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RESEARCH ARTICLE

Musa balbisiana Colla, an Ethnomedicinal Plant use as Abortifacient among Karbi Tribe of Assam, North East India: An *In Silico* Characterization

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Abstract

Background: *Musa balbisiana* Colla is an important ethnomedicinal plant known among the Karbi tribe of Assam for managing unintended pregnancy. **Aim:** The present study highlighted the physicochemical properties and bioactive compounds present in the medicinal extract along with its ADME properties that predicts the potential compounds as having drug-likeness properties. **Methods:** Physicochemical analysis was done by simple determination techniques; qualitative and quantitative test for the presence of bioactive compounds in methanol extract were done following standard procedure. Functional groups and potential bioactive compound were characterized by FTIRand GC-MS analysis. The physicochemical, lipophilicity and drug-likeness properties of the compounds, identified from the extract were studied using an online server for ADME. **Results:** Analysis of physicochemical properties (pH, LOD, extractive value, flowability properties) reveals the nature and quality of the powders drugs. Bioactive compounds like flavonoid, tannins, phenolic content, saponin, alkaloid and steroid were present; the plant have good amount of total flavonoid contents (162.733 mg QE/g), phenolic content with 396.33 mg GAE/g and total alkaloid content as 44 mg/g. Bioactive compounds like 1,2-Benzenedicarboxylic acid, Butyl octyl ester; Dibutyl phthalates;1,2-Benzenedicarboxylic acid, Dipropyl ester; Phthalic acid, Decyl isobutyl ester *etc*. were reported to show reproductive toxicity. *In silico* SwissADME analyses revealed the identified compounds follows Lipinski's rule and shows drugs like characteristics with good bioavailability score. The bioavailability radar revealed the compounds to have drug-like property. **Conclusions:** Here from the study, we can conclude that rhizomes of *M. balbisiana* are potential source of phytomedicines. The presence of important bioactive compounds with its ADME properties and bioavailability radar can predict a compound as having drug-likeness and may consider as pharmaceutically important plant based oral drugs in the near future.

Major Findings: Rhizome of *M. balbisiana* Colla is enriched with potential bioactive compounds with efficient abortifacient activities. The identified compounds, as per SwissADME parameters suggest the compounds as having drug-like property and may be considered as pharmaceutically important plant based drugs (orally active) in the near future.

Keywords: Abortifacient, Bioactive Compound, *In Silico*, Medicinal Plants, Rhizome, SwissADME

Abbreviations: GC-MS = Gas Chromatography- Mass Spectroscopy; RT = Retention Time; SIF = Sophisticated Instrumentation Facility; TFC = Total Flavonoid Content; TPC = Total Phenolic Content; TAC = Total Alkaloid Content; ADME = Absorption, Distribution, Metabolism, and Excretion

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1. Introduction

In silico analysis helps in identification of compound and its structure; thereby optimized the primary activities of Absorption, Distribution, Metabolism and Excretion (ADME)[1]. Evaluations of phytocompounds from medicinal plants are the most important in medicinal plant research[2,3]. *M. balbisiana* Colla, belong to the family *Musaceae* and in Assam it is commonly known as *Athiakol* or *bhimkol*, a cultivated clone without fertile seeds[4]. The Karbi, an indigenous tribe of Assam is well known for their therapeutic utilization of plants drugs for various purposes[5]. In Karbi dialect, it is called as *nusador* and the rhizome's extract have been used as an abortifacient for unintended pregnancy. The knowledge of medicinal plants therapy for reproductive health care among the Karbitribehas been in practiced since time immemorial[6]. The present study deals with analysis of physicochemical and bioactive compounds along with *in silico* characterization of the compounds present in *M. balbisiana,* traditionally used as abortifacient among Karbi tribe of Assam, North East India.

2. Materials and Methods

2.1 Collection of Plant Material and Sample Preparation

Fresh rhizome and inflorescence of *M. balbisiana* Colla, were collected from Karbi Anglong district, Assam, India (25°55' 29.658" N and 93° 30'31.9968" E) for the experiments. It is widely distributedin the studied areas and because of its economic utilities; it is also grown in their home stead garden. The plant morphology was studiedfor identifications purposes and the flowering twigs along with its inflorescences and rhizome were collected from the study area following guidelines for plant collections. The plant was identified following standard literatures and the herbarium specimen was deposited in the Herbarium of Gauhati University, Guwahati, Assam, India[4,7]. The rhizome was cut into pieces and dried properly and after drying it was grounded into powder and stored for further analysis. The powders were used for the analysis of various physicochemical properties using simple determination techniques[8].

