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Evaluation of bioactive compouds through qualitative, quantitative and HPLC analysis of *Commelina maculata* leaves

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Abstract
Plants have basis of many traditional medicines throughout the world for thousands of years and continue to provide new remedies to mankind. Keeping in view, present study to investigate the phytochemicals and HPLC analysis of *Commelina maculata* leaves extract. The qualitative phytochemical analysis of aqueous and methanol extract of *Commelina maculata* leaves showed the presence of tannin, saponins, flavonoids, steroids, terpenoids, triterpenoids, anthroquinonesm Coumarins and polyphenol in both extract while emodins, anthocyanins were absent in aqueous extract. Alkaloids and anthocyanins were present only methanol extract. Quantitative analysis revealed that methanolic extract of Commelina maculata contain significant amount of total phenol, flavonoids, terpenoids, tannin and saponin. Among the two extract, methanol extract has rich source of phytochemicals were found. HPLC profiles of *Commelina maculata* were analysed and four phenolic compounds are Gallic Acid, Hypersoide, Catechol and Delphinidin -3-O-retinoside. The present study provides evidence that ethanol extract of *Commelina maculata* leaves contains medicinally important bioactive compounds and this justifies the use of plant species as a traditional medicine for treatment of various diseases.

Keywords: *Commelina maculate*, qualitative and quantitative analysis, HPLC profiles

Introduction
The therapeutic efficacy of many indigenous plants, for various diseases has been described by traditional herbal medicinal practitioners (Gami Bharat and Parabia, 2010) [1]. Natural products are the source of synthetic and traditional herbal medicine. They are still the primary health care system in some parts of the world (Shaukat Mahmud *et al.*, 2010) [2]. In India, local empirical knowledge about medicinal properties of plants is the basis for their uses as a home remedies. It is generally accepted by many Indians and elsewhere in the world that beneficial medicinal effects can be obtained by ingesting plant products. Plants have basis of many traditional medicines throughout the world for thousands of years and continue to provide new remedies to mankind (Patel, 2009) [3]. The plant is traditionally used for the treatment of epilepsy, dysentery, cardiac problems, worm infestation, constipation, haemorrhage, antibacterial infection, dysuria, fever, and ulcer. It also has antifertility, antitumour and abortifacient properties (Asolkar *et al.*, 1992; Vohora *et al.*, 1975; Yoganarasimhan, 2000; Seetharaman, 1986; Atique *et al.*, 1985; Topno, 1997) [4, 5, 6, 7, 8, 9].
Studies of medicinal plants based on ancient literature and its investigation in modern light is under process The medicinal importance of a plant is due to the presence of some special substances like alkaloids, glycosides, resins, volatile oils, gums and Tannins etc. These active principles usually remain concentrated in the storage organs of the plants. Keeping in view, present study to investigate the phytochemicals and hplc analysis of *Commelina maculata* leaves extract.

Materials and Methods
Plant materials
The leaves of *Commelina maculata* was collected in January 2019 from Tamil University campus, Thanjavur District, Tamil Nadu, India from a single herb. The leaves was identified and authenticated by Dr. S. John Britto, The Director, the Rabiant Herbarium and centre for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

Preparation of alcoholic extract
The leaves of *Commelina maculata* was first washed well and dust was removed from the plant. Leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with aqueous and methanol for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used.

Phytochemicals and HPLC screening: Chemical tests were carried out on the alcoholic extract using standard procedures to identify the preliminary phytochemical screening following the methodology of Sofowara (1993) [10], Trease and Evans (1989) [11] and Harborne (1973) [12]. Determination of total phenols (Edeoga *et al.*, 2006) [13]. Saponin was determined by the method of Obdoni and Ochuko (2001) [14]. Flavonoid was determined by the

method of Bohm and Kocipai-Abyazan (1994) [15]. Total terpenoid estimated by Ferguson (1956) [16]. The powder were treated with specific chemicals and reagents. The treated plant powder further analysed in light microscope. Flavonoids were analysed using a RP-HPLC method (Weerasak Samee, 2007) [17].

Results and Discussion

The qualitative phytochemical analysis of aqueous and methanol extract of *Commelina maculata* leaves showed the presence of tannin, saponins, flavonoids, steroids, terpenoids, triterpenoids, anthroquinonesm Coumarins and polyphenol in both extract while emodins, anthocyanins were absent in aqueous extract. Alkaloids and anthocyanins were present only methanol extract. Quantitative analysis (Table 2) revealed that methanolic extract of *Commelina maculata* contain significant amount of total phenol (188.65±13.20mg/gm), flavonoids (120.35 ± 8.71 mg/gm) terpenoids (80.94 ± 4.46 mg/gm), tannin (31.78 ± 2.21 mg/gm) and saponin (35.84 ± 2.50mg/gm) were presented. Among the two extract, methanol extract has rich source of phytochemicals were found.

Table 1: Preliminary Qualitative phytochemical screening

S. No	Test analysis	Aqueous	Methanol
1	Tannin	+	++
2	Saponin	++	++
3	Flavonoids	++	++
4	Steroids	+	++
5	Terpenoids	++	++
6	Triterpenoids	+	++
7	Alkaloids	-	+
8	Anthroquinone	+	++
9	Polyphenol	++	++
10	Glycoside	+	++
11	Coumarins	++	+
12	Emodins	-	-
13	Anthocyanins	-	+

(-) Absent, (+) Present and (++) high concentration

Table 2: Quantitative analysis of phytochemical

S. No	Phytochemicals	Results (mg/gm)
1	Flavonoids	120.35±8.71
2	Saponin	35.84±2.50
3	Terpenoids	80.94±4.46
4	Total phenol	188.65±13.20
5	Tannin	31.78±2.21

Values expressed as Mean ± SD for triplicates

HPLC profiles of *Commelina maculata* were analysed and four phenolic compounds (Figure 1 and table 3) namely Gallic Acid, Hypersoide, Catechol and Delphinidin -3-O-retinoside having different elution times could be obtained.

