

**PRELIMINARY PHYTOCHEMICAL SCREENING AND HPLC  
ANALYSIS OF BARK AND LEAF EXTRACT OF *DOLICHANDRONE*  
*ATROVIRENS***

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**ABSTRACT**

The present paper deals with the phytochemical screening of bark and leaf extract of *Dolichandrone atrovirens* an important medicinal plant used in the Indian traditional system of medicine. This study involves the preliminary screening of carbohydrates, glycosides, protein, aminoacids, tannins, phenol, terpenoids, flavonoids, saponins, fixed oil and fats. *Dolichandrone atrovirens* leaf and bark extracts are further analyzed by HPLC using Shimadzu Class-VP V6.14 SP2 system. The preliminary screening test results in the detection of bioactive principles and HPLC analysis conform the phytochemical active ingredients like gallic acid, rutin, quercetin, and ferulic acids are present in the leaf and bark extracts of *Dolichandrone atrovirens*. This analytical technique used to find the pharmacologically active

constituents present in the aqueous methanolic bark and leaf extract of *Dolichandrone atrovirens*.

**KEYWORDS:** Aqueous methanolic, HPLC, bark and leaf extract, *Dolichandrone atrovirens*.

**INTRODUCTION**

Minority people only used the modern medical service in developing countries.<sup>[1]</sup> World Health Organization (WHO) estimated that 80% or more of the population living in rural areas are regularly use traditional medicine.<sup>[2, 3]</sup> China used 30-50 % herbal medicine to treat people. Through literature thousands of phytochemicals from plants are broadly effective and no or less adverse effect. Many plant materials are biological activities as antidiabetic

antimicrobial, antioxidant, anti-inflammatory, anticancer, analgesic and wound healing activity were reported. Throughout the world many people claim the good benefit of certain natural or herbal products.<sup>[4,5]</sup> Only a small proportion of plant has been investigated both phytochemically and pharmacologically. Even a single plant may contain up to thousands of constituents, the possibilities of making new discoveries become clear.<sup>[6]</sup>

The pharmacologically active chemical substances like phenolic compounds, flavonoids, resins, glycoside, and Tannins are increase the medical importance of the plants. Large number of plant species potentially available for study but the important factor for the ultimate success of an investigation into bioactive plant constituents is thus the selection of plant material. It is a common practice in isolation of these bioactive compounds that a number of different separation techniques such as column chromatography, Thin-Layer Chromatography (TLC), flash chromatography and High-Performance Liquid Chromatography (HPLC) used to obtain pure compounds.<sup>[7]</sup>

HPLC is a technique for isolate natural products and is used in phytochemical and analytical chemistry to identify, quantify and purify the individual components of the mixture.<sup>[8, 9]</sup> Considering all these facts, presents investigation is designed to find out phytochemical investigation of *Dolichandrone atrovirens* leaf and bark which evokes antioxidant and antidiabetic activity.<sup>[10, 11]</sup>

## MATERIALS AND METHODS

### Plant materials

*Dolichandrone atrovirens* form as a moderate tree and the leaves pinnate, opposite and obscurely toothed. Flower is white with terminal corymbs or panicles. Seeds are rectangular with broad wings on each side. The barks and leaves of *Dolichandrone atrovirens* were collected from Chitheri hills, Salem in the November 2009 and used for the current study.

### Preparation of extract

The shade dried coarse powders of the plant material (1.5 kg) are extracted with 80 %v/v aqueous methanol by maceration at room temperature for 72 h and the extract filtered, concentrated to dryness in a rotavapor under reduced pressure and controlled temperature (40-50 °C). The extractive values and nature of the leaf and bark extracts of *Dolichandrone atrovirens* administered orally to the experiments.

**Test for Carbohydrates**

Molisch's test: The extract mixed with a small amount of Molisch's reagent ( $\alpha$ -naphthol dissolved in ethanol) in a test tube and add small amount of concentrated sulfuric acid in the sides of the sloping test-tube to form a layer. Appearance of a purple ring at the user interface between the two layers indicates the carbohydrate presence.