2.2 Preparation of theExtract and Further Biochemical Analysis

10 g of samples powder were used for continuous hot extraction (Soxhlet) using methanol (250ml) and the extract obtained was further used for qualitative and quantitative analysis.

2.2.1 Qualitative and Quantitative Phytochemical Analysis

Preliminary phytochemical screening was done to analyses the bioactive compound present in the plant sample following standard procedure with a little modification to test for the presence of flavonoids, tannin,saponins, alkaloids,terpenoids, phlobatannins, steroids and phenolic compounds[9-11]. The qualitative result are expressed as + for the presence and as – for absence of phytochemical compound.Further quantitative determination were done for alkaloids, flavonoids and total phenolic content following standard proceduresince these compounds were mostly studied as having abortifacient and antifertility effect[12-15].

2.2.2 FTIR and GC-MS Analysis of Bioactive Compounds

The FTIR spectrum of dry powder of the plant samples were recorded using KBr pellet method, recorded in a FT-IR spectrometer (Bruker, Alpha 2, Germany), while GC- MS of the methanol extract was recorded in Perkin Elmer GC model Clarus 680 with softwareTurbo Mass ver 5.4.2 and library search with NIST-2008 which was performed at Sophisticated Instrumentation Facility (SIF), School of Advanced Sciences, Chemistry division, VIT, Vellore and the compounds were identified using the database of National Institute Standard and Technology library, 2008(NIST)[16].

2.3 *In Silico* ADME

The physicochemical, lipophilicity and drug-likeness properties of phytochemicalcompounds, identified from the methanol extract of the plant sample were studied usingan online server for ADME (absorption, distribution,metabolism, and excretion) prediction, SwissADME. Drug likeness properties of the selected compounds were evaluated using the bioavailability radar to understand their oral bioavailability. The

most significant features of ADME- Pharmacokinetics parameters (GI absorption, BBB permeability and skin permeation), Inhibitors (CYP1A2, CYP2C19 and CYP2C9) and drug-likeness properties (Bioavailability score and Lipinski's rule) were considered in this study (Table 3).

3. Result and Discussion

3.1 Traditional Use of the Ash Filtrate (Rhizome) of *M. balbisiana* Colla as an Abortifacient

Every parts of *M. balbisiana* (leaves, pseudostem, rhizomes, fruits, cortex) are considered important by the Karbi tribe since it serves many purposes- in rituals, religious ceremony, as packing materials, as food, as fodders and as medicines. Likewise the rhizomes were reported to be use as abortifacient among the Karbi tribe for managing unintended pregnancy. Fresh rhizomes were chopped into pieces, dried and charred into ashes. The ashes were then filtered through "phelobisir", a bamboo made handicraft use as distiller for alkali and the filtrate were collected (Figure 1). Thefiltrate, a highly concentrated alkaline solution were use as a measures for getting rid of unintended or risk pregnancy at early conception. Ash filtrates of 10 ml (approx.) weretaken orally for thrice a day for 2 to 3 days during the initial stage of pregnancy to induced abortion.According to traditional birth attendant, the observation of traces of blood indicates the start of termination of risk pregnancy.Although, the measures are not openly practiced but whenever required, this are made easily available by the local herbal practitioner, elderly women, birth attendant *etc.*

3.2 Physicochemical and Phytochemical Analysis of Rhizome Extract of *M. balbisiana,* Used an Abortifacient

Physicochemical analysis reveals the nature and quality of the powders drugs that paved way to understand its pharmaceutical consideration. The quality control of the extract formulation and dose accuracy is very crucial and analysis of physicochemical properties helped to understand the nature of the powdered drugs. The powder drug is slightly alkaline with pH value recorded at 8; it has moderate swelling index (2.42mg/g) and help in assessing the capacity of water uptake by the powder (Table 1). The moisture content is fairly high (15.9 %) since rhizome generally contains lots of water. The powder flowability properties was determined through bulk (0.20 g/ml) and tapped density (0.23 g/ml) and confirmed by carr's index (14.28 %) and Hausner ratio (1.15). These valueshow moderate flowability indicating moderate to good parameters for assessment of quality of medicinal plants as per WHO standard. Acarr's index below 15 % and Hausner's ratio below 1.25 is considered as good flowability[17,18]. The water-soluble extractable matter was fairly high (27.68 %) in comparison to methanol soluble extractive (27.46 %) and least in ethanol extract (1.1 %). The amounts of extracts obtainedare indicative of approximate measures of their chemical constituents of crude drugs and gives an idea of which solvent is to be use for extraction to yield the maximum extract. These are good measures, since, the Karbi tribe traditionally prepared the extract in water and no other solvent were used. Hence, the more the extractive value (in water), the higher would be the extract of biologically active compound.

