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HPLC AND GC-MS ANALYSIS OF *BIDENS BITERNATA* (LOUR.) MERR AND SHERIFF A WILD ETHNO MEDICINAL PLANT

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ABSTRACT

Bidens biternata (Lour.) Merr and Sheriff, belongs to the family Asteraceae, is a wide spread weed in Waynadu district of Kerala state. It is used as a leafy vegetable by Paniya, Chetti, Kani and Kattunaayika tribes of Waynadu Districts in Kerala and also to cure inflammation, hepatitis, fever, cough, dysentery, bronchitis etc. In the present study crude methanolic leaves extract of *B. biternata* was investigated using HPLC and GC-MS. Presence of bioactive molecules like quercetin, gallic acid, luteolin, ferrulic acid and rutin, were identified using HPLC. Quercetin was found in higher quantities among them. GC-MS analysis of crude methanolic extract showed the presence of four important compounds.

KEYWORDS

Bidens biternata, Western Ghats, HPLC and GC-MS.

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INTRODUCTION

Plants used for traditional medicine contain a wide range of compounds that can be used to treat chronic as well as infectious diseases[1]. Due to the development of adverse effects and microbial resistance to the chemically synthesized drugs, men turned to ethnopharmacognosy[2]. They found literally thousands of phytochemicals from plants as safe and broadly effective alternatives with less adverse effect. Many beneficial biological activity such as anticancer, antimicrobial, antioxidant, anti-inflammatory, analgesic and wound healing activity were reported. In many cases the people claim the good benefit of

certain natural or herbal products[3]. However, clinical trials are necessary to demonstrate the effectiveness of a bioactive compound to verify this traditional claim[4]. Although there are several hundred-thousand plant species around the globe, only a small proportion has been investigated both phytochemically and pharmacologically. When one considers that a single plant may contain up to thousands of constituents, the possibilities of making new discoveries become evident[5]. The crucial factor for the success of an investigation of bioactive plant constituent is thus the selection of plant material. It is essential to have efficient systems available for the rapid chemical and biological screening of the plant extracts selected for investigation[5]. An attempt has been made to identify and evaluate the bioactive molecules that might be responsible for the medicinal properties of green leafy vegetable *B.biternata*, since the plant possess a tremendous medicinal properties to help the people to overcome the deadly diseases of modern society. HPLC and GC-MS has importance in the bioactive molecular analysis of plant extracts. The qualitative analysis which produces a "fingerprint" chromatogram obtained under standard conditions can be very useful for quality control of phytochemicals. Although TLC is a powerful and simple technique used for this purpose, there are situations in which it can produce doubtful results. HPLC and GC-MS can also be a useful tool in chemosystematics i.e.to characterizes species on the basis of their secondary metabolite contents[6]. So the present study aimed to investigate the HPLC and GC-MS analysis of crude methanolic extracts of leaves of *B.biternata*.

MATERIALS AND METHODS

Collection and preparation of sample

Leaves of *B.biternata* were collected fresh from Kalpetta of Waynadu, Kerala. These were shade dried, ground well using mechanical blender in to fine powder and transferred to airtight containers for future studies.

Extraction from plant parts

The fine powder was used for extraction by using solvent like methanol. Fifty gram of sample powder was covered with cotton cloth and kept into the soxhlet apparatus for distillation. Methanol (300 ml) was taken into the round bottom flask and heated in a mantle for 8 hours at 70°C. After completing the process, extract was collected in beaker and was kept in oven at 37°C-40°C for evaporation. The crude concentrated extract was again weighed and used for further HPLC and GC-MS investigations.

High Performance Liquid Chromatographic (HPLC) Analysis

To evaluate the presence of bioactive molecules crude methanol extract of *B.biternata* leaves were analyzed using HPLC and compared with standards like quercetin, gallic acid, luteolin, ferrulic acid and rutin.

