Study of Phytochemical Content and Antioxidant Properties of *Musa Balbisiana* Corm Extract

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Daimari *et al.*: Phytochemical investigation of *Musa balbisiana* corm extract

***Musa balbisiana* colla is an important plant native to India and many other Asian countries. Parts of this plant such as seeds, fruit pulp, inflorescence, pseudo-stem, and corm have been known to possess several medicinal values. In Kokrajhar district of Assam, the decoction of corm part of the plant is traditionally used as antidiabetic medicine. The present study was aimed to investigate the phytochemicals, antioxidants, trace element, and major compounds of the corm part of *Musa balbisiana*. Phenolic and flavonoid contents were estimated following standard protocols. The antioxidant activity of the plant was studied by ferric reducing antioxidant power assay, total antioxidant capacity, 1,1-diphenyl-2-picryl-hydrazyl, and lipid peroxidation scavenging assay. The phytochemical study revealed that the corm extract is rich in protein, carbohydrates, phenolics, and flavonoid content. Antioxidant study revealed strong free radical scavenging property of the crude corm extract of *Musa balbisiana*. The elemental analysis showed highest Zn content (0.2993 ppm) followed by Ni, Cu, and Mn. Pb, Cd, and Cr were not detected in the extract. GC-MS analysis showed difluroisocyanotophosphine to be the major compound of *Musa balbisiana* corm extract**

Key words: Phytochemicals, antioxidants, trace elements, gc-ms, musa balbisiana, kokrajhar

Natural products play an important role in the treatment of various diseases and drug discovery processes. Plants have been used in ethnomedicine system since ancient times to cure many diseases including diabetes[1]. Many therapeutically active plants are known to be used in the preparation of herbal medicine. Plants are rich sources of pharmacologically active substances which can be helpful in designing therapeutically active medicines for treating various ailments. Phytochemical content such as phenols or their oxygen substituted derivatives such as tannins while some may contain nitrogen or sulfur that are biologically active and useful for the treatment of diseases and preserve well-being in humans and animals[2]. It is estimated that about 70-80% population of tropical countries rely on medicinal plant as the source of medicine, and the tendency to use ethnomedicine is also gradually increasing in other developed countries because of its healthy effects[3]. In many developing countries like India, several plants and its derivatives have been used traditionally for the treatment of many diseases[4,5]. The state of Assam is one of the 29th states of India blessed with rich flora and fauna. With the geographical location 89°50/ E to 96°10/ E and 24°30/ N to 28°10/ N Assam state is one among the richest biodiversity zones of North-East India[6]. Most of the people, especially ethnic tribal groups living in this state perform various traditional health-care practices and rely on traditional medicines as the primary source of healthcare needs. According to World Health Organisation (WHO), the use of traditional herbal medicine has spread not only in the developing countries but also in the industrialized ones, as a complementary way to treat and prevent illness[7]. Natural products could be a potential source of drugs for humans or livestock, and also the products and their analogs can act as intermediates for the synthesis of useful drugs[8]. Plant possesses many phytochemicals with various bioactivities including antioxidant, anti-inflammatory, anticancer, antiviral, antidiabetic, anthelmintic, etc[9-11]. *Musa balbisiana* colla belonging to the family Musaceae is an important monocotyledonous herb having several religious and medicinal values[12]. Among the different varieties of Musa species, *M. balbisiana* is native to India and has been utilized as folk medicine since ancient times[13]. Various parts of this plant are reported to be used for the treatment

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of diseases including diabetes, diarrhea, scabies, helminthiasis, stomach problem, and inflammation[14]. Several studies have reported the pharmacological properties of different parts of *M. balbisiana*. However, we did not find any literature regarding the corm extract of the plant. Therefore, the present study has been designed to study the phytochemical profile and antioxidant activities of *Musa balbisiana* corm extract. Sample plant was collected from Kokrajhar district of Assam and identified in the department of Botany, Bodoland University. The sample plant was identified as *Musa balbisiana* Colla and the identification number is BUBH2018067.