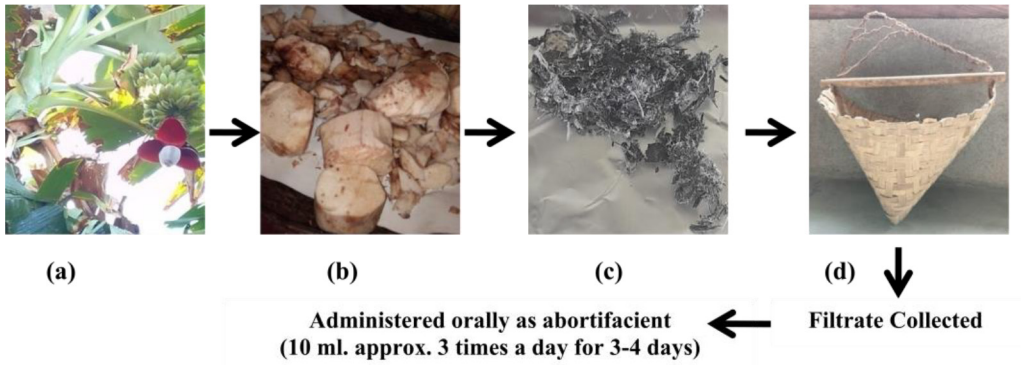


Figure 1. Traditional method for obtaining ash filtrate from rhizome of *Musa balbisiana* Colla to be used as abortifacient.

Qualitative analysis of the methanol extract reveals the presence of phytocompounds like flavonoids, tannins, saponins, alkaloids and terpenoids which are proven efficacy having potential abortifacient as well as other remarkable pharmacological activities (Table 2).The rich phytochemical compounds along with potassium and chloride accumulation in the plant provide potential medicinal uses including combating metabolic disorders and oxidative stress[19,20]. Quantitatively, the plant's rhizome have good amount of total flavonoid (162.733 mg QE/g), phenolic (396.33 mg GAE/g) and alkaloid content with 44mg/g respectively. It acts as important contributors to antioxidant activity exhibiting promising biological effect due to their ability to scavenge reactive oxygen species[21].

3.3 Analysis of Potential Bioactive Compounds Present in the Rhizome Extract Identified through GC-MS along with its Biological Activities

FTIR and GC-MS revealed the presence of potential bioactive compounds in methanol extract of *M. balbisiana*, S3-(19ES-0597) and its related functional groups. The potential compounds showing reproductive toxicity and other biological activities are depicted in Table 3. The compound presented in Table 3 shows one or the other biological effects on reproductive health. The most important bioactive compounds that shows acute reproductive toxicity identified from the plant is Dibutyl Phthalate (DBP). It is one of the most widely-used phthalates as a plasticizer known to have cytotoxic effects on developing fetus, induces abortion and shows abnormal fetal growth showing reproductive toxicity, fetotoxicity and teratogenicity[22-25].Other compounds identified in the extract includes, 1, 2-Benzenedicarboxylic acid, Butyl methyl ester (RT 19.480), 1,2-Benzenedicarboxylic acid, Butyl octyl ester (RT 20.035) and 1,2-Benzenedicarboxylic acid, Dipropyl ester (RT 21.521); these are the derivatives of phthalates or phthalic acid and reported to show one or the other biological functions[26,27]. Exposure to phthalates during pregnancy produced serious adverse effects like miscarriage and encounter with the derivatives of phthalates ester can cause reproductive and developmental toxicity[28,29]. Phthalates are known as potential reproductive toxicants as it has endocrine-disrupting properties and *in utero* phthalate exposure is associated with a shorter duration of pregnancy[30]. These exposures are of particular concern during pregnancy, as it may affect both maternal and fetal health[31]. In modern societies, the effects of Environmental Endocrine Disruptors (EEDs) on fertility and reproduction are growing concerns and DBP is a member of EEDs with the estrogenic property and may affect the steroidogenic capacity in human ovaries, thus affecting the female reproductive system[32,33]. Hence, as the phytocompounds showing potential reproductive toxicity are present in the rhizome of *M. balbisiana*, so its use during pregnancy is not recommended. The folk using the rhizome extract rightly claimed as ethnomedicines for abortifacient.

