ISSN: 0976-3031

*Available Online at http://www.recentscientific.com*

**CODEN: IJRSFP (USA)**

*International Journal of Recent Scientific Research*
*Vol. 9, Issue, 1(F), pp. 23249-23252, January, 2018*

**International Journal of Recent Scientific Research**

**DOI: 10.24327/IJRSR**

**Research Article**

# QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ESTIMATION OF LEAVES EXTRACTS OF PLANT *PLUMBAGO ZEYLANICA*

**Anjali Jijhotiya., Madhuri and Sadhna Goyal**

Department of Chemistry, Motilal Vigyan Mahavidhyalay Bhopal M.P

**DOI: http://dx.doi.org/10.24327/ijrsr.2018.0901.1429**

**ARTICLE INFO**

***Article History:***

Received 17th October, 2017
Received in revised form 21st November, 2017
Accepted 05th December, 2017
Published online 28th January, 2018

***Key Words:***

Phytochemicals, secondary metabolites, flavonoids, phenolic, saponin content.

**ABSTRACT**

Plants are being widely used either as single drug or in combination in health care. The Indian herbs are broadly utilized as source of drugs mentioned in the traditional systems of medicine. *Plumbago zeylanica* Linn. (Plumbaginaceae) is one of the well-known herbal plant. It is commonly known as chitramula and chitrack. This is perennial herb grow in shady places in the garden and found in Sri Lanka (Ceylon) and parts India, which include Bengal, Uttar Pradesh, and Southern India. The objective of the present study is to evaluate scientific data for presence of various phytochemicals in the leaves extract of methanol, aqueous and petroleum benzene. The three different extracts of leaves were found to contain triterpenoids, flavonoids, phenolic tannins, saponins & carbohydrate. The total flavonoid content was 213.3±0.577 and 177.3±0.577 in methanolic and aqueous extract, The total phenolic content in the methanol and aqueous extract was found to be 358.1.±1.963 and 174.8±0.2, saponin content in the methanolic and aqueous extract was found to be 326.5±0.173 and 134.8±0.230 in mg/g. Presence of such secondary metabolites makes this plant useful for mankind. In future we can prepare herbal drug from the leaves of this plant.



## INTRODUCTION

The role of plant is very useful for life of mankind. There is a mutual relation among plants and human beings. Plants have been used as medicine since long time. Plants contain many chemical components which are useful in biological functions. These chemical components work on the human body in exactly the same way as pharmaceutical drugs. Secondary metabolites which are produced by plants play an important role to cure many diseases.

*Plumbego zeylanica* is from plumbaginaceae family.It is an important herb which is revealed in charak samhita [Vishnukanta Rana AC 2010]. *Plumbego zeylanica* also recognized as white leadwort and its trade name is chitrak. It has been used in Indian and Chinese medicinal system for more than 3000 years also used by African healers. The family plumbaginaceae consist of 10 genera and 280 species. Its genus consists 3 different species, namely *P.indica*, *P.capensis* and *P.zeylanica* which are distributed in several parts of India, as wild or in cultivation due to its more therapeutic uses [Chetty, K. M. 2006]. *Plumbago zeylanica* is well known plant in India. The flowers of this plant reminiscent with jasmine. *Plumbago zeylanica* is semi-climbing, sub-scandant shrub of warm temperature tropical and subtropical region of the world [Nadkarni KM 1982] and expand in various part of India especially in Bengal, Uttar Pradesh, Andhra Pradesh and Sri Lanka [Kirtikar KR and Basu BD 2000].

Leaves show anti-inflammatory, antinociceptive [Sheeja E, *et.al*., 2010], larvicidal activity [Maniafu BM *et al.,* 2009]. Stem show inhibition of immediate allergic reactions [Yue D *et al.,* 2004]. Plumbagin, naphthoquinone which is obtained from *Plumbago zeylanica* root inhibits cell growth and potentiates in human gastric cancer cell [Li J. *et al.*, 2012] inhibits ultraviolet radiation induced development of squamous cell carcinomas [Sand JM *et al.,* 2012] and genotoxic damage induced by potassium canrenoate in culture human peripheral blood lymphocytes [Siddique YH, *et al.,* 2012]. Root also shows anti-fertility activity [Sandeep G *et al.,* 2011]. Neo and 1-epineo-isoshinanolones which is isolated from roots of *Plumbego zeylanica* shows antimicrobial activity [Jetty A *et al.,* 2010]. Based on the above study was design to evaluate the phytochemicals presence in this plant also the quantity of flavonoids, phenolic and saponins present in the plant.

