The potential of antioxidant activity of methanolic extract of *Coscinium fenestratum* (Goetgh.) Colebr (Menispermaceae)

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**A B S T R A C T**

To explore the possible bioactive compounds and to study the antioxidant capacity of *Coscinium fenestratum* (Goetgh.) Colebr (Menispermaceae), the qualitative and quantitative phytochemical screening for various secondary metabolites were evaluated. Using the GC–MS analysis, a total number of 30 phytochemical compounds were predicted with their retention time, molecular weight, molecular formula, peak area, structure and activities. The most prevailing heterocyclic compound was Bis(2,4,6- triisopropylphenyl) phosphinic azide (6.70%). The antioxidant activity was evaluated by spectrophotometric methods using the reducing power assay and the DPPH\(^*\) and ABTS\(^*\) scavenging assays. The activity was determined to be increased in all the test samples with the increase in the volume of the extract. *C. fenestratum* possess a good source of many bioactive compounds that are used to prevent diseases linked with oxidative stress.

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1. Introduction

The natural products from plant origin are safer than the synthetic drug molecules, and are widely recognized in the pharmaceutical industries for their broad structural diversity and the pharmacological activities (Newman and Cragg, 2016; Thenmozhi et al., 2018). *Coscinium fenestratum* (Goeth.) Colebr. (commonly known as ‘tree turmeric’, belongs to the family Menispermaceae), is a medicinally important dioecious threatened liana (Tushar et al., 2008), distributed in Vietnam, Singapore, Thailand, Sri Lanka and in isolated regions of the Western Ghats of India (Ved et al., 2015). The stem and root of *C. fenestratum* are used in the traditional system of medicine (Tushar et al., 2008). The active chemical berberine (-a natural isoquinoline alkaloid), ceryl alcohol, hentriacontane, palmitic acid, sitosterol, saponyin with some resinous material and oleic acid have earlier been reported from *C. fenestratum* (Rojsanga et al., 2006) which possess variety of pharmacological activities including antidiabetic, anti-inflammatory, thermogenic and antiseptic activity (Kashyap et al., 2016). The free radicals and the other reactive oxygen species (ROS) generated within the living cells as a result of physiological and biochemical processes of the cells causes oxidative damage to the macromolecules of the cells, which lead to liver diseases (Arteel, 2003), asthma (Lobo et al., 2010; Bharathi et al., 2018), cancer (Kinnula and Crapo, 2004), chronic inflammation, diabetes, multiple sclerosis (Lobo et al., 2010; Bharathi et al., 2018), neural disorders (Sas et al., 2007), rheumatoid arthritis (Lobo et al., 2010; Bharathi et al., 2018), cardiovascular disease (Singh and Jialal, 2006), Alzheimer disease (Smith et al., 2000), Parkinson's disease (Bolton et al., 2000), ulcerative colitis (Ramakrishna et al., 1997), and aging (Hyun et al., 2006). The free radicals and other reactive oxygen species can be scavenged by the protective role of antioxidants from the natural products of wild and medicinal plants (Pietta et al., 1998). Hence, the objective of the present study was to investigate the phytochemical constituents and antioxidant activity of *C. fenestratum*.
2. Materials and methods

2.1. Collection of the plant sample and the preparation of methanolic crude extract

The fresh leaves of *C. fenestratum* were collected from Vellian-giri hills of Western Ghats, Coimbatore, Tamil Nadu, India. The semidry methanolic crude extract [MeOHCF, test compound] was prepared from 50 g of shade dried powdered leaves using soxhlet extractor.

2.2. In vitro antioxidant activity

There are various *in vitro* and *in vivo* methods available for the evaluation of the antioxidant activity of natural products (Alam et al., 2013). The reducing ability (Yildirim et al., 2001), DPPH radical scavenging activity (Blois, 1958), the total phenolic and tannin contents were performed (Harbone, 1973; Trease and Evans, 1983). The total content of flavonoids was determined spectrophotometrically using a standard curve rutin (Zhishen et al., 1999). MeOHCF was then subjected to the gas chromatography–mass spectrometry (GC–MS) analysis using 5975C Agilent Technologies GC systems equipped with DB-5 ms Agilent fused silica capillary column (30 × 0.25 mm ID, 0.25 μm film thickness) operating with electron impact mode at 70 eV. Finally MeOHCF was assigned for comparison of their retention indices and the mass spectra fragmentation patterns with chemical library of NIST (National Institute of Standards and Technology).