Fehling's test: The extract taken in the test tube and add equal volumes of Fehling A & Fehling B and place it in a boiling water bath for few minutes and see change in color. The brick-red precipitate indicates carbohydrate presence.

**Test for Glycosides**

Add 1 ml of the extract solution to 1 ml of dilute Sulphuric acid. Boil, filter and shake with equal volume of dichloromethane or chloroform. Separate the lower layer of dichloromethane or chloroform and shake it with half of its volume of dilute ammonia. Formation of rose-pink to red color of the ammonical layer shows the presence of glycosides and this test is known as Borntrager's test. In some cases ferric chloride used this test may call modified bontrager's test.

**Test for Proteins and Amino Acids**

Ninhydrin Test: Add two drops of 0.2% ninhydrin reagent (0.1% solution in n-butanol) to the small quantity of extract solution and heat. The presence of proteins or amino acids produce purple color.

Biuret Test: In the plant extract add equal amount of 5% sodium hydroxide solution and 2 drops of 1% CuSO<sub>4</sub> solution till a blue color is produced. Formation of pinkish or purple violet color indicates the presence of proteins.

**Test for Tannins and Phenolic Compounds**

Braemer's test: Add 1ml of the plant extract to ferric chloride solution. The formation of a dark blue or greenish black color product shows the presence of tannins.

**Test for Terpenoids**

Add 0.5 gm of plant extract in to 2 ml of chloroform and 3 ml concentrated sulphuric acid to form a layer. A reddish brown color of the user interface indicates the presence of terpenoids.

**Test for Flavonoids**

Shinoda's Test: Add the alcoholic plant extract with piece of magnesium and concentrated hydrochloric acid give intense cherry red colour indicates the presence of flavonones or orange-red color indicates the presence of flavonols.

**Test for Saponins**

Foam test/ Frothing test: Add small quantity of extract to 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1cm layer of foam indicates the presence of saponins.

**Test for Fixed Oils and Fats**

Add small quantity of plant extract to alcoholic potassium hydroxide (0.5 N) along with a drop of phenolphthalein. Heat the mixture on a water bath for 1-2 hours and formation of soap indicates the presence of fixed oils and fats.

**Test for Alkaloids**

Mayer's reagent producing cream precipitate, Dragendroff's reagent producing orange brown precipitate, Hager's reagent producing yellow precipitate and Wagner's reagent producing reddish-brown precipitate while adding into the plant extracts containing alkaloids.

**Test for Phytosterols**

Salkowski test: Add Small quantities of plant extracts in to 5 ml of chloroform and few drops of concentrated sulphuric acid were added. Brown color shows the presence of phytosterols.

**Test for Gums and Mucilage**

Add the plant extract to 25ml of absolute alcohol with constant stirring. Filter the precipitate and dry in air. Examine the precipitate for its have swelling properties indicates the presence of gum and mucilage.

**Study of leaf and bark extract of *Dolichandrone atrovirens* by HPLC**

The HPLC analysis performed using Shimadzu Class-VP V6.14 SP2 system consisting of an auto-sampler and using C18 reverse phase column (4.6 mm X 250 mm) packed with 5 µm diameter (temperature keep up at 40 °C with) with binary gradient dual pump (0.00 kgf/cm<sup>2</sup> – 400.0 kgf/cm<sup>2</sup>- flow rate of 1.00 ml/min). Gallic acid obtained from Loba Chemie (Mumbai, India). Ferulic acid, caffeic acid, rutin and quercetin were purchased from Sigma (MO, USA). HPLC grade solvents obtained from Sigma-Aldrich Chemicals (Dombivil,

India). The standard stock solutions filtered and injected by autosampler. The dried sample extracts dissolved in 100 ml of mobile phase and filtered then extract subjected to HPLC. The plant extract eluted in HPLC using a binary gradient.

## RESULT AND DISCUSSION

The results of phytochemical screening shown in Table: 1 which reveals carbohydrates, glycosides, proteins, amino acids, tannins, phenolics, terpenoids, flavonoids and saponins are present in aqueous methanolic bark and leaf extract of *Dolichandrone atrovirens*. Thus, the preliminary screening test useful to detect bioactive principles and named on these results, HPLC analysis employed to define for the content of the methanolic bark and leaf extract of *Dolichandrone atrovirens*.