**Corresponding author:* **Anjali Jijhotiya**
Department of Chemistry, Motilal Vigyan Mahavidhyalay Bhopal M.P

**Fig 1**

**Fig 2**

## MATERIALS AND METHODS

### *Collection of Plant Material & authentication*

Leaves of plant collected from the Sanjivani Bhopal. Dr. Zia Ul Hasan Professor, Department of Botany, Safia College of Art & Science, Bhopal, identified and authenticated this plant.

### *Preparation of Plant Extracts*

The leaves of *plumbego zeylanica* was collected and air dried at room temperature. The leaves were crushed to make fine powdered. This power of leaves was subjected to successive extraction by maceration process and different extracts from non-polar to polar solvents such as petroleum benzine, methanol and water used. Each filtrate was concentrated and dried at 40$^{n}$C. These crude extracts then weighed and kept in refrigerator for further phytochemical investigation.

### *Phytochemical analysis of different Crude extracts*

### *Qualitative analysis*

Extracts were tested to identify presence or absence of phytochemicals such as Triterpenoids, Steroids, Saponins, Alkaloids, Flavonoids, Tannins, Proteins, glycosides and Carbohydrates by using the standard procedures [Kokate C.K. *et al.,* 2006].

### *Quantitative analysis*

### *Estimation of Flavonoids*

Total flavonoids were measured by a colorimetric assay. An aliquot of diluted sample was added to a 75 µl of 5% $NaNO_2$ solution, and mixed for 6 min, before adding 0.15 mL 10 % $AlCl_3$ (100 g/L). After 5 min, 0.5 mL of 4% NaOH was added. The final volume was adjusted to 2.5 ml with distilled water and thoroughly mixed. Absorbance of the mixture was taken at 510 nm against the same mixture, without the sample, as a blank. Total flavonoid content was expressed as mg rutin/g dry weight (mg rutine/g DW), through the calibration curve of Rutin. All samples were analysed in three replications [Zou Y.P. *et al.,* 2004], [ Zengin H.W. *et al.*, 2011].

### *Estimation of phenolic compound*

The amount of total phenolic in extracts was determined with the Folin Ciocalteu reagent. Gallic acid was used as a standard and the total phenolic were expressed as mg/g gallic acid equivalent (GAE). Concentration of 0.1and 1mg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced in to test and mixed with 2.5ml of a 10 fold dilute folin Ciocalteu reagent and 2ml of 7.5% sodium carbonate and allowed to stand for 30 minutes at room temperature before the absorbance was taken at 760 nm spectrometrically. All determination was performed in triplicate. The folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols. It produce a blue colour upon reaction. This blue colour was measured spectrophotometrically [Maurya S. and Singh D. 2010].

### *Estimation of saponin*

Standard saponin solution prepared by dissolving10 mg of diosgenin add (16 mL) methanol and distilled water (4 mL). To the aliquots for each tube, vanillin reagent (8%, 0.25 mL) was added and sulphuric acid (72% v/v, 2.5 mL) added slowly on the inner side of the wall. The solutions were mixed well and the tubes were transferred to a 60 $^{0}$C water bath. After 10 mins incubation, the tubes were cool in ice cold water bath for 3 – 4 mins. The absorbance was measured at 544 nm against the reagent blank. 0.1 g of freeze dried sample was dissolved in aqueous methanol (80%, 0.1 mL). 0.25 mL of aliquot was taken for spectrophotometric determination for total saponins at 544 nm [Sim E.E. W.E.I. 2011].

## RESULTS

The present study was carried out on the *plumbego zeylanica* revealed that the presence of active phytochemical constituents. The bioactive components *of plumbego zeylanica* were qualitative and quantitatively analysed from different extracts of leaves and the results are mentioned in Table 1 and Table 2 respectively.

