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# GC-MS profiling of phytocomponents from hydromethanolic fraction of *Bambusa tulda* leaf: a potential candidate for anti-diabetic activity

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***Abstract:*** ***Bambusa tulda*** **(Poaceae) being the most useful bamboo species in terms of construction is also associated with the health-promoting effects. The aim of this study is to carry out the identification of bioactive compounds from the hydomethanolic extract of** ***Bambusa tulda*** **leaf by Gas Chromatography and Mass Spectrometry (GC-MS). GC-MS analysis of hydromethanolic extract was done by standard photocol using MRM method in GC-MS revealing the presence of seven bioactive compounds like** ***p*****-hydroxy benzoic acid, salicylic acid,** ***p*****-coumaric acid,** ***o*****-coumaric acid, vanillic acid, ferulic acid and 2,4-Dihydroxybenzoic acid. All the identified phytocompounds except** ***o*****-coumaric acid have proven anti-diabetic activity. Hence the presence of these phytoconstituents substantiates the pharmacological activities of** ***Bambusa tulda*** **leaf particularly as a potential candidate for anti-diabetic activity. However, detailed studies are needed to elucidate their exact mechanism of action in various disorders.**

***Keywords:*** **Anti-diabetic;** ***Bambusa tulda*****; GC-MS; phytoconstituents.**

## I. INTRODUCTION

Plants have great potential sources for producing new drugs of benefit to mankind. There are many approaches in the research for new biologically active principles in higher plants. During the time frame 1/1981 – 6/2005, roughly 70% of new approved drugs for the treatment of human diseases were isolated directly from plants or related to natural product. An estimate of World Health Organisation (WHO) stated that around 85-90% of world population consumes traditional herbal medicines. It is thus clear that that impact of natural product research on drug development is extremely important. Plants are the rich sources of antioxidant which contain secondary metabolites such as phenolic and flavonoids compounds commonly which act as antioxidants with redox and metal chelating properties. Although the plants are used in Ayurvedic medicine for the treatment of ailments, there are only a few scientific evidences to support the extract compound responsible and the mechanism of action. The potential of bamboo species as a source of natural antioxidants has been documented by a number of researchers such as *Phyllostachys edulis* [1], *Sasa borealis* [2], *Bambusa vulgaris* 'Vittata' [3], [4], *Dendrocalamus strictus* [5], *D. hamiltonii* [6], *Bambusa balcooa* [7]. On the other hand only a few studies have been conducted to ascertain the anti-diabetic effect of bamboo [7], [8], [9], [10], [11].

*Bambusa tulda* Roxb. (Poaceae) designated locally as *Owa gubwai* (*Bodo*) and *Jati banh* (*Assamese*) [12]. The culms are dark green in color attaining a diameter of about 16.51 cm and attain a maximum height of about 17 m [13]. The tender shoots are edible [14]. Though commonly found in Southern Asia, it is endemic to North Eastern region and West Bengal in India [15]. Due to myriad documented uses of this species as raw materials for building purpose, furniture, handicrafts, paper and pulp, fishing rods, flute making, wind breakers etc, it not only grows in wild in the forest but also domesticated

in the villages [16], [17]. Traditionally *B. tulda* has a number of documented medicinal uses in Northeast India [18]. The fresh juice of shoots is effective in nails injury while the fermented shoots is used as an ingredient in formulations used in the management of tumors [15].

Though, *B. tulda* leaf have been explored with respect to its antioxidant activity, preliminary phytochemical analysis and the phenolics and flavonoids content, till date no compound are detected in *Bambusa tulda*. With this back ground, the present study might be the first one to identify active compounds in *B. tulda* leaf by using GC-MS analysis.

## II. MATERIALS AND METHODS

### *A. Plant material collection and extraction*

Leaf of *Bambusa tulda* were collected from the forests of Kokrajhar District, BTAD, Assam, India during July, 2016. After authentication by a plant taxonomist and a voucher specimen (Voucher No. DBT/BU/Bamboo/007) was been deposited at Bodoland University, Kokrajhar, BTAD, Assam, India.