**Table: 1 Preliminary Phytochemical screening aqueous methanolic bark and leaf extract of *Dolichandrone atrovirens*.**

Phytochemical constituents	Leaf Extract	Bark Extract
Carbohydrates	+	+
Glycosides	+	+
Proteins & Aminoacids	+	+
Tannins & Phenolics	+	+
Terpenoids	+	+
Flavonoids	+	+
Saponins	+	+
Fixed oils & Fats	+	-
Alkaloids	-	-
Phytosterols	-	-
Gums & Mucilages	-	-

The results of HPLC analysis of bark extract (Table: 2) showed that the gallic acid<sup>[12-17]</sup> was found to be major compound. Rutin<sup>[18-20]</sup>, Quercetin<sup>[21-24]</sup>, and ferulic acid<sup>[25, 26]</sup> were also found in lesser amount. In other leaf extract (Table: 3) contains rutin, quercetin, ferulic acid and gallic acid. Among these compounds, gallic acid was found to be a major compound.

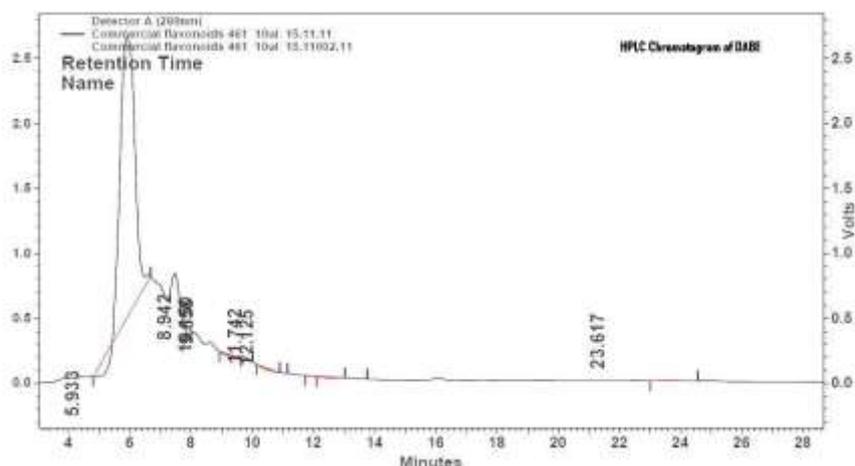


Fig: 1 *Dolichandrone atrovirens* bark extract (HPLC chromatogram)

Table: 2 Compounds Identified by HPLC from *Dolichandrone atrovirens* bark extract

Retention Time	Area	Height	Name
5.933	68405677	2159318	Gallic acid
9.658	867322	16726	Caffeic acid
10.150	388130	0	Rutin
12.125	292179	4369	Quercetin
23.617	226261	4474	Ferulic acid

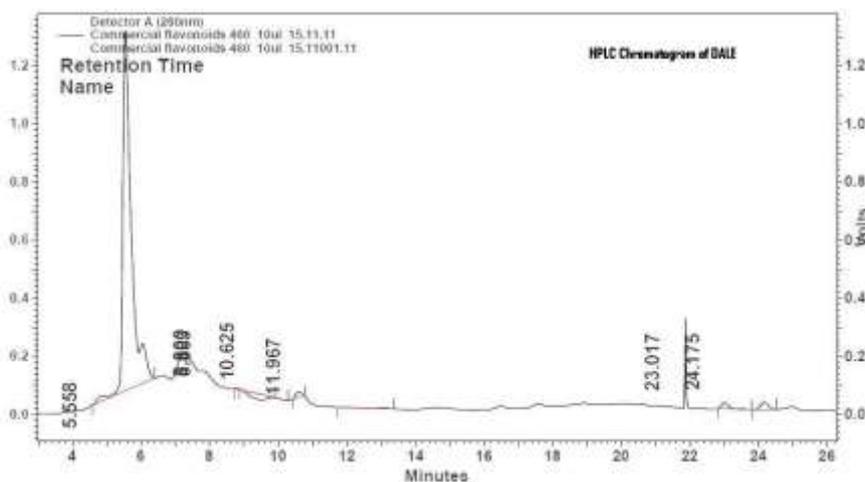


Fig: 2 *Dolichandrone atrovirens* leaf extract (HPLC chromatogram)