**Table 1**

| Chemical Test | Pet. ether | Aqueous | Methanolic |
|---|---|---|---|
| **1.Test for Triterpenoids & Steroids:** | | | |
| Liebermann Burchard Test | + | _ | + |
| Salkowski test | + | _ | + |
| **2.Test for Glycosides** | | | |
| Keller Killiani Test | _ | _ | _ |
| Legal test | _ | + | + |
| **3.Test for Saponins** | | | |
| Foam test | _ | + | + |
| **4.Test for Alkaloids** | | | |
| Hager's Test | _ | _ | + |
| wagner Test | _ | + | + |
| Mayer Test | _ | _ | _ |
| **5.Test for Flavanoids** | | | |
| Lead acetate test | + | + | + |
| Alkaline reagent test | _ | + | + |

| | | | |
|---|---|---|---|
| Shinoda test | – | – | – |
| **6.Test for Tannins and phenolic** | | | |
| Ferric chloride test | + | + | + |
| Lead acetate test | – | – | – |
| Dil. $I_2$ test | – | – | – |
| **7.Test for Proteins** | | | |
| Biuret test | – | – | – |
| **8.Test for Free amino acids** | | | |
| Ninhydrin Test | – | – | + |
| **9.Test for Carbohydrates** | | | |
| Molish test | | + | |
| Benedict test | – | + | + |
| Barfoed | – | – | – |

The quantitative estimation of primary metabolites revealed that the various phytochemical constituents present in the plant extract (Table-2).

**Table 2**

| S.No. | Phytochemicals | Methanol extract | Aqueous extract |
|---|---|---|---|
| 1 | Total Flavonoid content in mg/g equivalent of rutin | 213.3±0.577 | 177.3±0.577 |
| 2 | Total phenolic content in mg/g equivalent of galic acid | 358.1.±1.963 | 174.8±0.2 |
| 3 | Total saponin content in mg/g equivalent of Diosgenin | 326.5±0.173 | 134.8±0.230 |

