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Short communication

Anti-oxidant activity, phytochemical screening and HPLC profile of rare endemic *Cordia diffusa*

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ABSTRACT

The present study deals with the investigation of phytochemical screening, anti-oxidant activity and HPLC analysis of methanol and petroleum ether extract of *Cordia diffusa* leaves. The invitro anti-oxidant activity of *Cordia diffusa* leaf samples were investigated spectrophotometrically using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Methanolic extract showed significant anti-oxidant activity compared to petroleum ether extract. Determination of phytochemical constituents like phenols, flavonoids, tannins, saponins, alkaloids, steroids, terpenoids, anthocyanin, betacyanin and glycoside tests were carried out. HPLC chromatogram supported the presence of 1–8 phytocompounds. In conclusion, *Cordia diffusa* leaves might be used as a potential antioxidant source in pharmaceutical and food industries.

1. Introduction

The scientific attention towards nature for the use in food and medicine has increased considerably in recent years due to synthetic ones are restricted in various countries based on food safety regulation (Michielin et al., 2011). Antioxidants are substance which protects the human immune system against free radicals that may cause pathological conditions such as anaemia, asthma, inflammation, ischemia, ageing process (Makari and Patil, 2008). In living cells, free radicals are continuously produced as a result of abnormal metabolisms or external factors. Free radicals cause single and double strand break. This nucleotide break or damage leads to mutagenesis and aging (Bursal and Gülçin, 2011).

Naturally plants have the ability to produce secondary metabolites such as phenols, essential oils, flavonoid, tannins, saponins, which act as a protective agents against free radicals and pathological attack (Bajalan et al., 2017). Secondary metabolites are generally not important for the growth and reproduction of plants but they play significant role in pharmaceutical and food industries (Makari and Patil, 2008; Casuga et al., 2016).

The *Cordia* genus contains more than 300 species distributed worldwide, mostly in the warm region of the world (Al-Musayeib et al., 2011). Biological studies related to the different extract and compounds isolated from *Cordia* genus reported as antiviral, cytotoxic activities in tumour cells, anti-inflammatory and free radical scavenging properties (Ficarra et al., 1995; Rapisarda et al., 1992). Some substance from *C. corymbosa* and *C. goetsei*, are mentioned in the literature for their fungicidal activity against phytopathogens (Ficarra et al., 1995). Phytochemicals isolated from *Cordia dichotoma* leaves showed significant cytotoxic activity against human prostate carcinoma cell line (Rahman et al., 2017)

Cordia diffusa Jacob (Boraginaceae) is an endemic plant mainly distributed in southern parts of the Tamilnadu State, India (Ahmedullah and Nayar, 1987). The population of this species from *Cordia* genus is found rare and becoming endangered. Nair and Henry (1983) have included this species in their list of rare and threatened flowering plants of Asia. Medicinal and pharmacological value of the genus Cordia (Prasad et al., 2013) represents the importance of this plant. However, to date, less attention has been paid to study the biological properties of this genus *Cordia*. To the best of our knowledge, this is the first report on phytochemical screening and antioxidant free radical scavenging activity of the methanol and petroleum ether extract of *Cordia diffusa* leaves.

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2. Materials and methods

2.1. Chemicals and reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, methanol (HPLC grade), Petroleum ether, quercetin were purchased from sigma Aldrich. All other chemicals and reagents used in this study were of analytical grade.

2.2. Plant collection and authentication

The leaves of *Cordia diffusa* (Fig. 1) were collected from Institute of Forest Genetic and Tree Breading (IFGTB) campus, Coimbatore, Tamilnadu, India. It was identified at Botanical Survey of India (Ref. BSI/SRC/23/01/15/T/0011-02), TNAU, Coimbatore, Tamilnadu, India.

2.3. Preparation of methanol and petroleum ether extract

Plant leaf extract was prepared using methanol and petroleum ether by soxhlet extractor. It was performed as described by Redfern et al. (2014). After completion of extract, the methanol and petroleum ether solvents were evaporated by using rotatory evaporator. Prepared extract were concentrated, dried and kept at 4 °C until further analysis.