Table: 3 Compounds Identified by HPLC from *Dolichandrone atrovirens* leaf extract

Retention Time	Area	Height	Name
5.558	21427784	1240133	Gallic acid
10.625	241335	18919	Rutin
11.967	87500	1161	Quercetin
24.175	395578	24090	Ferulic acid

## CONCLUSION

Herbs are commonly used in developing county and Western County through traditional and western medicine.<sup>[27]</sup> Hence it is essential to analyze the phytochemicals constituents present in the plant through a various analytical technique. The HPLC chromatograph will help to find the presence of important medical constituents in the aqueous methanolic bark and leaf extract of *Dolichandrone atroviren*. The study concluded that the plant has the antidiabetic and antioxidant property may due to the above mentioned phytochemicals.

## REFERENCES

1. Bodeker G. Lessons on integration from the developing world's experience. *BMJ.*, 2001; 322: 164–7.
2. Bannerman RH. *Traditional Medicine and Healthcare Coverage*. Geneva: World Health Organization, 1983.
3. World Health Organization. *Traditional Medicine*. WHO Fact Sheet No. 134. Geneva: WHO, revised 2003, Available at: <http://tinyurl.com/5mrd5> (accessed 11 December 2008).
4. Barbosa Filho VM, Waczuk EP, Kamdem JP, Abolaji AO, Lacerda SR, Costa JGM, et al. Phytochemical constituents, antioxidant activity, cytotoxicity and osmotic fragility effects of Caju (*Anacardium microcarpum*). *Industrial Crops and Products.*, 2014; 55: 280-288.
5. Boligon AA, Kubica TF, Mario DN, de Brum TF, Piana M, Weiblen R, et al. Antimicrobial and antiviral activity-guided fractionation from *Scutia buxifolia* Reissek extracts. *Acta Physiol Plant.*, 2013; 35: 2229-2239.
6. Janovik V, Boligon AA, Athayde ML. Antioxidant activities and HPLC/DAD analysis of phenolics and carotenoids from the barks of *Cariniana domestica* (Mart.) Miers. *Res J Phytochem.*, 2012; 6: 105-112.
7. Martin M, Guiochon G. Effects of high pressure in liquid chromatography. *J Chromatogr A.*, 2005; 1090: 16-38.
8. Cannell RJP. *Natural Products Isolation*. Human Press Inc. New Jersey., 1998; 165-208.
9. Piana M, Zadra M, de Brum TF, Boligon AA, Gonçalves AF, da Cruz RC, et al. Analysis of rutin in the extract and gel of *Viola tricolor*. *J Chromatogr Sci.*, 2013; 51: 406-411.
10. Kavimani S, Saminathan K, Senthil Kumar R. In vitro antidiabetic activity of *Dolichandrone atrovirens* – an Indian medicinal plant. *Inter. J. of Pharmacotherapy.*, 2014; 4(3), 107-113.