2.3. Identification of the phytochemical components of MeOHCF by GC–MS analysis

The qualitative phytochemical analysis of MeOHCF for the phytochemicals viz., alkaloids, cardiac glycosides, glycosides, flavonoids, phenols, resins, steroids, saponins, tannins, triterpenoids terpenoids, were performed (Blos, 1958). The total antioxidant activity (Siddhuraju and Manian, 2007) of MeOHCF were determined using the standard method in order to evaluate the *in vitro* antioxidant activity. One way analysis of variance (ANOVA) test was carried out for statistical analysis using SPSS 10.0.

2.4. Antimicrobial activity

The isolation and identification of **C. fenestratum** were performed (Karthika et al., 2014; Thenmozhi et al., 2015) which may serve as significant indicator for the potential antimicrobial activity. The extract showed significant scavenging effect on the sample of the concentration of the extract from 50 to 250 μg/mL, which might be due to abundance of the flavonoid (42 mg of QE/g extract) content, the most

### Table 1

| Phytochemical constituents                  | Trace Qualitative phytochemical analysis |
|--------------------------------------------|------------------------------------------|
| Yield (%)                                  | 15.8 ± 0.02                               |
| Alkaloids                                  | ++ Total alkaloids (mg of dry powder): 52.00 ± 0.19 |
| Flavonoids                                 | +++ Total flavonoids (mg of QE/g extract): 42.01 ± 0.06 |
| Terpenoids                                 | ++ –                                      |
| Triterpenoids                              | ++ –                                      |
| Glycosides                                 | – –                                      |
| Cardiac glycosides                         | ++ Total phenols (mg of GAE/g extract): 35.11 ± 0.04 |
| Phenols                                    | ++ –                                      |
| Saponins                                   | +++ –                                     |
| Steroids                                   | +++ –                                     |
| Tannins                                    | ++ Total tannins (mg of GAE/g extract): 34.46 ± 0.02 |
| Resins                                     | + –                                      |

+: Present, ++: Moderately present, +++: Highly present, GAE: Gallic Acid Equivalent, QE: Quercetin Equivalent. Values were performed in triplicates and represented as mean ± SD.

### Table 4

| Percentage yield and qualitative phytochemical analysis of MeOHCF. |
|-------------------------------------------------------------------|
| **Yield (%)** | 15.8 – |
| **Alkaloids** | ++ Total alkaloids (mg of dry powder): 52.00 ± 0.19 |
| **Flavonoids** | +++ Total flavonoids (mg of QE/g extract): 42.01 ± 0.06 |
| **Terpenoids** | ++ – |
| **Triterpenoids** | ++ – |
| **Glycosides** | – – |
| **Cardiac glycosides** | ++ Total phenols (mg of GAE/g extract): 35.11 ± 0.04 |
| **Phenols** | ++ – |
| **Saponins** | +++ – |
| **Steroids** | +++ – |
| **Tannins** | ++ Total tannins (mg of GAE/g extract): 34.46 ± 0.02 |
| **Resins** | + – |

The antioxidants molecules helps in preventing diseases by neutralize the effects of ROS (Sindhi et al., 2013). The antioxidant property of MeOHCF was determined using various methods. In reducing power assay, MeOHCF displayed significant activity which was found to increase with the increase in the concentration (Table 3) which may serve as significant indicator for the potential antioxidant activity. The results of the recent study were in accordance with the previous reports (Karthika et al., 2014; Thenmozhi et al., 2015). The percentage of scavenging activity on the DPPH radical varies from 32.54% (50 μg/mL of extract) to 64.80% (250 μg/mL of extract). The *IC*$_{50}$ value of MeOHCF was 182.48 μg/mL. The extract showed significant scavenging effect on the DPPH which was increasing with the increase in the concentration of the sample from 50 to 250 μg/mL, which might be due to abundance of the flavonoid (42 mg of QE/g extract) content, the most

### Table 2

| Phytochemical constituents                  | Trace Qualitative phytochemical analysis |
|--------------------------------------------|------------------------------------------|
| Yield (%)                                  | 15.8 ± 0.02                               |
| Alkaloids                                  | ++ Total alkaloids (mg of dry powder): 52.00 ± 0.19 |
| Flavonoids                                 | +++ Total flavonoids (mg of QE/g extract): 42.01 ± 0.06 |
| Terpenoids                                 | ++ –                                      |
| Triterpenoids                              | ++ –                                      |
| Glycosides                                 | – –                                      |
| Cardiac glycosides                         | ++ Total phenols (mg of GAE/g extract): 35.11 ± 0.04 |
| Phenols                                    | ++ –                                      |
| Saponins                                   | +++ –                                     |
| Steroids                                   | +++ –                                     |
| Tannins                                    | ++ Total tannins (mg of GAE/g extract): 34.46 ± 0.02 |
| Resins                                     | + –                                      |

+: Present, ++: Moderately present, +++: Highly present, GAE: Gallic Acid Equivalent, QE: Quercetin Equivalent. Values were performed in triplicates and represented as mean ± SD.