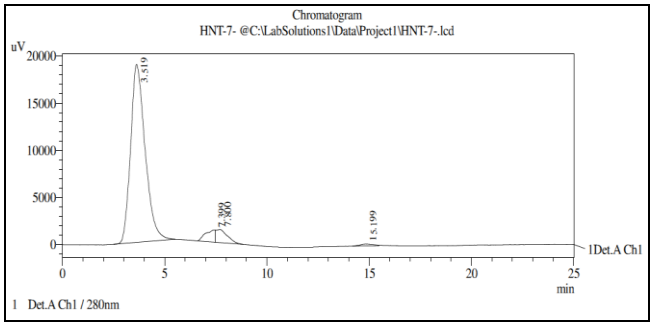

Fig 1: HPLC chromatogram of *Commelina maculata*

Table 3: HPLC profiles of *Commelina maculate*

Peak#	Ret. Time	Area	Height	Area %	Height %	Compounds identified by literature **
1	3.519	931735	8886	89.925	86.811	Gallic Acid
2	7.399	42780	1267	4.129	5.822	Hypersoide
3	7.800	52477	1395	5.065	6.411	Catechol
4	15.199	9137	208	0.882	0.956	Delphinidin -3-O-retinoside
Total		1036129	21756	100.000	100.000	

Phenolic compounds are phytochemicals that have one or more aromatic rings with at least one hydroxyl group. Flavonoids are low molecular weight polyphenolic antioxidants naturally present in fruits, vegetables, and beverages such wine and astea (Middleton *et al*., 2000) [18]. Flavonoids are known to improve cardiac function, decrease anginas and lowers cholesterol levels. These compounds act by regulation of inflammation mediators t has been shown that the flavonoid and flavonoid glycosides cause pancreatic beta cell regranulation and have been used in clinical treatment of diabetes due to improved sensitivity of insulin (Iwase *et al*., 2000) [19].

Anthocyanins are known to inhibit formation of free radicals thus protecting cardiomyocytes after ischemic episodes (Ali *et al.*, 2003) [20]. Anthocyanins have vasolidating and antiaggregative activities and also lower levels of oxidized LDL. Tannins are polyphenols that are obtained from various parts of different plants belonging to multiple species. Tannin has been shown to stimulate the receptor cells to utilize carbohydrate and management of glucose level in blood (Knox *et al*., 2000) [21].

Terpenoids are compounds synthesized from five carbon isoprene units mainly isopentenyl pyrophosphate and its isomer dimethylallyl pyrophosphate by terpene synthases (Salminen *et al*., 2008) [22]. Terpenoids have antioxidant properties and also interact with most regulatory proteins. Plant extracts have been used both traditionally and in modern medicine in the treatment of cancer and inflammatory diseases. Terpenes are used as inhibitors of NF-kB in modern medicine (Wagner and Elmadfa, 2003) [23].

Alkaloids are phytochemicals that contain nitrogen and are derived from various amino acids. Alkaloids are known to have blood glucose lowering activity. Alkaloids with therapeutic effects mainly act by affecting chemical transmitters of the nervous system like dopamine, γ-aminobutyric acid, acetylcholine and serotonin. Alkaloids are also known to be anti-arrythmic effects, antihypertensive effects, anticancer and antimalarial activity. Alkaloids are believed to have neuro-protective, cholinergic and antioxidant activities in Alzheimer's disease (Chiu-Yin *et al*., 2002) [24].

Saponins are plant compounds that occur either as steroid alkaloids, glycosides of triterpenoids or steroids. These phytochemicals are known to have hypocholesterolaemic, immunostimulant, hypoglycemic effect and anticarcinogenic properties (Mujoo *et al*., 2001) [25]. Cardiac glycosides are plant secondary metabolites that have a glycoside unit and act on the contractile action of the cardiac muscle. These compounds have been used traditionally for the treatment of cardiac arrhythmias and congestive heart failure as they increase contractile force (Francis *et al.*, 2002) [26].

Phytosterols are subgroup of steroids that have structures and functions similar to cholesterol (Yamada *et al*., 1983) [27]. Phytosterols in plants act as substrates for the synthesis

of secondary metabolites, regulate permeability and fluidity of cell membranes and also act as biogenic precursors of growth factors. These compounds are also believed to have insulin releasing effect, anti-complement and antipyretic activity (Katan *et al*., 2003) [28]. β-sitosterol and its glycoside together have immune modulating activities on non-infectious conditions like rheumatoid arthritis and allergies and chronic infectious diseases like tuberculosis and Human Papilloma Virus. A mixture of the two with higher concentrations of β-sitosterol is reported to influence the proliferation of T-lymphocytes after these cells are activated by mitogens *in vitro* (Harshal *et al*., 2014) [29].

Conclusion

The plant extract evaluated for phytochemical and chromatographic analysis. The plant extract contains phenols, flavonoids, alkaloids, tannins, terpenoid, and steroid. The present study provides evidence that ethanol extract of *Commelina maculata* leaves contains medicinally important bioactive compounds and this justifies the use of plant species as a traditional medicine for treatment of various diseases.

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