Assessment of Antioxidant and Phytoactive Compounds of ethanolic extract of *Mukia maderaspatana* (L.) leaves

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Abstract: Plants are the basis for the development of modern drugs. Medicinal plants are rich sources of pharmacological active molecules. This study investigated the antioxidant activity, GC-MS analysis of ethanolic extract of *Mukia maderaspatana* (EEMM). DPPH assay, Superoxide radical scavenging, reducing power assay were used for determination of free radical scavenging activity of extracts, GC-MS analysis is a unique technique for analysis and measures the quantity of organic volatile, medicinally active compounds which is present in medicinal plants. The results obtained showed that the extract has an excellent antioxidant activities in DPPH (IC_{50} =100.81 ± 1.53 µg/ml), Superoxide (IC_{50} = 146.76 ± 1.54 µg/ml), Nitric oxide radical scavenging assay (IC_{50} = 153.76 ± 3.82 µg/ml), GC-MS analysis showed that the presence of many medicinally active compounds.

Keywords: *Mukia maderaspatana*, DPPH, GC-MS analysis, Antioxidants.

1. INTRODUCTION

Plants are the traditional source for many of the chemicals used as pharmaceuticals, biochemicals, fragrance, food colours and flavours. Most valuable phytochemicals are the products of secondary metabolism and possess sufficient chemical or structural complexity. Many plants synthesize substances that are useful to the maintenance of health in humans and other animals [1]. All biological systems have antioxidant defence mechanism that protects against oxidative damages and repairs enzymes to remove damaged molecules. However, this natural antioxidant mechanism can be in efficient; hence dietary intake of antioxidant compounds is important. Consumption of medicinal plants is known to lower the risk of several diseases which are caused by Oxidative damages and such health benefits are mainly imposed due to the presence of phytochemicals, such as polyphenols, carotenoids and vitamins [2]. *Mukia maderaspatana belongs* to the family Cucurbitaceae. The plant is a tendril climber/prostate herb. The plant was reported to have activities such as hepatoprotective, antirheumatic, diuretic, stomachic (a digestive tonic), gentle aperients, antipyretic and antiflatulent, antiasthmatic, anti-inflammatory, antidiabetic and antibronchitis and is used for tooth-ache besides its use in vertigo and biliousness [3]. It is called as Musumusukkai in Tamil and it is used in Siddha medicine against a variable disease [4].

Phytotherapy is the oldest form of health care known to mankind and bioactive substances present in plants are well-known for their antimicrobial, antioxidant and immunomodulatory properties. Previous studies report that more than 400,000 species of tropical flowering plants possess medicinal properties and that is the reason for traditional medicine to be cheaper than modern medicine, particularly in the developing countries. Profiling of bioactive metabolites from the plants delivers valuable information about their chemical diversities, toxicity concerns and medicinal potentials that are relevant to various fields. Green extraction processes are considered as an important tool in discovering and extracting phytochemicals possessing beneficial properties with non-toxic nature. GC–MS offers a reliable and reproducible analytical protocol for the profiling of the bioactive principles from the extracts among other hyphenated techniques described elsewhere [5]. Therefore, the present work is aimed to assess the *Mukia maderaspatana (L.)* leaves extracts in terms of their preliminary phytochemical testing, antioxidant efficiency. Furthermore, the phytoactive compounds responsible for these activities were also analyzed.

2. MATERIALS AND METHODS

2.1 Collection of plant materials

The plant *Mukia maderaspatana* (Linn) was collected from their natural habitat around local areas of Coimbatore District during the month of January 2019 and the leaves were kept in sterile bags and then taken to laboratory for further purposes.

2.2 Processing of plant sample

The leaves were washed with tap water, air dried in shed at room temperature (26°C) for 2 weeks and homogenized to a fine powder and stored in airtight bottles under low temperature in a refrigerator which were then used for fresh preparation of extract.

2.3 Solvent extraction

The dried powdered sample of the leaves and roots are weighed 30g, dissolved in 150 ml of ethanol in a soxhlet apparatus by continuous heat exposure for 48 hours till the solution becomes clear and then the extract were collected in a tube. The extracts were concentrated under reduced pressure 22-26 mmHg at 45°C and the residue obtained was stored at 4°C for further use.

2.4 Phytochemical Analysis

2.4.1 Qualitative Phytochemical Analysis

The phytochemical screening of EEMM is assayed by standard methods [6]. The screening was carried out to discover the significant bioactive components such as tannins, saponins, favonoids, phenols, terpenoids, alkaloids, glycosides, steroids.

2.5 *In-Vitro* Antioxidant Activities Different concentrations

(50-250 µg/ml) of EEMM were tested for various types of radicals scavenging potential. Ascorbic acid was used as standard reference compounds for all in vitro antioxidant assays.

2.5.1 Free Radical Scavenging Activity on DPPH

The antioxidant activity of the sample was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH, according to the method of Blois (1958) [7].