### Table 3

| Percentage yield and qualitative phytochemical analysis of MeOHCF. |
|-------------------------------------------------------------------|
| **Yield (%)** | 15.8 – |
| **Alkaloids** | ++ Total alkaloids (mg of dry powder): 52.00 ± 0.19 |
| **Flavonoids** | +++ Total flavonoids (mg of QE/g extract): 42.01 ± 0.06 |
| **Terpenoids** | ++ – |
| **Triterpenoids** | ++ – |
| **Glycosides** | – – |
| **Cardiac glycosides** | ++ Total phenols (mg of GAE/g extract): 35.11 ± 0.04 |
| **Phenols** | ++ – |
| **Saponins** | +++ – |
| **Steroids** | +++ – |
| **Tannins** | ++ Total tannins (mg of GAE/g extract): 34.46 ± 0.02 |
| **Resins** | + – |

The antioxidants molecules helps in preventing diseases by neutralize the effects of ROS (Sindhi et al., 2013). The antioxidant property of MeOHCF was determined using various methods. In reducing power assay, MeOHCF displayed significant activity which was found to increase with the increase in the concentration (Table 3) which may serve as significant indicator for the potential antioxidant activity. The results of the recent study were in accordance with the previous reports (Karthika et al., 2014; Thenmozhi et al., 2015). The percentage of scavenging activity on the DPPH radical varies from 32.54% (50 μg/mL of extract) to 64.80% (250 μg/mL of extract). The *IC*$_{50}$ value of MeOHCF was 182.48 μg/mL. The extract showed significant scavenging effect on the DPPH which was increasing with the increase in the concentration of the sample from 50 to 250 μg/mL, which might be due to abundance of the flavonoid (42 mg of QE/g extract) content, the most
| S. no. | Name of the compound                                                                 | RT    | Molecular formula | Molecular weight | Peak area (%) | Category of the compound | Activity                  |
|-------|--------------------------------------------------------------------------------------|-------|-------------------|------------------|---------------|---------------------------|----------------------------|
| 1     | EthylN-(p-tolylsulfinyl)(α-trifluoromethyl)-α-allylglycinate                          | 4.16  | C15H18F3NO3S      | 349              | 2.56          | Cyclic compound           | Antiproliferative and antitumor properties |
| 2     | Trimethylester of(4r,5s;4s,5r)-5-(methoxycarbonylmethyl)-1-methyl-2-pyrazolin-3,4,5-tricarboxylic acid | 4.98  | C15H18NO5         | 330              | 1.76          | Heterocyclic compound     | No activity reported         |
| 3     | 2-Thienylmethylo-(3’-t-butyl > amino-2’-hydroxypropyl) ketoxime                      | 12.60 | C6H4N2O5S         | 283              | 1.74          | Heterocyclic compound     | Antioxidant activity         |
| 4     | Benzaldehyde, 4-hydroxy-3-methoxy-(CAS)                                             | 14.11 | C6H8O3            | 152              | 2.12          | Phenolic aldehyde         | Anticancer, antioxidant, antimutagenic agents |
| 5     | D-friedoolean-14-en-3-one (CAS)                                                     | 21.72 | C22H22O5          | 424              | 2.41          | Triterpenoid derivatives  | Antifungal and antioxidant agents |
| 6     | Ethyl N-benzylanthranilate                                                          | 22.03 | C6H12N2O2         | 255              | 2.55          | Coumarin                  | Antiinflammatory activity    |
| 7     | (E)-α-[2-hydroxyphenylethylene]benzeneethanol-D2                                     | 23.38 | C10H12O2          | 226              | 1.64          | Heterocyclic compound     | No activity reported         |
| 8     | Himacalol                                                                           | 25.23 | C6H12O2           | 222              | 2.63          | Heterocyclic compound     | Insecticidal activity, Antitumor activity |
| 9     | 1,5,6-Tetrahydro-8,9-dimethoxy-10b-(p-methoxyphenyl)-2-methylene-2H-isoaxazole[3,2-al]soiquinol ne-1-carbonitrile | 25.64 | C22H22N4O4        | 378              | 1.54          | Heterocyclic compound     | No activity reported         |
| 10    | 1,9-Dimethoxy-10-methyl-2-(carbamoylmethylcarbonyl)-3-(methoxycarbonylmethyl)-10-methyl-anthracene | 26.15 | C24H24N4O         | 409              | 2.82          | Heterocyclic compound     | Antimicrobial and used for tranquilizing large animals in veterinary medicine. |
| 11    | α-Cyperone                                                                          | 26.78 | C16H12O2          | 218              | 5.81          | Aromatic compound         | Antiinflammatory activity    |