2.4. Qualitative phytochemical analysis

Phytochemical screening of methanol and petroleum ether extract of *C. diffusa* leaf samples were performed for the qualitative detection of different phytochemicals such as alkaloids, flavonoids, saponins, glycosidas, phenols, steroids, anthocyanin, betacyanin, tannin and terpenoids. All phytochemical tests were performed according to the stranded methods described by Siddiqui and Ali (1997), Trease (1989), Sithara et al. (2016).

2.5. Quantitative determinations of phytochemicals

2.5.1. Estimation of total phenol and flavonoids content

Total polyphenol content of *Cordia diffusa* leaves were estimated according to the protocol prescribed by Bursal and Gülçin (2011). The amount of phenols in both extracts was expressed as μg of catechol equivalent per mg of extract. Total flavonoid content of methanol and petroleum ether extract was estimated according to lamien-meda et al. (2008). The result was expressed μg of quercetin equivalent per mg of extract.

Fig. 1. Leaf sample of *C. diffusa.*

2.6. HPLC analysis

High Performance Liquid Chromatography (HPLC) was used to identify the presence of phytocompounds in leaf extracts of *Cordia diffusa.* HPLC analyzed with Hitachi instrument and L-4000 UV detector was used. HPLC analysis was performed according to Lone et al. (2015).

Methanolic and petroleum ether extract of *Cordia diffusa* and mobile phase solvents (acetonitrile with HCI) were filtered through 0.45 μm membrane filter. Prepared solvents and extracts were degassed by ultrasonic method. 23 Polyphenol standards such as resorcinol, vanillin, vanillic acid, phloroglucinol, gallic acid, pyrocatechol, salicylic acid, benzoic acid, syringic acid, syringaldehyde, pyrogalcol, tannic acid, morphillin, catecol, cinnamic acid, catechin, picric acid, phenyl acetic acid, elagic acid, ferulic acid, chlorogenic acid, hydroxyl benzoic acid and oxalic acid were used for characterization and identification of the phenolic compounds in *Cordia diffusa* leaf samples. Identification of each compound was done by comparison of their retention's time and UV absorption spectrum with those of the standards.

2.7. Invitro anti-oxidant activity

2.7.1. Determination of DPPH anti-oxidant activity

The DPPH free radical scavenging activity of leaf extracts of *Cordia diffusa* were carried out spectrophotometrically according to the method reported by Govindappa et al. (2013). Different concentration (20, 40, 60, 80, 100 μg/mL) of methanol and petroleum ether extract was added to an equal volume, to 0.002% methanolic DPPH solution. Samples were incubated for 20 min at room temperature and spectral absorbance was measure at 517 nm. Ascorbic acid is used as a reference stranded. Percentage of inhibition was calculated using the following equation

$$\% \text{ inhibition} = ([\text{control absorbance} - \text{Blank absorbance}]/\text{Control Absorbance}) \times 100$$

2.8. Statistical analysis

All experiments were carried out in triplicate and results were expressed as mean ± SEM (standard error of mean). All statistical analysis were performed using MS excel 2007.

3. Results and discussion

3.1. Qualitative phytochemical screening

The results of qualitative phytochemical screening were present in Table 1. Phytochemical screening revealed the presence of alka-

Table 1
Phytochemical screening of *C. diffusa.*

S.No.	Test(s)	Extract	
		Methanol	Petroleum ether
1	Phenols	+	+
2	Alkaloid	+	+
3	Glycosides	+	–
4	Saponins	+	–
5	Flavonoids	+	+
6	Steroids	+	–
7	Terpenoids	+	–
8	Anthocyanin	–	–
9	Betacyanin	+	–
10	Tannins	–	+

Notes: + denotes positive and – denotes negative.

loid, flavonoids, saponins, glycosidas, betacynin, phenols, steroids in methanolic extract and petroleum ether contain phenolic acids, flavonoids, tannins, alkaloids. Presence of these phytochemicals in plants greatly determined the antimicrobial, anti-oxidant and other biological activities (Zheng and Wang, 2001). Among the phytochemicals, phenols, flavonoids and terpenoids have been proven to be of great importance because they play important role in immune defense activity (Idris et al., 2017). The presence of these phytochemical in the both methanol and petroleum ether leaf extract of *C. diffusa* could possibly play a significant role in the anti-oxidative activity observed in this present study. Similarly, it has been reported that the other species of this genus *C. myxa* leaves contain various phytochemicals such as alkaloids, phenols, tannins, saponins and glycosides (Jasiem et al., 2016). The difference observed in the results of phytochemical constituents between the methanol and petroleum ether extract may be attributed to the solvent of extraction, nature of processing method and micro change in lab environment (Odeyemi et al., 2017).