The corm parts of *M. balbisiana* were collected from Kokrajhar district of Assam with the help of local people. The plant part was brought to the laboratory and washed with distilled water and dried in hot air oven below 50° and processed for preparation of crude extract. Dried plant parts were ground into powdered form and soaked into 80% methanol. Solution was filtered after 72 hrs of soaking and fresh solvent was added. The process was repeated three times and the filtrate obtained was evaporated in a rotary evaporator. Dry, semi-solid *M. balbisiana* methanolic extract (MBME) obtained was kept at -20º for further use. The process was followed as per the method described in our earlier publication[6].

The protein content of the plant extract was estimated following the Lowry method[15]. The presence of total carbohydrate content in MBME was estimated following the Anthrone method[16]. The total phenolic content (TPC) was estimated using Folin-Ciocalteu reagent[17]. The amount of TPC was calculated from a calibration curve of gallic acid and results were expressed as µg gallic acid equivalent (GAE)/mg plant extract. The total flavonoid content (TFC) was determined following the method of Ordonez *et al.*[18]. TFC was calculated from the standard curve of quercetin, and the values were expressed as µg quercetin equivalent (QE)/mg plant extract.

The total antioxidant capacity (TAC) of MBME was done by phosphomolybdate method using ammonium molybdate[19]. The reaction mixture was incubated at 95° for 30 min and absorbance measured at 695 nm against a blank solution. TAC was expressed as µg ascorbic acid equivalent (AAE)/mg plant extract.

Ferric reducing antioxidant power assay (FRAP) was performed following the method of Iloki-Assanga *et al*[20]. The FRAP activity of MBME was compared with the standard ascorbic acid and values were expressed as µg Fe^{2+} equivalent (FE)/mg plant extract. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity of MBME was estimated using DPPH as described by Mamta *et al.*[21]. Lipid peroxidation inhibitory activity was studied following the modified thiobarbituric acid reactive species (TBARS) assay to measure the lipid peroxide formation using egg yolk homogenates as lipid-rich media[22]. The colouration of the assay mixture was measured at 532 nm. The 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) free radical scavenging activity of MBME was measured following the method of Re *et al*[23] using gallic acid as the standard reference. Seven elements such as lead (Pb), chromium (Cr), nickel (Ni), cadmium (Cd), copper (Cu), manganese (Mn), and zinc (Zn) were analyzed using Atomic Absorption Spectroscopy (AAS) following the method of Zheljazkov and Nielson with slight modification[24]. Briefly, 1 g of plant powder was digested with concentrated HNO_3 at 90º for 45 min. The temperature is then slowly increased up to 100º and boiled for 6-7 h by the addition of HNO_3 till complete digestion. The process was continued till the solution became colourless and the end volume was maintained at 10 ml. The solution was then diluted to 100 ml of distilled water and then filtered using Whatman filter no. 1. The solution was then analysed for trace elements at AAS, Shimadzu AA-7000. The chemical composition of MBME was determined using the GC-MS system (TQ-8030 Shimadzu Corporation Kyoto, Japan) as described by Kalita *et al.*[12]. All the statistical calculations were carried out in Microsoft excel. IC_{50} (concentration of plant extract at 50% inhibition of activity) values were calculated using SPSS software. Correlation study and figures were drawn using OriginPro software. All the experiments were represented with mean ± standard deviation (SD). Statistical significance was calculated at $P \leq 0.05$ level.