| 12    | 17-(Cyclopseudynamilk)-(α-1’-dimethylethyl)-4,5-epoxy-18,19-dihydro-3-hydroxy-6-methoxy-α-methyl-5,14-ethenomorphinan-7-methanol | 27.19 | C22H20N4O4        | 425              | 1.68          | Heterocyclic compound     | Antitumor activity           |
| 13    | 1-P-menth-8-yl acetate                                                              | 27.74 | C10H16O2          | 196              | 1.76          | Alkaloid                  | Flavor and fragrance agent   |
| 14    | 6-Bromohexanoic acid, 10-undecenyl ester                                           | 28.51 | C11H18BrO2        | 451              | 2.54          | Aromatic aldehyde         | No activity reported         |
| 15    | 2-[Diacetylamino]-6-(3’-methyl-5’-oxo-1’-phenyl-2’-pyrazolin-4’-yl)-4-phoenylpyridine-3-carbonitrile | 30.32 | C11H12N3O4        | 451              | 4.80          | Aromatic aldehyde         | No activity reported         |
| 16    | Pyranthrene                                                                         | 31.02 | C10H16O2          | 376              | 5.16          | Alkaloid                  | Antiinflammatory activity    |
| 17    | Methyl 2-N-cyclohexylamino-2,3-dideoxy-4,6-o-(phenylmethene)-3-c-phenylsulfonyl-α-D-glucopyranoside | 31.73 | C24H24N4O4        | 486              | 5.99          | Alkaloid                  | No activity reported         |
| 18    | 1-Pyrilidino-benzanthra-9,10-quinnone                                               | 32.03 | C25H22N4O2        | 331              | 2.95          | Heterocyclic compound     | Antibiotic agent             |
| 19    | (22E)-3ÁAcetoxy-7-alpha, -hydroxyprostigmast-a-5, 22-diene                           | 32.69 | C10H16O2          | 486              | 1.54          | Alkaloid                  | Piscicidal activity          |
| 20    | 1-Diphenylphosphino-1-dichlorophosphino-[1]-ferrocene                               | 33.34 | C22H18Cl2F2P2     | 470              | 4.24          | Alkaloid                  | No activity reported         |
| 21    | Bis(2,4,6-trisopropylphenyl)phosphinicacide                                        | 34.15 | C25H40N4O2        | 495              | 6.70          | Alkaloid                  | No activity reported         |
| 22    | 6-[N-(Cyanoamino)]-3Ámethoxymethoxy-cholestan                                             | 36.62 | C10H16N2O2        | 470              | 3.85          | Alkaloid                  | No activity reported         |
| 23    | 3,3-Bis(3’,4’-dimethoxyphenyl)-5,6-difluorobenzene[b]Furan                           | 36.96 | C11H12F2O3        | 426              | 4.02          | Alkaloid                  | No activity reported         |
| 24    | 5ÁAndrost-16-en-3Áol-(1-butyl)dimethylsilyl ether                                      | 37.43 | C22H40Si          | 388              | 2.88          | Alkaloid                  | Antidepressant               |
| 25    | 3-(4-Chlorobenzoyl)-7-methyl-(2-methylphenyl)indole                                 | 38.00 | C21H16ClNO        | 374              | 4.62          | Alkaloid                  | Antifungal and antioxidant properties |
| 26    | Cyclohexane, 1,4-dimethyl-2-ocdecetyl-(CAS)                                          | 38.37 | C12H18          | 364              | 5.82          | Alcohol                   | Anticancer agent             |
| 27    | 10-[3’5’-Bis(trifluoromethyl)phenyl]-3-(ethoxycarbonylmethyl)isoalloxazine          | 38.61 | C22H24F6N4O4     | 512              | 2.20          | Tricyclic compound        | No activity reported         |
| 28    | 13-Docosanamide, (z)-                                                              | 39.00 | C24H48N2O        | 337              | 4.46          | Carboxylic acid           | Used as a detergent, fabric softener, anti-static agent, anti-sedating agent, germicide, lubricant, ore floating agent, emulsifer, water treatment agent and insecticide. |
| 29    | ([Thorium-(pentamethylcyclopentadieny1)-tris(trimethylsilylamo)]: 1’2’-ethylideneamino]) | 39.43 | C12H24N4Si1Th     | 726              | 4.80          | Aromatic compound         | No activity reported         |
| 30    | Methyl-3-deoxy-6-isothiocyanato-2,3,4-tri-ethoxycarbonyl-1-D-galactopyranoside        | 39.82 | C19H27NO3Si1      | 451              | 2.40          | Organic compound          | No activity reported         |

* Source: Dr. Duke's Phytochemical and Ethnobotanical Databases.
Fig. 1. GC–MS chromatogram of methanolic leaf extract of Coscinium fenestratum.