2.5.2 Superoxide Radical Scavenging Activity

Superoxide radicals were generated by a modifed method of Beauchamp and Fridovich (1971) [8].

2.5.3Nitric Oxide Radical Scavenging Activity

The nitric oxide scavenging activity of the sample was measured according to the method of Sreejayan and Rao (1997) [9].

2.5.4 Reducing Power Assay

The reducing power of the sample extract was determined by the method reported by Siddhuraju et al. (2002) [10].

2.6 Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The main phytocomponents of EEMM were identified by using GC–MS detection system. The samples were suspended with ethanol and subjected to GC–MS analysis. Elucidation of phytocomponents was assayed by comparison of their retention times and mass with their regular authentic standard spectra using computer searches in NIST08.L and Wiley7n.l libraries [11].

3 RESULTS AND DISCUSSION

3.1 Qualitative analysis

Qualitative estimation of phenol, flavanoids, alkaloids, tannins, terpenoids, proteins, carbohydrates, pytosterols, cardiac glycosides was carried out in the EEMM. Among these constituents flavonoids, tannins, proteins, carbohydrates, phytosterols are confirmed the presence which is depicted with positive sign in Table 1.

Table 1.Qualitative phytochemical screening of EEMM

TEST	OBSERVATION
Phenols	-
Flavanoids	+
Tannins	+
Terpenoids	-
Proteins	+
Carbohyrates	+
Phytosterols	+
Cardiac glycosyl	-
Alkaloids	-

3.2 Antioxidant activity

Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants through their scavenging power are useful for the management of those diseases. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of specific compound of plant extracts.

The results subjected in DPPH Assay showed maximum free radical scavenging activity of 54.8 ± 1.05 at a concentration of 400 µg/ml when compared with standard ascorbic acid levels thus elucidating importance of EEMM a potent anti-oxidant agent (Table 2).The naturally available anti-oxidants from the medicinal plants because that are having lower side effects and comparatively safe [12].

Superoxide anion (O_2^-) is an extremely reactive compound synthesized when oxygen is reduced by a single electron and may be produced during the regular catalytic role of various enzymes. It is well known that superoxide anions damage biomolecules directly or indirectly by forming H2O2, ¯OH, peroxy nitrite or singlet oxygen during aging and pathological events such as ischemic reperfusion injury [13]. In the analysis of superoxide the results indicate that the radical scavenging ability of EEMM was found to be 38.33 % at a concentration of 400 µg with an IC 50 value of 146.76 ± 1.54 (µg/ml) (Table 2).

Nitric oxide is a free radical component generated by endothelial cells, macrophages, and neuron, etc., and involved in the modulation and regulation of various physiological processes [14]. Excess concentration is connected with the onset of several diseases. It reacts with oxygen to produce its stable products of nitrate and nitrite through intermediates NO_2, N_2O_4 and N_3O_3.(Table 2) The result subjected in this analysis, nitric oxide radical scavenging ability of EEMM was found to be 44.87 % at a concentration of 400 µg with an IC 50 value of 153.76±3.82(µg/ml).

Reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron [15]. The Reduction ability (Fe3 to Fe2 transformation in terms of increasing absorbance) was found to increase with rising concentration. The result indicates the reducing power radical scavenging potential was found to be 0.188 at a concentration of 400 µg (Table 3).

Table 2. *In-vitro* Antioxidant activity of EEMM

Antioxidant activity	Sample concentration (µg/reaction volume)	Inhibition (%)	IC50 (µg/ml)
DPPH radical scavenging activity	80	14.95±1.93	
	160	22.59±1.03	
	240	26.75±1.18	100.81±1.53
	320	41.88±1.72	
	400	54.87±1.05	
Super oxide radical scavenging activity	80	14.44±0.53	
	160	22.01±0.33	
	240	24.65±0.51	146.76±1.54
	320	34.31±0.43	
	400	38.33±0.97	
Nitric oxide radical scavenging activity	80	13.29±0.46	
	160	21.52±0.92	
	240	30.80±1.22	153.76±3.82
	320	36.88±1.04	
	400	44.87±0.82	