Values are expressed by mean±SD of three readings, SD: Standard deviation,

***Standard curve of rutin***

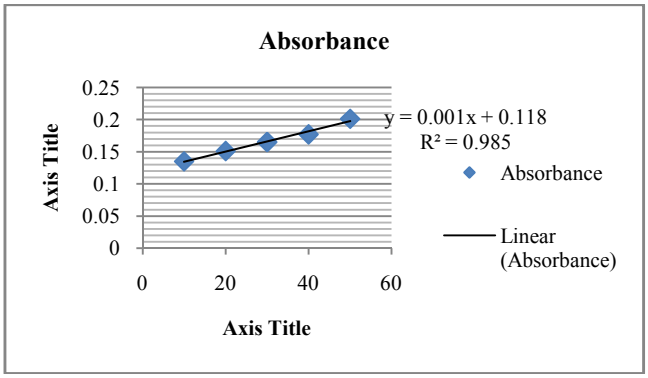


**Fig 3**

***Standard curve of gallic acid***

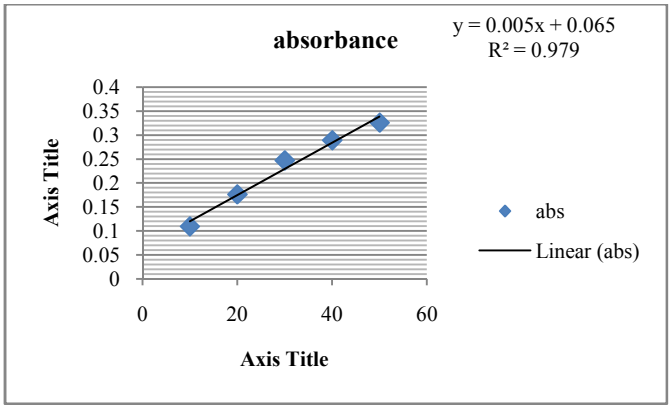


**Fig 4**

***Standard curve of diosgenin***

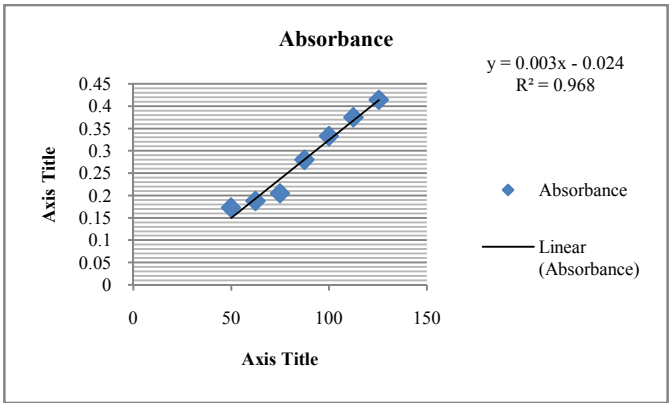


**Fig 5**

## DISCUSSION

Flavonoids have been reported to contains wide range of biological activities. The total flavonoid content was determined using aluminum tri chloride reagent. Rutin used as a standard compound and the total flavonoid content was expressed as mg/g rutin equivalent using the standard curve equation: $y = 0.001x + 0.118$, $R^2 = 0.985$ (Figure 3); where y is absorbance at 510 nm and x is total flavonoid content in the extracts of *Plumbego zeylanica* leaves expressed in mg/g. The total flavonoid content was 213.3±0.577 and 177.3±0.577 in mg/g equivalent of rutin in the methanolic and aqueous extract, respectively (Table 2). The higher amount of phenolic component is important in the regulation of plant growth, development and disease resistance. Consumption of diets rich in plant polyphenols offers protection against the development of many diseases.

Folin-Ciocalteu reagent used for determining total phenolic content. Gallic acid standard curve and equation: $y = 0.005x + 0.065$, $R^2 = 0.979$(Figure 4); where y is absorbance at 760 nm and x is total phenolic content in the extracts of *plumbego zeylanica* expressed in mg/g equivalent of Gallic acid used for determination. The total phenolic content in the methanol and aqueous extract of *Plumbego zeylanica leaves* was found to be 358.1.±1.963 and 174.8±0.2 in mg/g equivalent of Gallic acid (Table 2).

The saponins are used in hypercholestrolaemia, hyperglycemia, antioxidant, anticancer, anti inflammatory activity and weight loss [Manickam M. and Veerabahu R.M. 2014]. The total saponin content was determined using vanillin reagent. Diosgenin was used as a standard compound and the total saponin content were expressed as mg/g diosgenin equivalent using the standard curve equation: $y = 0.003x - 0.024$, $R^2 = 0.968$(Figure 5); where y is absorbance at 544 nm and x is total saponin content in the extracts of *Plumbego zeylanica* leaves expressed in mg/g. The total saponin content in the methanolic and aqueous extract of *Plumbego zeylanica* leaves was found to be 326.5±0.173 and 134.8±0.230 in mg/g equivalent of diosgenin (Table 2).

Methanolic extract of leaves showed highest amount of total phenolic content (358.1.±1.963 mg/g equivalent of Gallic acid) and total flavonoid content (213.3±0.577 mg/g equivalent of rutin) total saponin content (326.5±0.173 mg/g equivalent of diosgenin). The presence of such important secondary

metabolites in *Plumbego zeylanica leaves* indicates its therapeutic importance in men and animals.

## CONCLUSION

The presence of phytoconstituents make the plant useful for treating different malady and have a potential of providing useful drugs of human use. In the present study, we have found that methanolic and aqueous extracts of leaves of *Plumbego zeylanica* consist high amount of flavonoid, phenolic and saponins it can be considered beneficial for further investigation. These phytoconstituents can act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital role for good health.

**Aknowledment**

The authors are grateful to Pinnacle Biomedical Research Institute Bhopal for proceeding this work in their lab.