Medicinal plants are rich sources of phytochemicals and secondary metabolites. In the present study, phytochemical content of methanolic crude extract of *M. balbisiana* corm was analyzed. Fig. 1 showed the moisture content, crude extract obtained, phenolic, and flavonoid content of MBME. The plant part (corm) was found to contain high moisture content (75.30%) while the crude extract obtained was only 2.35% after three rounds of extraction process. The methanolic extract of corm was found to contain high protein content (79.65±1.22 µg/mg extract) while the carbohydrate content was found to be almost half of

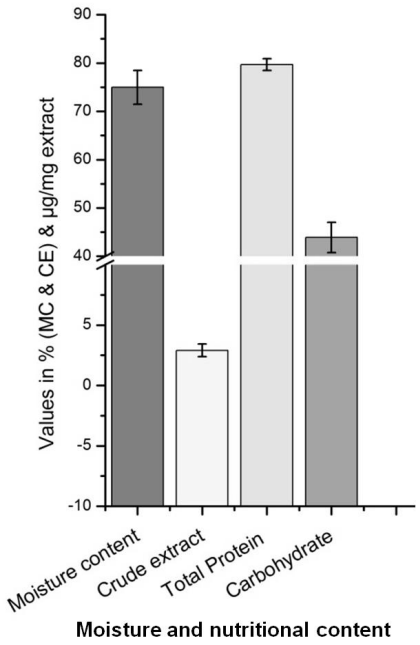

Fig. 1: Moisture content (MC), crude extract (CE) and phytochemical contents of *Musa balbisiana*. MC and CE were represented as percentage (%) of moisture per 100 g plant and % of methanolic extract obtained per 100 g of dry plant powder. Values were represented as mean ± SD.

the protein (43.9±3.13 µg/mg extract). Statistical analysis showed significant difference (at P≤0.05 level) between the protein and carbohydrate content of *M. balbisiana.* MBME also showed considerable amount of phenolic and flavonoid content. *M. balbisiana* corm extract showed higher TPC content than TFC. The TPC and TFC were found to be 10.93±1.71 µgGAE/mg and 4.72±0.33 µgQE/mg plant extract, respectively. Similarly, the TAC and FRAP activity was found to be 24.43±2.42 µgAAE/mg extract and 87.69±3.27 µg FeE/mg extract, respectively (fig. 2a). The methanolic crude extract of *M. balbisiana* was found to possess strong free radical scavenging activity. The IC_{50} values for DPPH, ABTS, and TBARS assays were found to be 287±17.45 µg/ml, 102.89 ±4.16 µg/ml, and 60.11±0.86 µg/ml, respectively. The standard reference chemical showed 3.64±0.365 µg/ml, 1.76 ±0.05 µg/ml, and 37.65±0.91 µg/ml for DPPH, ABTS, and TBARS antioxidant assays, respectively (fig. 2b). Fig. 3 showed the metallic content of the methanolic crude extract of *M. balbisiana*. A total of seven trace elements were analysed out of which Zn was found to be in highest concentration (0.2993 ppm) followed by Ni (0.03 ppm), Cu (0.0124 ppm), and Mn (0.0121 ppm). On the contrary, three toxic elements, Cd, Pb, and Cr were not detected in the analysis. All the metallic contents were found to be in the permissible limit.

GC-MS analysis identified five compounds from the corm extract of *M. balbisiana*. Table 1 showed the GC-MS parameters of all the five compounds identified from the plant. The five major compounds identified were difluoroisocyanatophosphine (1), 2'-methoxy-2,3',4,4'-tetrabromodiphenyl ether (2), isophthalic acid, ethyl 6-ethyloct-3-yl ester (3), phthalic acid, 2-(4-chlorophenoxy)ethyl hexylester (4), and pseudodiosgenin diacetate (5). The chromatograms of retention time and m/z intensities are presented in fig. 4a & b. The compound which shows highest peak was identified as Difluoroisocyanatophosphine and has the molecular formula CF_2NOP and molecular weight of 110.987 with the retention time of 5.785 (Table 1).