Table 1. Analysis of physicochemical properties rhizome's extractof *M. balbisiana* Colla

S. No.	Physicochemical Parameter	Value
1	pH	8
2	Swelling index	2.42 ml/g
3	Loss on drying	15.9 %
4	Total ash	34.0 %
5	Water soluble ash	37 mg/g
6	Water soluble extractive	27.68 %
7	Methanol soluble extractive	27.46 %
8	Ethanol soluble extractive	1.1 %
9	Bulk density	0.20 g/ml
10	Tapped density	0.23 g/ml
11	Hausner ratio	1.15
12	Carr's index	14.28 %

Table 2. Analysis of bioactive compounds present in rhizome's extract of *M. balbisiana* Colla

Type and Nature ofTest	Flavonoid	Phenolic	Alkaloid	Saponin	Tannin	Terpenoid	Steroid
Qualitative	+	+	+	-	-	+	+
Quantitative	TFC (162.733 mg QE/g)	TPC (396.33 mg GAE/g).)	TAC (44 mg/g)				

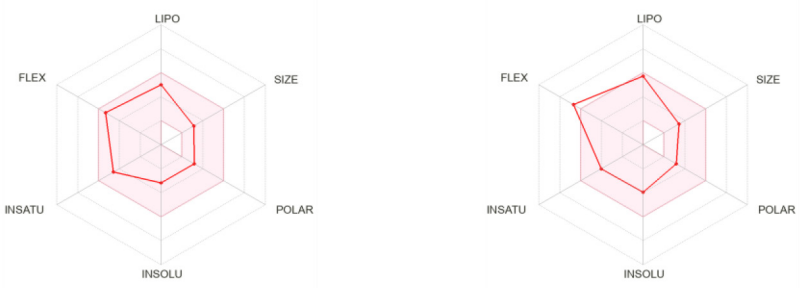
+ indicate presence of the compound and- indicate absence of the tested bioactive compound

3.4 SwissADME Properties of the Compounds Identified in the Rhizome Extract

The SwissADME properties of the identified compounds were studied to identify the most promising compounds with limited risk of drugs attribution in the later phase[35]. The analysis of ADME properties of bioactive compounds illustrated good oral absorption with good bioavailability score (0.55), signifying the orally administered compounds reaches systemic circulation based on Lipinsk's rule (Table 3)[36]. The pharmacokinetics parameters evidences most of the compounds having high Gastro-Intestinal (GI) absorption characteristics while Phthalic acid, Methyl nonyl ester and Phthalic acid, Decyl isobutyl ester have low GI absorption properties. The compounds presented in Table 3 have abilities to cross Blood Brain Barrier (BBB) except Phthalic acid, Methyl nonyl ester and Phthalic acid, Decyl isobutyl ester whereas Phthalates can cross the placental and BBB which can directly influences the physiological and anatomical development of fetus[37]. Drug-likeness is the property that qualitativelyassesses the chance for a molecule to become an oral drug with respect to bioavailability, advanced enough to considered oral drug-candidates[22]. One of the parameter of drug-likeness properties, the Lipinski's rule, in Table 3, showed that 5 compoundsshow 0 violations while 3 compounds (Phthalic acid, Methyl nonyl ester; Phthalic acid, Decyl isobutyl ester and Phthalic acid, Isobutyl octyl ester) have 1 violation (MLOGP>4.15) which means it has high lipophilicity (logP>5).