Preparation of standard and sample solutions

Ten mg of quercetin, luteolin, ferrulic acid, gallic acid and rutin were accurately weighed in to 10 ml volumetric flask dissolved in 5 ml methanol and the solution was made up to 10 ml. *B.biternata* leaves extract was accurately weighed (10 mg) in to a 10 ml of volumetric flask and shaken on a mechanical shaker for 10 minutes, filtered through What man filter paper No.42 and the filtrate was used for analysis. A supercritical fluid extractor SFE-2 (Applied Separation, USA) which is capable of pressure up to 680 bar and temperature up to 240ºC, static and dynamic extraction with flow from 0 to 10 L/min (gaseous carbon dioxide) and extraction vessels from 5 ml to 11 were used. An Agilent 1200 liquid chromatography system (Agilent technologies, CA, USA) consisting of binary pump, an auto-sampler and diode-array detector was used. Agilent Zorbas Extend reversed-phase C18 column (250 mm × 4.6 mm, 5 μm) was used. Detection wavelength was set at 220 nm. The mobile phase consisted of a (methanol) and B (deionized water), using a linear gradient 0-40 min (85% A), 40-60 min (85% A-95% A). The flow rate was 1.0 ml/min and the column temperature was maintained at 30ºC. The area of sample and standards were compared and the percentage of quercetin, gallic acid, luteolin, ferrulic acid and rutin content were calculated from the peak[7] using the formula,

$$\textbf{Percentage of compound} = \frac{\textbf{Area of test}}{\textbf{Area of sample}} \times \textbf{100}$$

Investigation using GC-MS

Crude methanol extract of *B.biternata* leaves were used for GC-MS investigation. The GC-MS analysis was carried out using a Clarus 500 Perkin-Elmer (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold-Perkin Elmer Turbo mass 5.2 spectrometer with an Elite-5MS (5% Diphenyl/95% Dim ethyl poly siloxane), 30 m x 0.25 µm DF of capillary column. The instrument was set to an initial temperature of 110ºC and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 280ºC, at the rate of an increase of 5ºC/min and maintained for 9 min. Injection port temperature was ensured as 200ºC and Helium flow rate as 1 ml/min. The ionization voltage used was 70 eV. The samples (crude methanolic extract of *B.biternata*) were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 m/z[8].

RESULTS AND DISCUSSION

HPLC analysis of methanolic extract of *B.biternata* leaves

HPLC is a suitable method for estimation of chemical constituents present in plant materials[9]. The retention time of standard quercetin was found to be 3.4981 and for gallic acid was 2.969 minutes (Figure No.1, 2 and Table No.1). The amount of quercetin and Gallic acid in *B.biternata* was found to be 4.480 and 0.80 mg ml^{-1} respectively. Other compounds such as luteolin, ferrulic acid and rutin were present in very low amount. Quercetin was found in higher quantities among them. Quercetin and Gallic acid is reported for its anti-malarial, anti-allergic, anti-hepatic, antitumor and immunomodulatory activities[7].

GC-MS investigations in leaf methanolic extract of *B.biternata*

Methanolic extract of *B.biternata* leaves was investigated by GC-MS and results showed the presence of four important compounds. Using computer searches on a NIIST Version–Year 2011 MS data library and comparing the spectrum obtained through GC-MS, 4 major compounds present in the plants extract were identified (Figure No.3 and Table No.2) as Fluorine, 5,9-Tetradecadienedioic acid, Pentadecanoic acid and 9-Octadecenoic acid. The presence of various phytochemicals contributed to the medicinal activity of this plant. The presence of bioactive compounds justified the use of *B.biternata* for various ailments. Many of the compounds identified using GC-MS have medicinal properties like anti-inflammatory, ant cancerous, hepatoprotective activity etc.[10]. Tetradecanoic acid and octadecanoic acidare reported to have potential antibacterial, antifungal and anti-inflammatory [11, 12] properties which could be effective in the management of bacterial, fungal and viral infections. The anti-inflammatory[13] activity would give relief in cases of sprained joints and general body pains as acclaimed by herbal medical practitioners. The presence of various bioactive compounds justified that the use of *B.biternata* for various ailments by traditional practitioners. So it is recommended as a plant of phytopharmaceutical importance. However further studies will need to be undertaken to ascertain its bioactivity fully. HPLC and GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant *B.biternata*.