Phytochemical analysis revealed high protein, carbohydrate, phenolic, and flavonoid content in the methanolic corm extract of *M. balbisiana*. Similar to our study, Mahmood reported high content of TPC

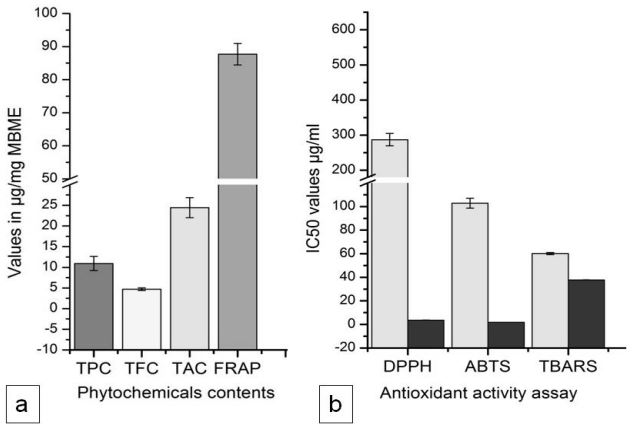

Fig. 2: Phytochemical and antioxidant properties of methanolic extract of *Musa balbisiana*. Values of TPC, TFC, TAC and FRAP were expressed as µg/mg extract and (a) DPPH, ABTS, and TBARS assays were represented in IC_{50}.

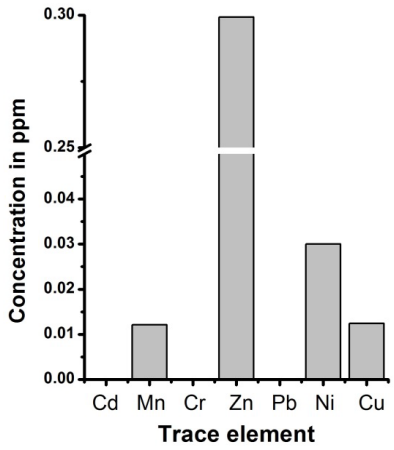

Fig. 3: Composition of trace elements of corm extract of *musa balbisiana*. Cd - cadmium, pb - lead, cr - chromium, zn- zinc, cu - copper, ni - nickel, Mn - manganese

and less content of TFC in *M. paradisiaca*[25]. The presence of phenolic, flavonoid, and glycosides was also reported in *M. acuminata*[26]. Free radicals are generated continuously in our bodies as a result of several metabolic processes. Our body has an innate capacity to neutralise these free radicals. However, our innate antioxidant capacity to neutralize free radicals is limited to certain concentration and beyond that concentration our body fails to neutralize it. With rich phytochemicals and secondary metabolites, plants act as a source of antioxidant molecules which can increase the antioxidant property of our body[27,28]. Several studies have reported the antioxidant and free radical scavenging properties of several medicinal plants[29,30].

Metallic content plays a vital role in our day to day life. It can be harmful or toxic if it crosses the permissible level. In our analysis, we have found that the toxic heavy metals such as Pb and Cd were below the WHO permissible limits[31]. The study thus suggests that the corm extract do not contain metallic content at the range that is toxic to human consumption. Trace elements are important for several biological activities and human health. Trace elements such as Zn, Cu, Fe, and Mg functions as cofactors for many proteins and enzymes[32,33]. On the other hand, heavy metals like Pb, Cd, Ni, and Cr do not have any beneficial roles but are known to be toxic to the health[34]. GC-MS study of corm extract of *M. balbisiana* showed five major compounds from the plant. All the compounds were reported for the first time from *M. balbisiana* corm extract. We did not find any literature about the pharmacological properties of the identified compounds. Similarly, several ester compounds were reported from the fruit peel extract of Musa species[35]. Recent studies by Yingyuen *et al.*[36] reported the presence of rutin as the major compound in the leaves of *M. balbisiana*. Similarly, Kumari *et al.*[37]