11. Kavimani S, Saminathan K, Senthil Kumar R. Antioxidant and free radical scavenging activities of *Dolichandrone atrovirens* using various in vitro assay models. *International Journal of Phytopharmacology.*, 2014; 5(4): 293-300.
12. Ganesh Muguli PR, Rao Vadaparathi, Ramesh B, Vishakante Gowda, Rangesh Paramesh, Atul N, Jadhav, and Suresh Babu K. *Pharmacogn Mag*, 2015; 11(Suppl 1): S66–S72.
13. Basu S, Vandana BP, Snehasis J, Patel HS. Liquid chromatography tandem mass spectrometry method (LC-MS/MS) for simultaneous determination of piperine, cinnamic acid and gallic acid in rat plasma using a polarity switch technique. *Anal Methods*, 2013; 5: 967–76.
14. Pushkar M Ketkar, Shraddha U Nayak, Sandeep R Pai, and Rajesh K Joshi. Monitoring seasonal variation of epicatechin and gallic acid in the bark of *Saraca asoca* using reverse phase high performance liquid chromatography (RP-HPLC) method. *J Ayurveda Integr Med*, 2015; 6(1): 29–34.
15. Patel MG, Patel VR, Patel RK. Development and Validation of Improved RP-HPLC method for Identification and Estimation of Ellagic and Gallic acid in Triphala Churna. *Int Chemtech Res*, 2010; 2: 1486–96.
16. Saminathan Kayarohana, S.Kavimani. Quantitative Phytochemical and GC-MS analysis of leaf and bark extract of *Dolichandrone atroviren*. *International research journal od pharmacy*, 2015; 6(3): 219-222.
17. Kumara Prasad S. A , Subrahmanyam E.V.S, Shabaraya A.R. Design and biological screening of some novel formazan derivatives from schiff bases of gallic acid, 2014; 3(2): 2741-2752.
18. Gaurav Mahesh Doshi, Sandeep Prabhakar Zine, Pratip Kashinath Chaskar, Hemant Devidas Une. Solicitation of HPLC and HPTLC Techniques for Determination of Rutin from *Polyalthia longifolia* Thwaites. *Pharmacognosy Res*, 2014; 6(3): 234–239.
19. Shengnan Li, Minglei Tian, and Kyung Ho Row. Effect of Mobile Phase Additives on the Resolution of Four Bioactive Compounds by RP-HPLC. *International Journal of Molecular Sciences*, 2010; 11(5): 2229-2240.
20. Han D, Row KH. Determination of four bioactive compounds in *Herba artemisiae scopariae*. *Asian J. Chem.*, 2010; 22: 2711–2716.
21. Dongmei Wang, Fengyuan He, Zhenjiang Lv, Dengwu L. Phytochemical Composition, Antioxidant Activity and HPLC Fingerprinting Profiles of Three *Pyrola* Species from Different Regions. *Chamani J,ed.PLoS ONE*, 2014; 9(5): e96329.

22. Hemayet Hossain, Shaikh Emdadur Rahman, Probity Nayeeb Akbar, Tanzir Ahmed Khan, Md. Mahfuzur Rahman, Ismet Ara Jahan. Determination of antioxidant activity and HPLC profile of *Euphorbia cotinifolia linn.* Leaf extract growing in Bangladesh, 2014; 3(7): 93 -104.
23. Gomathy Subramanian, Subramania Nainar Meyyanathan, Yamjala Karthik, Anjana Karunakaranair and Dhanabal S Palanisamy. Development and validation of HPLC Method for the simultaneous estimation of Quercetin and Rutin in aganosma dichotoma [Roth] K. Schum. International Journal of Pharmacy and Pharmaceutical Sciences, 2014; 6(2): 606-608.
24. Ujjwala Supe, Prarthna Daniel. Preliminary phytochemical analysis and quantitative analysis of Quercetin by HPLC of *Momordica charantia.* World Journal of Pharmaceutical Research, 2014; 4(01): 848-853.
25. Kareparamban JA, Nikam PH, Jadhav AP, Kadam VJ. A Validated High-performance Liquid Chromatography Method for Estimation of Ferulic Acid in Asafoetida and Polyherbal Preparation. Indian Journal of Pharmaceutical Sciences, 2013; 75(4): 493-495.
26. Nadal JM, Toledo M da G, Pupo YM, Padilha de Paula J, Farago PV, Zanin SMW. A Stability-Indicating HPLC-DAD Method for Determination of Ferulic Acid into Microparticles: Development, Validation, Forced Degradation, and Encapsulation Efficiency. Journal of Analytical Methods in Chemistry, 2015; 2015: 286812.
27. Kong Jin-Ming, Goh Ngoh-Khang, Chia Lian-Sai, Chiatet-Fatt. Recent advances in traditional plant drugs and orchids. Acta Pharmacol Sin., 2003 Jan; 24(1): 7-21.