The leaves were air dried and ground to powder using mechanical grinder. Ten grams of the each powdered samples were extracted in a Soxhlet apparatus using 70% aqueous methanol (v/v) separately (the ratio of plant material to solvent was 1:15 m/v) [19]. The extraction was carried out at boiling temperature for 6 h. The extract obtained was evaporated under pressure at 50°C to a constant weight and stored at 4°C until required. Before use, the extract was dissolved in double-distilled water (DDW) in desired concentrations [18].

### *B. GC-MS Analysis*

GC-MS analysis was carried out as per the standard protocol of Thomas et al [20] on a Perkin Elmer Turbo Mass Spectrophotometer (Norwalk, CTO6859, and USA) which includes a Perkin Elmer Auto sampler XLGC. Perkin Elmer Elite - 5 capillary column measuring 30cm × 0.25mm with a film thickness of 0.25mm composed of 95% dimethyl polysiloxane column was used. The carrier gas used was Helium at a flow rate of 0.5mL/min. 1µl sample injection volume was utilized. The inlet temperature was maintained at 250°C. The oven temperature was programmed initially at 110°C for 4 min, then an increased to 240°C for a 90 min run time. The MS transfer line was maintained at a temperature of 200°C. The source temperature was maintained at 180°C. GC-MS was analyzed using electron impact ionization at 70eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library. Measurement of peak areas and data processing were carried out by Turbo-Mass-OCPTVS-Demo SPL software.

### *C. Standard phenolics used*

Phenolic acid standards namely ferulic acid, 2,4-dihydroxybenzoic acid, o-coumaric acid, p-coumaric acid, p-hydroxybenzoic acid, salicylic acid, vanillic acid were acquired from Sigma Chemical Co., USA. The standard solutions in the range of 1mg per ml were prepared in 80% ethanol. The organic solvents used for the analysis were of chromatographic/MS grade and all the other reagents were of analytical grade. Water purified in the Milli-Q (Millipore) system was used to prepare the mobile phases. All mobile phases were filtered through membranes with a pore size of 0.45 µm.

### *D. Calibration Curve*

The calibration curve for total and individual phenolic acids was made by using different concentrations. Total phenols were expressed in gallic acid equivalents. Individual phenolic acids and flavonoid were identified and quantified by MRM method in GC-MS-MS knowing their parent mass m/z and most abundant fragmented daughters.

## III. RESULTS AND DISCUSSIONS

Plants have been a source of medicine since the time immemorial. Different plants are key ingredients of many herbal formulations that fight against various human related health ailments. Therefore, detailed study of plant materials is of prime focus for the advancement and quality control of folk formulations. Keeping this in mind, the present study was undertaken identify the bioactive compounds present in the hydromethanolic extract of *Bambusa tulda* leaf native to Bodoland, India using GC-MS.

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The phytoconstituents identified from *Bambusa tulda* leaf are represented in figure 1-5 and table 1. Among the identified phyto-compounds, the anti-hyperglycemic activity of p-hydroxybenzoic acid [21], salicylic acid [22], ferulic acid [23], vanillic acid [24], p-coumaric acid [25] is already documented. p-hydroxybenzoic acid was also known to have anti-fungal, anti-mutagenic, anti-microbial estrogenic activities [26]. 2,4-Dihydroxy benzoic acid have proven thyroid peroxidase inhibitory effect. Vanillic acid or 4-Hydroxy-3-methoxybenzoic acid, (E)-3-(4-hydroxy-3-methoxy-phenyl) prop-2-enoic acid possess astringent, antineoplastic and bacteriostatic activities [26]. (E)-3-(4-hydroxyphenyl)-2-propenoic acid or Benzothiophene derivatives are known to be estrogen receptor degraders [27].