Fig. 2a. Mass spectrum of Bis(2,4,6-triisopropylphenyl) phosphinicazid.

Fig. 2b. Mass spectrum of Methyl2-N-cyclohexylamino-2,3-dideoxy-4,6-O-(phenylmethylene) -3-C-phenylsulfonyl-ß,D-glucopyranoside.
Table 3
Reducing power activity of MeOHCf compared with certain standard antioxidants.

| Sample concentration (μg/mL) | Leaf extract (absorbance at 700 nm) | Sample concentration (μg/mL) | Rutin | BHA | Quercetin | BHT |
|-----------------------------|-------------------------------------|-------------------------------|-------|-----|-----------|-----|
| 50                          | 0.610 ± 0.03<sup>a</sup>            | 20                            | 0.238 ± 0.003<sup>a</sup> | 0.236 ± 0.016<sup>b</sup> | 0.359 ± 0.012<sup>a</sup> | 0.224 ± 0.001<sup>a</sup> |
| 100                         | 0.645 ± 0.02<sup>a</sup>            | 40                            | 0.350 ± 0.013<sup>a</sup> | 0.396 ± 0.017<sup>a</sup> | 0.632 ± 0.023<sup>b</sup> | 0.368 ± 0.009<sup>b</sup> |
| 150                         | 0.723 ± 0.06<sup>b</sup>            | 60                            | 0.408 ± 0.013<sup>c</sup> | 0.496 ± 0.028<sup>b</sup> | 0.718 ± 0.019<sup>c</sup> | 0.478 ± 0.013<sup>c</sup> |
| 200                         | 0.816 ± 0.04<sup>d</sup>            | 80                            | 0.476 ± 0.006<sup>b</sup> | 0.593 ± 0.008<sup>b</sup> | 0.833 ± 0.044<sup>d</sup> | 0.517 ± 0.017<sup>d</sup> |
| 250                         | 1.060 ± 0.07<sup>c</sup>            | 100                           | 0.557 ± 0.014<sup>c</sup> | 0.644 ± 0.011<sup>b</sup> | 0.973 ± 0.029<sup>bc</sup> | 0.584 ± 0.012<sup>c</sup> |

Values were performed in triplicates and represented as mean ± SD. Mean values followed by different superscript in a column are significantly different (p < 0.05).

Table 4
DPPH scavenging activity of MeOHCf compared with certain standard antioxidants.

| Sample concentration (μg/mL) | % of inhibition | IC<sub>50</sub> value (μg/mL) | Standard antioxidants | IC<sub>50</sub> value (μg/mL) |
|------------------------------|-----------------|-------------------------------|-----------------------|-------------------------------|
| 50                           | 32.54 ± 0.05<sup>a</sup> | 182.48                        | Rutin                 | 15.75 ± 0.01                  |
| 100                          | 32.74 ± 0.04<sup>a</sup> | 182.48                        | Quercetin             | 20.72 ± 0.05                  |
| 150                          | 44.21 ± 0.03<sup>b</sup> | 182.48                        | BHA                   | 21.42 ± 0.11                  |
| 200                          | 50.09 ± 0.07<sup>b</sup> | 182.48                        | BHT                   | 34.74 ± 0.26                  |
| 250                          | 64.89 ± 0.04<sup>c</sup> | 182.48                        |                       |                               |

Values are performed in triplicates and represented as mean ± SD. Mean values followed by different superscripts in a column are significantly different (p < 0.05).
required bio compounds for scavenging activity. Similar trend of this activity was also documented previously in our laboratory (Karthika et al., 2014). MeOHCf exhibited higher ABTS+ scavenging activity. The 2,2’-azinobis (3-ethylbenzothiazoline sulphonate) radical cation (ABTS+) scavenging activity was 2453.7 μmol trolox equivalent/ g extract (Table 4). This high activity could be due to abundance of secondary metabolites in the plant extracts (Rojsanga et al., 2006; Tushar et al., 2008).

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Conflict of interest

The authors report no conflicts of interest in this work.

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