3.2. Estimation of total phenol and flavonoids content

Total phenolic content of *C. diffusa* leaves were measured by Folin Ciocalteu reagent in terms of catechol equivalent and stranded quercetin equivalent were used for flavonoid estimation. The total phenol content of methanolic extract was 5.03 ± 0.50 μg/mL and petroleum ether contains 4.00 ± 0.21 μg/mL. The total flavonoid content of methanolic and petroleum ether extract was measured as 5.65 ± 0.43 μg/mL and 4.03 ± 0.57 μg/mL. Flavonoids play a major role in anti-oxidant activity but depend on their molecular structure and position of OH groups (Iqbal et al., 2015). Result obtained the present study revealed that the level of phenolic compounds in the both extract of the *C. diffusa* leaves was considerably higher in methanol extract then that in petroleum ether extract. This is due to different degree of solvents used for the extraction of polyphenols in plant (Bouaziz et al., 2015).

3.3. HPLC chromatogram analysis

The HPLC analysis of both extracts of *Cordia diffusa* showed the presence of 1–8 compounds such as morpholin, tannic acid, ellagic acid, ferulic acid, phloroglucinol, catechin, benzoic acid and syringaldehyde. Chromatogram of a methanol and petroleum ether extracts are shown in Fig. 2.

The HPLC chromatogram of the *C. diffusa* leaf methanol extract showed the presence of eight peaks of which they identified as morpholin, phloroglucinol, tannic acid, catechin, ferulic acid, benzoic acid and the identity of 2 peaks are unknown which needs detailed analysis. The HPLC chromatogram of petroleum ether extract showed the presence of morpholin, ellagic acid, syringic acid, syringaldehyde and two unknown compounds. It was noticed that morpholin compound are highly presented in the both solvent extract. Morpholin compound has a potential effect against pathogenic bacteria and fungus (Bektaş et al., 2013). Phloroglucinol, tannic acid, catechin, ferulic acid and syringaldehyde are phenolic compounds which has potential biological and pharmacological activities. Presence of benzoic acid represents the antiseptic value of the plant leaves (Lillard, 1904).