**TABLE I: PHYTOCHEMICAL CONSTITUENTS OF *BAMBUSA TULDA* LEAF USING GC-MS ANALYSIS**

| Sl. No | IUPAC Name | Chemical Formula | Common Name | RT (min) | Height | Area | Peak Area% |
|---|---|---|---|---|---|---|---|
| 1 | 4-Hydroxybenzoic acid | $C_7H_6O_3$ | p-Hydroxy benzoic acid | 2.25 | 1219 | 212.95 | 37.53 |
| 2 | 2-Hydroxybenzoic acid | $C_7H_6O_3$ | Salicylic acid | 6.27 | 828 | 354.41 | 62.47 |
| 3 | (2E)-3-(2-hydroxyphenyl)-prop-2-enoic acid | $C_9H_8O_3$ | o-Coumaric acid | 5.51 | 1315 | 150.48 | 8.31 |
| 4 | (2E)-3-(4-hydroxyphenyl)-prop-2-enoic acid | $C_9H_8O_3$ | p-Coumaric acid | 5.22 | 9985 | 1660.15 | 91.69 |
| 5 | 2,4-dihydroxybenzoic acid | $C_7H_6O_4$ | β-Resorcylic acid | 4.81 | 165 | 25.25 | 100 |
| 6 | 4-Hydroxy-3-methoxybenzoic acid | $C_8H_8O_4$ | Vanillic acid | 3.32 | 115 | 16.63 | 100 |
| 7 | (2E)-3-(4-hydroxy-3-methoxy-phenyl)prop-2-enoic acid | $C_{10}H_{10}O_4$ | Ferulic acid | 5.78 | 329 | 80.55 | 100 |

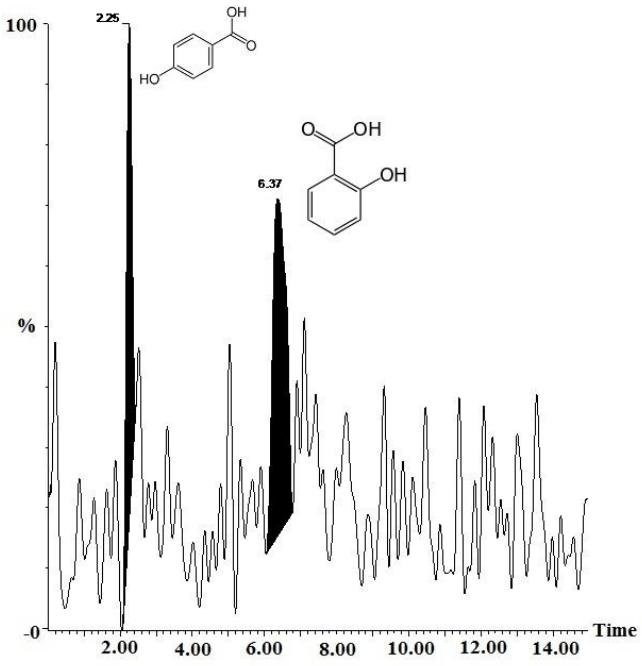


**Fig 1: GC–MS of hydromethanolic fraction of *Bambusa tulda* showing the presence of P- hydroxy benzoic acid and Salicylic acid**

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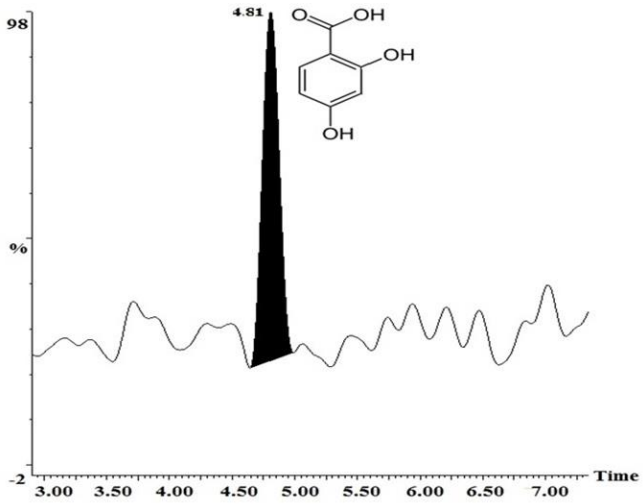