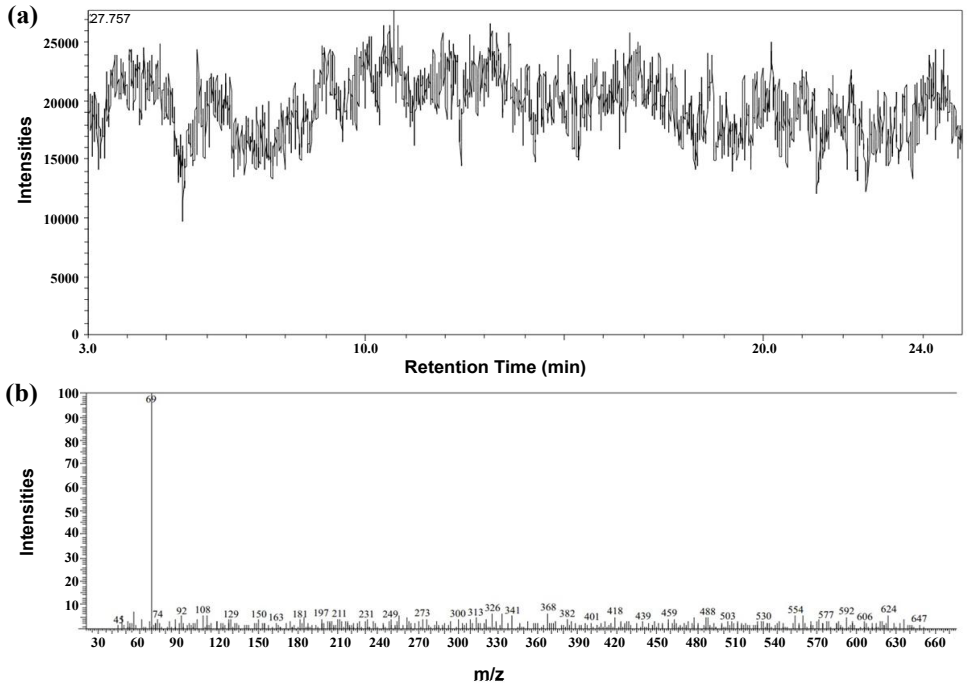

Fig. 4: GC-MS chromatogram of *Musa balbisiana* corm extract; a) Retention time and b) m/z spectrum of *Musa balbisiana* compounds

TABLE 1: GC-MS PROFILES OF THE COMPOUNDS IDENTIFIED FROM CORM EXTRACT OF *MUSA BALBISIANA*

Name of the compound	Retention time	m/z	Area (%)	Height (%)	Mol. weight (g/mol)	Mol. Formula
1. Difluoroisocyanatophosphine	5.785	69.25	8.77	24.94	110.987	CF_2NOP
2. 2'-Methoxy-2,3',4,4'-tetrabromodiphenyl ether	9.793	569.00	10.42	20.49	515.8	$C_{13}H_8Br_4O_2$
3. Isophthalic acid, ethyl 6-ethyloct-3-yl ester	15.100	177.00	30.20	21.14	334.4	$C_{20}H_{30}O_4$
4. Phthalic acid, 2-(4-chlorophenoxy)ethyl hexyl ester	20.460	193.00	29.98	16.67	404.9	$C_{22}H_{25}ClO_5$
5. Pseudodiosgenin diacetate	23.588	81.00	20.63	16.76	498.7	$C_{31}H_{46}O_5$

reported seven major phenolic content from the fruit pulp of *M. balbisiana* and also revealed that the extract contains cardioprotective activity.

Medicinal plants own the impression of significant success in the traditional system of disease treatment. This paper sheds a light on the phytochemical profile and antioxidant properties of the plant. Considering the different activities of plants, it therefore, justifies the traditional use of medicinal plants in the treatment of different health disorders. *Musa balbisiana* corm extract showed rich phytochemical and antioxidant properties as well as low toxic elements which are indicative enough for rich medicinal values of the plant. However, proper pharmacological studies and characterisation of bioactive compounds need to be carried out to understand the detailed mode of action.

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Conflict of Interest:

Authors declares no conflict of Interest.

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