Table No.1: HPLC peak results in *B.biternata* leaf methanol extract

S.No	Compounds	RT (Retention Time)	Area	Height	Amount (mg ml^{-1})
1	Quercetin	3.498 min	5042616	487893	4.480
2	Gallic acid	2.969 min	225087	19488	0.8

Table No.2: Compounds identified in *B.biternata* by GC-MS analysis

S.No	RT	Name of compound	Molecular formula	Class	Quan Lons	Peak Area	Amount/ RF
1	17.305	Fluorine	$C_{13}H_{10}$	Monoterpenoids	165.0	1.396 + 6	1395879 Counts
2	22.203	5, 9-Tetradecadienedioic acid	$C_{20}H_{34}O_4$	Diterpenoids	108.9	954289	954289 Counts
3	25.984	Pentadecanoic acid	$C_{16}H_{32}O_2$	Sesquiterpenoids	142.9	666252	666252 Counts
4	29.768	9-Octadecenoic acid	$C_{36}H_{71}NO_2$	Triterpenoids	264.0	334392	334392 Counts

Fig. 1 HPLC of Quercetin in Bidens biternata leaves methanolic extract

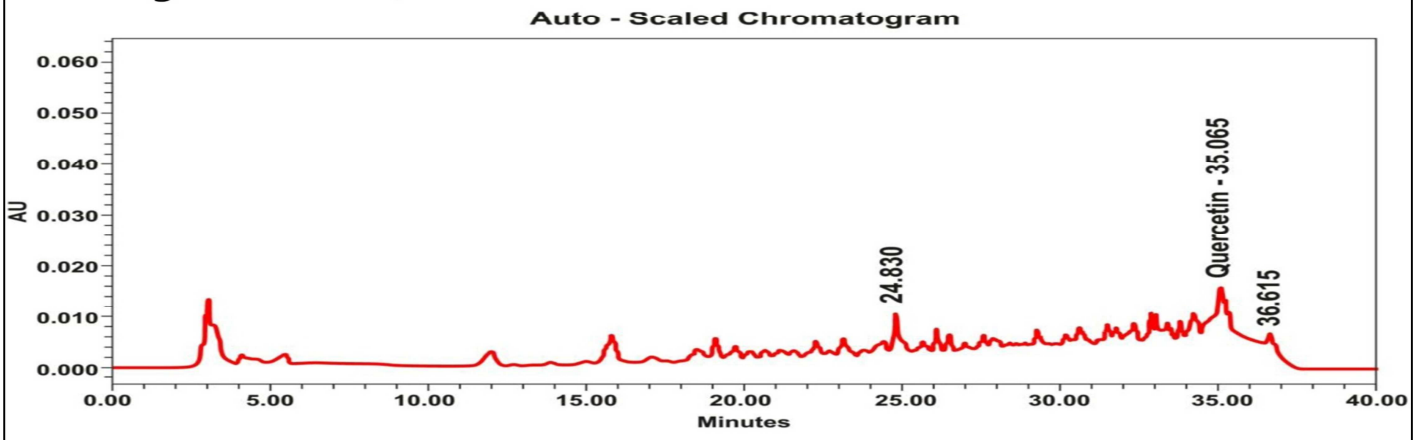

Fig. 2 HPLC of Gallic acid in Bidens biternata leaves methanolic extract

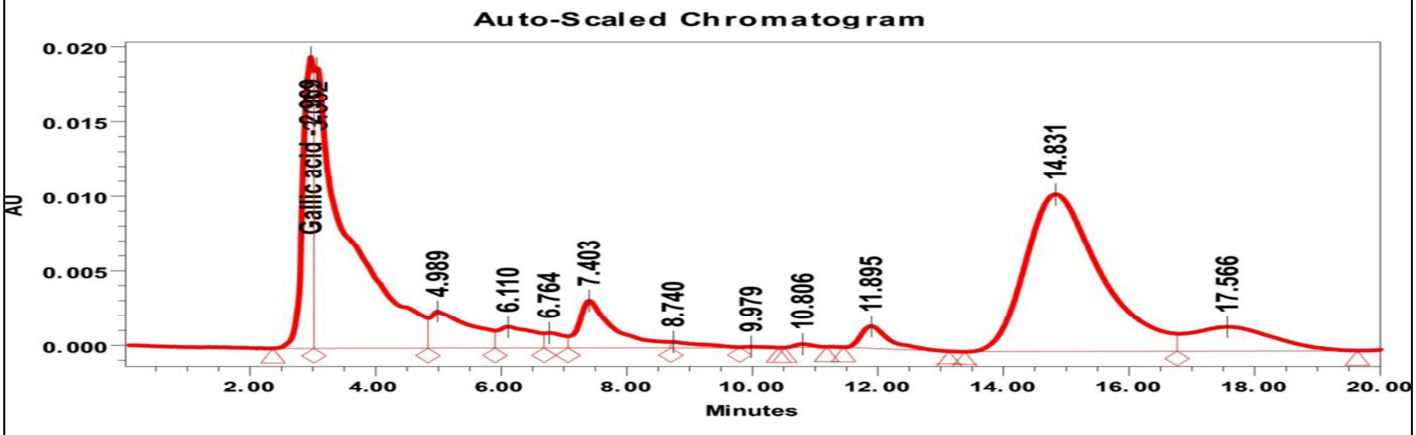

Figure No.1, 2: HPLC of quercetin and Gallic acid in *bidens biternata* leaves methanolic extract