Values are expressed as mean ± SD of triplicates

Table 3. Reducing power assay of EEMM

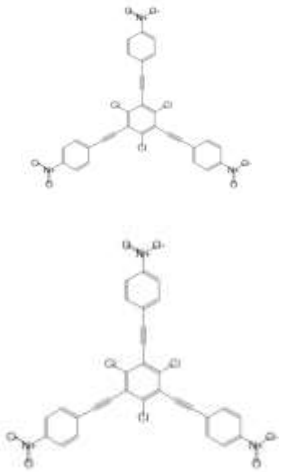
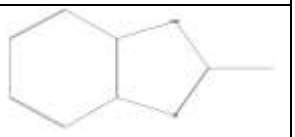
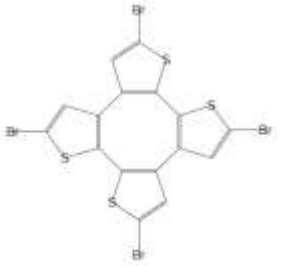
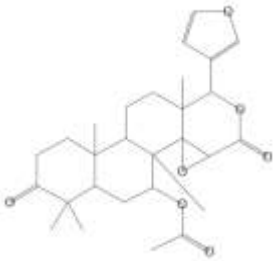
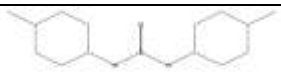
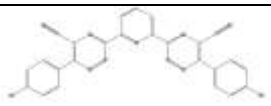
Sample	Sample concentration (µg/reaction volume)	OD
Mukia maderaspatana	80	0.092±0.005
	160	0.108±0.005
	240	0.140±0.006
	320	0.172±0.004
	400	0.188±0.003

Values are expressed as mean ± SD of triplicates

3.2 GC-MS Analysis

The GC-MS analysis of ethanolic extract of *Mukia maderaspatana* leaf shows different peaks (Fig 1). Each peak is representing a constituent present in the extract. These peaks are further analyzed and the fractions obtained at different retention time is characterized by MS, which is represented in Table.3 It shows that, among all compounds, compound with retention time 3.88 shows highest concentration (4.79 %), followed by compound with retention time 20.34 (3.44 %), compound with retention time 9.89 (3.38 %), compound with retention time 4.75 (3.10 %) and concentrations of remaining compounds are <2 %. These are probable compounds based on GC-MS compound library search.

Table 3.Components identified in the ethanolic extract of *Mukia maderaspatana* leaf sample

Compound Name	Area %	Molecular formula	Molecular Weight	RT (min)	Structure
1,3,5-Trichloro-2,4,6-tris benzene	4.79	C30H12C13N3O6	615	3.88	
1 H-Benzimidazole, 2-methyl-(CAS)	2.44	C8H8N2	132	4.29	
2,7,12,17-tetrabrom-cyclotetrathiophen	3.10	C16H4Br4S4	640	4.75	
d-Homo-24-nor-17-oxachola-20,22-diene-3,16-dione	2.51	C28H36O7	484	9.07	
Sulfurous acid,di-(p-tolyl)ester	3.38	C14H14O3S	262	9.89	
2,6-Bis[5-cyano-6-(4-bromophenyl)-1,2,4-triazin-3-yl]pyridine	3.44	C25H11Br2N9	595	20.34	
Astaxanthin	2.91	C40H52O4	596	35.38	

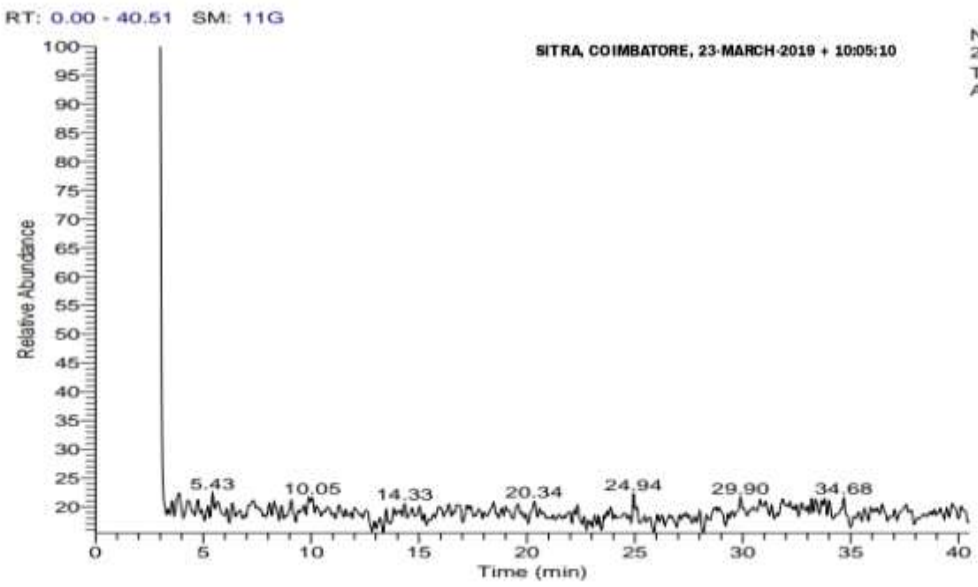


Figure 1: GC-MS analysis spectrum of *Mukia maderaspatana* leaf extract

4 CONCLUSION

The present investigation revealed that EEMM was comprised of a variety of metabolites which possess strong antioxidant activities. GC–MS analysis revealed the presence of 7 major components in EEMM. These substances could be isolated and empirically evaluated further to confirm their biologic and medicinal activities. The analysed GC-MS result showed the presence of sterol precursors may leads to presence of vitamin D, so it may help the human resource as the supplement of vitamin D. However, a detailed study is needed to elucidate the mechanism of chemical constituents available in EEMM for their better utility in various applications.

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