to stand for 15 min and absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve and the result was expressed as mg Quercetin equivalent per g dry weight.

Total Alkaloid Content

The plant extract 1ml of 2 N HCl was added and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 5 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/g of extract.

Total Terpenoid Content

1g of Plant powder was taken in a conical flask and soaked in ethyl alcohol for one day. Then it was filtered and the filtrate was extracted with petroleum ether. The ether extract was taken as the measure of total terpenoid.

Total terpenoid content =

$$\frac{\text{Final weight of the sample} - \text{Initial weight of the extract}}{\text{Weight of the Sample}} \times 100$$

Total Steroid Content

The extract was re-suspended in 20 ml of chloroform and the volume adjusted to 50 ml with the same solvent. Aliquots were transferred to 10 ml volumetric flasks and 2 ml of LB reagent (50 ml of acetic anhydride and 5 ml of sulfuric acid) was added and was incubate for 5 min. The volume was adjusted to 10 ml with chloroform. The absorptions were measured in a spectrophotometer at a wavelength of 625 nm against blank. A standard calibration curve was constructed using Cholesterol as standard and the amount of steroids present in the samples was calculated using standard calibration curve.

RESULTS

Alkaloids were detected in the aqueous leaf extract of *Diospyros chloroxylon* only. The stem and bark extracts did not test positive for the alkaloids. Therefore, the aqueous leaf extracts were considered for the estimation of alkaloids in which the extraction quantity is 19.6% w/w (table 1). The absorbance of plant extracts was read and corresponding quantity of alkaloids was determined with the help of standard graphs. The absorbance of leaf extract was 0.117 and by which the alkaloids quantity present in leaf aqueous extract was calculated as 1.59 mg/g extract (Table 2).

Table. 1: Extraction quantity of *Diospyros chloroxylon* leaf, stem and bark

S. No.	Plant part	Solvent Name	Extract obtained in %
1	**Leaf**	Ethyl acetate	2.6% w/w
2		Methanol	25.7% w/w
3		Aqueous	19.6% w/w
4	**Stem**	Ethyl acetate	2.1% w/w
5		Methanol	17.3% w/w
6		Aqueous	20.8% w/w
7	**Bark**	Ethyl acetate	1.9% w/w
8		Methanol	18.5% w/w
9		Aqueous	16.2% w/w

Table 2: Quantity of total alkaloids, flavonoids, phenolic compounds, steroids and terpenoids estimated in different solvent extracts of *Diospyros chloroxylon* leaf, stem, and bark

S. No.	Name of the compound	Solvent name	Plant part	Absorbance	Quantity of compounds mg/g extract
1	Alkaloids(mg of AE/g extract)	Aqueous	Leaf	0.117	1.594
2	Flavonoids(mg of Q/g ectract)	Methanol	Leaf	0.517	15.266
		Aqueous		0.934	29.166
		Methanol	Stem	0.618	18.633
		Methanol	Bark	0.328	8.966
3	Phenolic compounds(mg of GA/g extract)	Methanol	Leaf	1.425	37.837
		Aqueous		0.343	8.594
		Methanol	Stem	0.586	15.162
		Methanol	Bark	0.279	6.864
		Aqueous		0.931	24.486
4	Steroids(mg of CH/g extract)	Ethyl acetate	Leaf	0.116	1.340
		Methanol		0.379	7.318
		Aqueous		0.208	3.431
		Ethyl acetate	Bark	0.295	5.409
5	Terpenoids(mg/g)		Leaf	-	9.25
		Stem	-	4.18	
		Bark	-	5.67	

The flavonoids were tested positive preliminarily in the leaf, stem and bark with methanol solvent. They were also detected in aqueous leaf extract of *Diospyros chloroxylon*. Therefore, the quantification of flavonoids was carried out in the leaf aqueous extracts and methanolic leaf, stem and bark extracts of *Diospyros chloroxylon* (Table 2). Aqueous leaf extract was found to contain the maximum amount of flavonoids (29.17 mg/g), followed by methanolic stem extract (18.63 mg/g) and bark extract (8.97 mg/g). The flavonoids content was the highest in leaf when compared to stem and bark. Hence, in terms of flavonoid content the plant parts are in the order of leaf > stem > bark in *Diospyros chloroxylon*.

The extraction quantity of phenolic compounds in leaf was the highest in methonolic solvent (25.7% w/w), followed by aqueous extract (19.6% w/w). It was only 2.6% w/w for ethyl acetate solvent. Therefore, the estimation of total phenolic compounds present in the leaf of *Diospyros chloroxylon* was only carried with methanolic and aqueous extracts only. The bark methanolic extracts of bark and stem were also evaluated for phenolic compounds quantification (Table 2). Out of the above extracts screened, methanolic leaf extracts contained the maximum quantity of phenolic compounds (37.84 mg/g) followed by aqueous bark extracts (24.49 mg/l) and methanolic stem extracts (15.16 mg/g).

The highest quantity of steroids (7.32 mg/g) was recorded in methanolic leaf extracts followed by aqueous leaf extracts (3.43 mg/g). The bark of *Diospyros chloroxylon* contained 5.41 mg/g steroids and the minimum quantity of steroids was observed (1.34 mg/g) in ethyl acetate leaf (Table 2). Leaf was found with the greater quantity (9.25 mg/g) of terpenoids, followed by bark (5.67 mg/g) and stem (4.18 mg/g).