## References

1. Vishnukanta Rana AC. [2010]. "Evaluation of anticonvulasant activity of *Plumbago zeylanica* Linn leaf extract." *Asian Journal of Pharmaceutical and Clinical Research* 3(1): 76-78.
2. Chetty, K. M. (2006). "Pharmaceutical Studies and Therapeutic Uses of Plumbago Zeylanica L. Roots (Chitraka, Chitramulamu)." *Ethnobotanical Leaflets*, 10, 294-304.
3. Nadkarni KM [1982]. "Indian Materia Medica." Edn. 3, Vol. I, Popular Prakashan, Bombay. 990-991.
4. Kirtikar KR, Basu BD [2000]." Indian Medicinal Plants." Vol.VII,Sri Satguru Publications, New Delhi; 1466-1468.
5. Sheeja E, Joshi SB, Jain DC. [2010]. "Bioassay guided isolation of anti-inflammatory and antinociceptive compound from Plumbago zeylanica leaf." *Pharm Biol*; 48(4):381-387.
6. Maniafu BM, Wilber L, Ndiege JO, Wanjala CC, Akenga TA [2009]. "Larvicidal activity of extracts from three Plumbago spp against Anopheles gambiae." *Mem Inst Oswaldo Cruz;* 104(6): 813-817.
7. Yue D, Li-Fei H, Yiu-Pong C, Ling C, Paul PHB [2004]. "Inhibition of immediate allergic reaction by ethanol extract from Plumbago zeylanica stems." *Biol Pharm Bull*; 27(3):429-432.
8. Li J, Shen L, Lu FR, Qin Y, Chen R, Li Y, Zhan HZ, He YQ [2012]. "Plumbagin inhibits cells growth and potentiates apoptosis in human gastric cancer cells in vitro through th NF-kB signalling pathway." *Acta Pharmacol Sin*; 33(2); 242-249.
9. Sand JM, Bin HB, Jamal MS, Witkowsky O, Siebers EM, Fisher J, Verma AK. [2012] "Plumbagin ( 5-hydroxy-2-methyl-1,4- napthoquinone), isolated from *Plumbago zeylanica,* inhibits ultraviolet radiation-induced development of squamous cell carcinomas." *Carcinogenesis*. 2012 Jan; 33(1): 184–190.
10. Siddique YH, Ara G, Beg T, Afzal M, Faisal M. [2012]. "Antigenotoxic effect of the *Plumbago zeylanica* extract against the genotoxic damage induced by poatassium canrenoate in cultured peripheral blood lymphocytes." *DrugChem Toxicol*; 35(2): 119-126.
11. Sandeep G, Dheeraj A, Sharma NK, Jhad D, Bharti A. [2011]. "Effect of Plumbagin free alcohol extract of Plumbago zeylanica Linn root on reproductive system of female Wistar rats." *Asian Pac J Trop Med*; 4(12): 978-984.
12. Jetty A, Subhakar C, Rajagopal D, Jetty M, Subramanyam M, Marthanda MM. [2010]. "Antimicrobial activity of neo- and 1-epineo-isoshinanolones from *Plumbago zeylanica* roots." *Pharm Biol*; 48(9): 1007-911.
13. Kokate C.K., Purohit A.P., and Gokhale S.B. [2006]. "Pharmacognosy" 23 ed., Nirali Prakashan: 493-497.
14. Zou Y.P., Lu Y.H. and Wei D.Z. [2004]. "Antioxidant Activity of a Flavonoid-rich Extract of *Hypericum perforatum*. *in vitro*" J. Sci. Food Agr.; Vol. 52: 5032–5039.
15. Zengin H.W., Aktumsek A., Guler G.O., Cakmak Y.S., and Yildiztugay E. [2011] **"**Antioxidant properties of methanolic extract and fatty acid composition of centaurea urvillei dc. Subsp., rec." *Nat. Prod*.; Vol. 5(2): 123-132.
16. Maurya S. and Singh D. [2010]. "Quantitative Analysis of Total Phenolic Content in Adhatoda Vasica Nees Extracts." *International Journal of Pharmtech Research*; Vol. 4: 2403-2406.
17. Sim EE W.E.I, [2011]. "Isolation and determination of anti-nutritional compounds from root and shells of peanut (*Arachis Hypogaea*)." A project report of Department of Chemical Science Faculty of Science Universiti Tunku Abdul Rahman: 34-35.
18. Manickam M., Veerabahu R.M. [2014]. " Phytochemical, FT-IR and antibacterial activity of whole plant extract of *Aerva lanata* (L.) Juss. Ex. Schult." *J. of Med. Plants Studies*, 2(3): 51-57.

**How to cite this article:**

Anjali Jijhotiya., Madhuri and Sadhna Goyal.2018, Qualitative And Quantitative Phytochemical Estimation of Leaves Extracts of Plant Plumbago Zeylanica. *Int J Recent Sci Res.* 9(1), pp. 23249-23252. DOI: http://dx.doi.org/10.24327/ijrsr.2018.0901.1429

*******