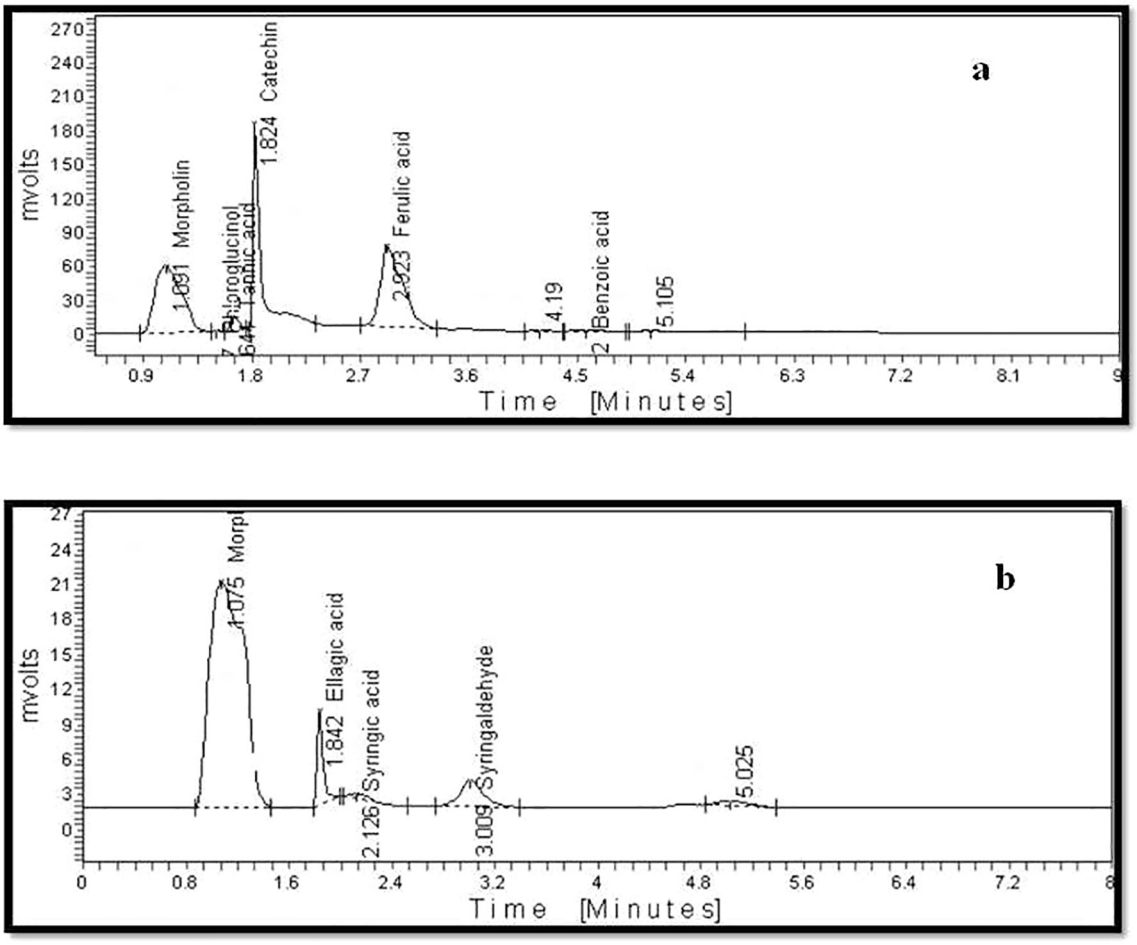

Fig. 2. HPLC chromatogram of *C. diffusa* leaf samples; a) methanol extract, b) petroleum ether extract.

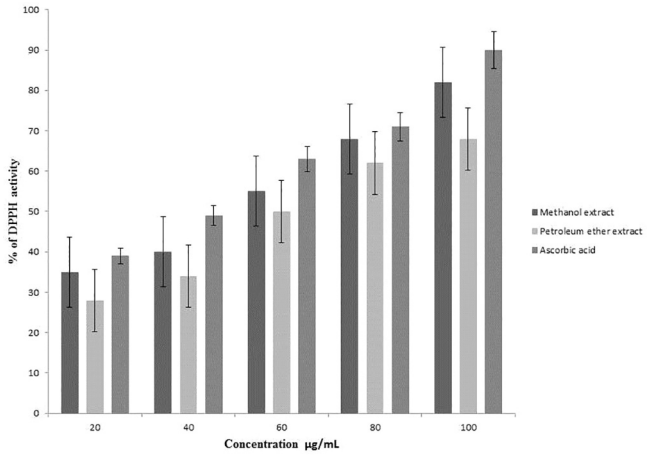

Fig. 3. DPPH anti-oxidant activity of methanol and petroleum ether extract of *C. diffusa*.

3.4. Invitro anti-oxidant activity

In this study, petroleum ether and methanol leaf extract of *C. diffusa* were investigated for the free radical scavenging activity with 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Result showed that maximum inhibition of anti-oxidant activity was noticed in methanol extract compared to petroleum ether (Fig. 3). It was observed that free radical scavenging of free radicals occurred in a concentration dependent manner. Methanolic extract of *C. diffusa* leaf extract has potential anti-oxidant activity that close to the ascorbic acid. This anti-oxidant capability might be due to the presence of polyphenolic compounds, especially phenols that have the ability to donate the hydrogen atoms in their hydroxyl groups (Bouaziz et al., 2015; Sagbo et al., 2017). It was reported that *Cordia wallichii* has 25.85% DPPH racial scavenging activity and also reported that methanolic extract of *C. wallichii* plant was found most effective than hexane (Makari and Patil, 2008).

4. Conclusion

The present study supported the anti-oxidant activity of methanol and petroleum ether extract of *Cordia diffusa* leaves. Moreover, presence of phytochemicals was analyzed by stranded method and chemical content of both methanol and petroleum ether extract were identified by HPLC. Further studies would involve separation and identification of individual components, anti-microbial and anti-cancer activity test.

Conflict of interest

The authors have declared no conflict of interest

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