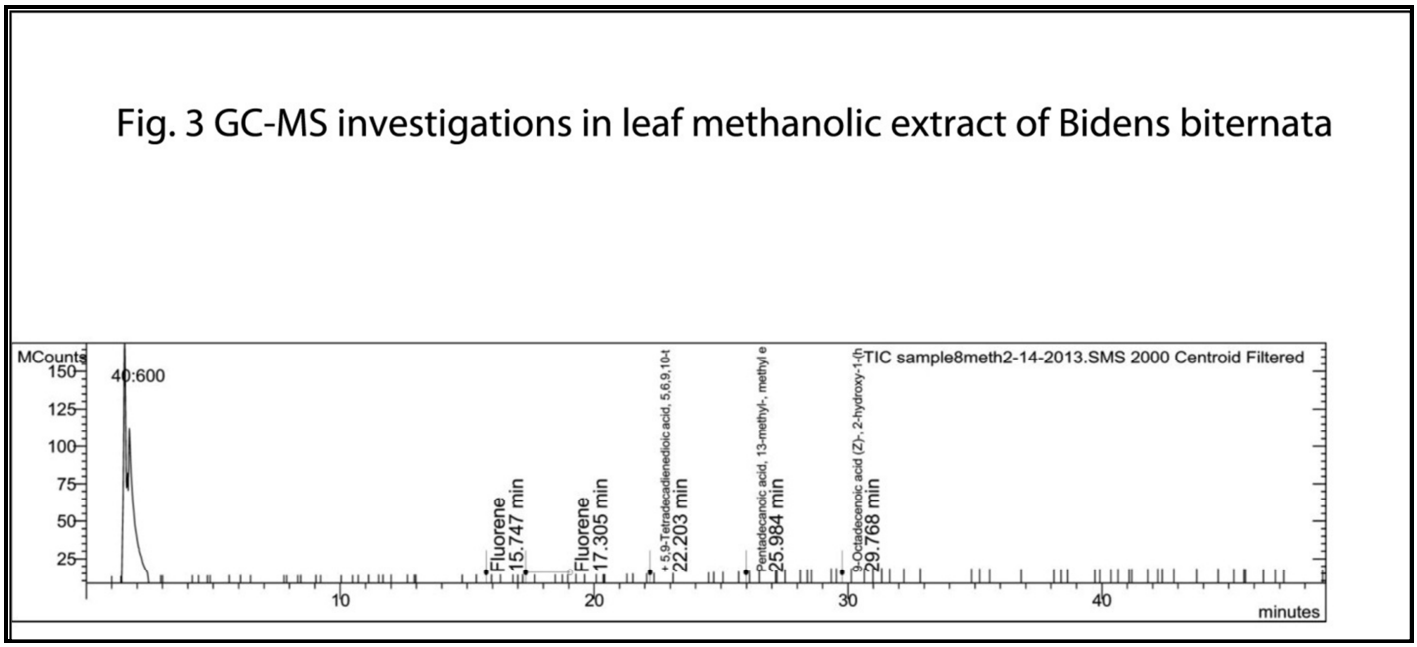

Figure No.3: GC-MS investigations in leaf methanolic extract of *B.biternata*

CONCLUSION

In the methanol extract of *B.biternata*, sufficient amount of quercetin and Gallic acid were identified using HPLC analysis, among them quercetin was found in higher quantities. GC-MS analysis of crude methanolic extract of *B.biternata* leaves showed the presence of four important compounds. *B. biternata* is a medicinal plant employed in the Indian traditional tribal system of medicine for curing inflammation, hepatitis and asthma due the presence of bioactive compounds. The bioactive compounds responsible for curing of various ailments and possess potential antimicrobial, antioxidant, anti-inflammatory, hepatoprotective and ant cancerous properties and leads to the isolation of new and novel compounds.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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