DISCUSSION

The secondary metabolites such as flavonoids and phenolic compounds constitute the two most pervasive groups from plants and are recognized with significant biological activities (Kim *et al.,* 2003). Flavonoids and phenolic acids are strong antioxidants capable of scavenging free radicals, anti-aging, anti-cancer activity and enhance human immunity (Atoui *et al.,* 2005). Phenolics along with flavonoids exhibit anti-cancer (Matsuda *et al.,* 2003), anti-inflammatoty (Araujo & Leon 2001), antioxidant (Ghasemzadeh & Ghasemzadeh 2011), cytotoxic and antitumor (Murakami *et al.,* 2004), antispasmodic (Ammon & Wahl 1991) and antidepressant activities (Yu *et al.,* 2002). Analgesic and antibacterial activities of steroids were reported by Sayyah *et al.,* (2004) and Malairajam *et al.,* (2006). Terpenoids were found to be very useful in the prevention and therapy of several diseases (Wagner & Elmadfa 2003). Terpenoids also showed antimicrobial, anti-hyperglycemic and antiviral activity (Rabi & Bishayee 2009). Van Vuuren *et al.,* (2007) commented that the plant extracts are often effective as antibacterial agents because of the compounds of extract interact additively or synergistically to bring out effective activity. Therefore, the quantification of such secondary metabolites from plant extracts would provide the correct assessment whether the plant has medicinal potential or not. In the present study, the quantification of various secondary metabolites viz., alkaloids, flavonoids, phenolic compounds, steroids and terpenoids in leaf, stem and bark of *Diospyros chloroxylon* were estimated. The alkaloids were present in aqueous leaf extracts of *Diospyros chloroxylon* (1.59 mg/g) where as in other solvent extracts alkaloids did not test positive. Flavonoids were also found maximum in aqueous leaf extracts (29.166 mg/g) followed by methanolic extracts of stem (18.63 mg/g) and bark (8.966 mg/g). Similarly phenolic compounds were also the highest in methanolic leaf (37.837 mg/g) followed by stem and bark. The steroids (7.32 mg/g) and terpenoids (9.25 mg/g) were also found in maximum quantity in methanolic leaf extract. The presence of alkaloids, flavonoids, phenolic compounds, steroids and terpenoids in *Diospyros chloroxylon* will assert its profound pharmacological importance together with other prominent *Diospyros* species.

References

Ammon HPT and Wahl MA 1991. Pharmacology of *Curcuma longa*. Planta Med. 57: 1-7.

Araujo CC and Leon LL 2001. Biological activities of Curcuma longa L. Mem. Inst. Oswaldo Cruz 96: 723-728

Atoui AK Mansouri A Boskou G and Kefalas P 2005. Tea and herbal infusions: their antioxidant activity and phenolic profile. *J. Food Chemistry* 89 : 27-36.

Feng Zhu Xinchen Yihua Yuan Meizhem Huan Huilisyn and Wenzhour xiang 2009. The chemical investigations of the mangrove plant *Avicennia marina* and its endophytes. *J. open natural products* 2: 24-32.

Ghasemzadeh A and Ghasemzadeh N 2011. Flavonoids and phenolic acids: role and biochemical activity in plants and human. *J. Med. Plant Res.* 531: 6697–6703.

Katiyar C Gupta A Kanjilal S and Katiyar S 2012. Drug discovery from plants sources an integrated approach. *J. Ayu.* 33: 10-19.

Kim D Jeond S and Lee C 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *J. Food Chem.* 81: 321-326.

Malairajan P Geetha G Narasimhan S Jessi KVR 2006. Analgesic activity of some Ind. Med. plants. *J. Ethnopharmacol.* 106 : 425-428.

Matsuda H Pongpiriyadacha Y Morikawa T Ochi M Yoshikawa M 2003. Gastroprotective effects of phenylpropanoids from the rhizomes of Alpinia galanga in rats: structural requirements and mode of action. *Eur. J. Pharm.* 471: 59-67.

Murakami A Tanaka T Lee JY Surh YJ Kim HW Kawabata K Nakamura Y Jiwajinda S Ohigashi H (2004). Zerumbone a sesquiterpene in subtropical ginger suppresses skin tumor initiation and promotion stages in ICR mice. *Int. J. Can.* 110: 481-490.

Rabi T and Bishayee A 2009. Terpenoids and breast cancer chemoprevention. *J. Breast Cancer Res.* Treat. 115: 223-239.

Rathore SK Bhatts S Dhyani S and Jain A 2012. Preliminary phytochemical screening of medicinal plant *Zizipus mauritiana Lam*. Fruits. *Int. J. Curr. Pharmaceutical Research* 4:160-162.

Sayyah M Hadidi N Kamalinejad M 2004. Analgesic and antiinflammatory activity of Lactuca sativa seed extract in rats. *J. Ethnopharmacol.* 92: 325-329.

Shoib Baba A and Shahid Malik A 2015. Determination of total phenolic and flavonoid content antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume *J. Taibah University for Science* 9 (4) : 449-454.

Sofowara AE 1996. Research on medicinal plants and traditional medicine in Africa. *J. Alt. Compl. Med.* 2 (3): 365-372.

Van Vuuren SF Sulimans S Viljoen AM 2007. The antimicrobial activity of four commercial essential oils in combination with conventional antimicrobial. *Lett. Appl. Microbial.* 48: 440-446.

Wagner KH and Elmadfa I 2003. Biological relevance of terpenoids. Overview of focusing on mono- di- and tetraterpenes. *J. Ann. Nutr. Metab.* 47: 95-106.

Yu ZF Kong LD Chen Y 2002. Antidepressant activity of aqueous extracts of Curcuma longa in mice. *J. Ethnoph.* 83: 161-165.

Received: 07-12-2020

Accepted: 06-01-2021