**Fig 2: GC–MS of hydromethanolic fraction of *Bambusa tulda* showing the presence of 2,4-dihydroxy benzoic acid**

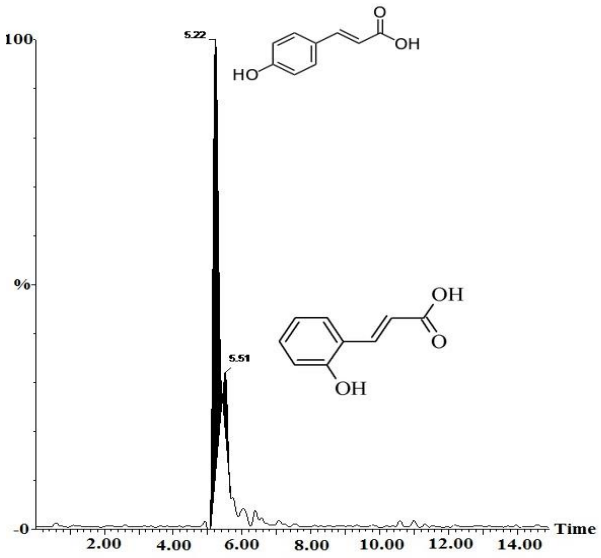


**Fig 3: GC–MS of hydromethanolic fraction of *Bambusa tulda* showing the presence of p-coumaric acid and o-coumaric acid**

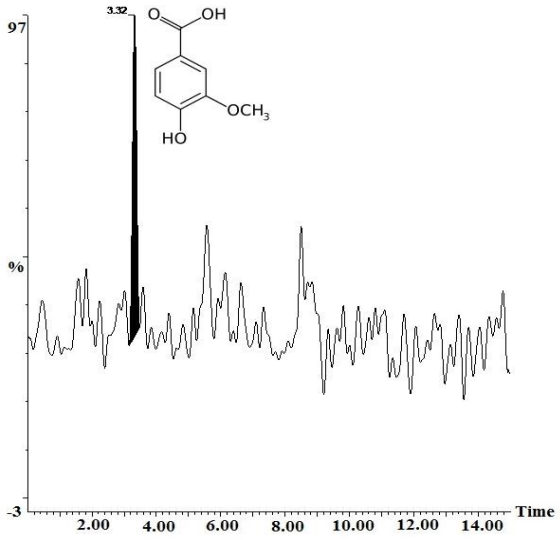


**Fig 4: GC–MS of hydromethanolic fraction of *Bambusa tulda* showing the presence of vanillic acid**

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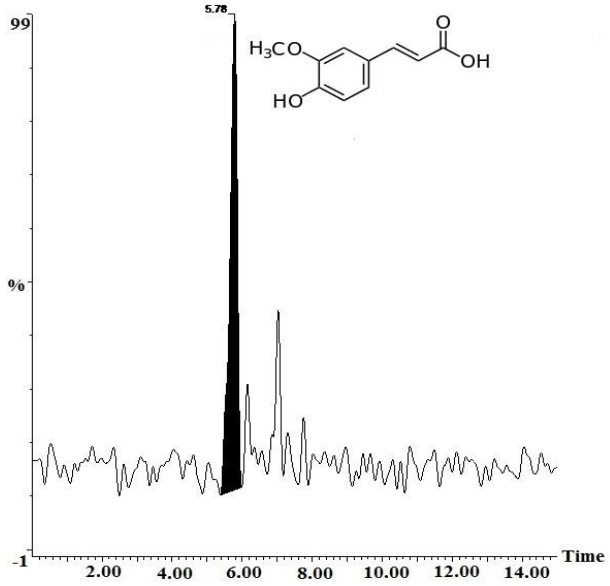


**Fig 5: GC–MS of hydromethanolic fraction of *Bambusa tulda* showing the presence of ferulic acid**

Gayathri and her co-workers [28] indicated that 2-hydroxy-4-methoxy benzoic acid isolated from *Hemidesmus indicus* is a promising compound for diabetes. Gallic acid possesses anti-melanogenic and antioxidant properties. 3-(4-Hydroxy-3-methoxyphenyl)-2-propenoic acid or Ferulic acid is known to have various therapeutic potentials such as cancer, diabetes, inflammatory diseases and in aging [29]. All identified compounds are known for their various therapeutic or pharmacological activities. As there are no reports found in public databases in case of *Bambusa tulda*. Although Goyal and his team [7] have reported the presence of gallic acids, gallic acid and few more compounds in *Bambusa balcooa*.

## IV. CONCLUSION

Thus, it can be inferred from the above study that *Bambusa tulda* leaf is rich source of various bioactive compounds which can either singly or in combination can help combat hyperglycemia and preventing diabetic complications occurring due to lipid peroxidation and free radicals. However, further studies at molecular level have to be carried out to prove its anti-diabetic efficiency and to bring out a drug molecule from the extract and to understand the molecular mechanism of function of the extract.

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