Table 3. Compounds identified in methanolic extract of *M. balbisiana* Colla and its biological activities

S.No.	Compound Name with Formula	Molecular Weight (MW) in g/mole	Retention Time (mins)	Biological Function
1	1,2-Benzenedicarboxylic acid, Butyl Methyl Ester ($C_{13}H_{16}O_4$)	236	19.480	Derivatives of phthalates; Reproductive toxicity[25,26]
2	Dimethyl Pthalate ($C_{10}H_{10}O_4$)	194	19.480	Fetotoxicity, Teratogenicity, and Testicular damage[26]
3	Phthalic Acid, Methyl Nonyl Ester ($C_{18}H_{26}O_4$)	306	19.480	Toxic to foetal development[25]
4	1,2-Benzenedicarboxylic acid, Butyl Octyl ester ($C_{20}H_{30}O_4$)	334	20.035	Derivatives of phthalates; Reproductive toxicity; Antimicrobial and Antifouling property[25,26]
5	Phthalic Acid, Decyl Isobutyl Ester($C_{22}H_{34}O_4$)	362	20.035	Toxic to fetal development[25]
6	Dibutyl Phthalate ($C_{16}H_{22}O_4$)	278	20.410	Fetotoxicity, Teratogenicity, and Testicular damage[26,34,35]
7	1,2-Benzenedicarboxylic acid, Dipropyl ester ($C_{14}H_{18}O_4$)	250	21.521	Derivatives of phthalates; Reproductive toxicity[25,26]
8	Phthalic Acid, Isobutyl Octyl Ester ($C_{20}H_{30}O_4$)	334	21.521	Toxic to fetal development[25]



(a) 1, 2- Benzenedicarboxylic acid, Dipropyl ester (b) Dibutyl phthalates

Figure 2. Bioavailability radar.

Table 4. SwissADME profiling of compounds identified in root extract of *M. balbisiana* Colla

Sl. No.	Compound Name	Pharmacokinetics				Inhibitors			Druglikeness	
		GI Absorption	BBB Permeant	P-gp Substrate	Log Kp (Skin Permeation)	CYP1A2 Inhibitor	CYP2C19 Inhibitor	CYP2C9 Inhibitor	Bioavailability Score	Lipinsk's Rule
1.	1,2-Benzenedicarboxylic acid, Butyl methyl ester	High	Yes	No	-5.43 cm/s	Yes	No	No	0.55	Yes; 0 violation
2.	Dimethyl pthalate	High	Yes	No	-6.35 cm/s	Yes	No	No	0.55	Yes; 0 violation
3.	Phthalic acid, Methyl nonyl ester	Low	No	Yes	-3.58 cm/s	No	No	No	0.55	Yes; 1 violation
4.	1,2-Benzenedicarboxylic acid, Butyl octyl ester	Low	Yes	No	-3.73 cm/s	No	No	No	0.55	Yes; 0 violation
5.	Phthalic acid, Decyl isobutyl ester	Low	No	No	-10.24 cm/s	No	No	No	0.55	Yes; 1 violation
6.	1,2-Benzenedicarboxylic acid, Dipropyl ester	High	Yes	No	-5.51 cm/s	Yes	Yes	No	0.55	Yes; 0 violation
7.	Phthalic acid, Isobutyl octyl ester	High	Yes	No	-4.07 cm/s	Yes	Yes	No	0.55	Yes; 1 violation
8.	Dibutyl phthalates	High	Yes	No	-4.80 cm/s	Yes	Yes	No	0.55	Yes; 0 violation

Most of the identified compounds were determined to be inhibitors of the cytochrome P450 enzymes (Table 3). The metabolism of drugs takes placeprimarily in the liver and intestines and Cytochrome P450 enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, UDP glucuronyl transferases and sulfo transferases) are involved in the metabolism of many drugs and pregnancy has differing effects on the apparent activity of the various enzymes[38-40]. When any of these enzymes get inhibited by any drugs, it has apparent effect on pregnancy and potentially increased risk of toxicity during pregnancy[38]. According to SwissADME Profile, few compound listed in Table 3 are inhibitors of CYP1A2 and hence evidences of toxicity to the fetus are established. Here from the cytochrome P450 enzymes mechanism, it can be inferred that compound like Dimethyl pthalate, Phthalic acid, Isobutyl octyl ester and 1,2-Benzenedicarboxylic acid, Butyl methyl ester can indirectly shows toxicity to the fetus since these compound inhibits the enzymes CYP1A2. Skin permeation values shows negative value which implies that they are not permeable through skin and hence are not suggested candidates for transdermal drug delivery.Here from the study, we can consider that rhizomes of *M. balbisiana* are potential source of phytomedicine having different bioactive compound and may considered as candidate drug as per Swiss ADME drugs profiling.

Bioavailability Radar is an important assessment tool for rapid prediction of drug likeness of a molecule[22]. From Table 3, twocompounds were represented for bioavailability radarand it showed that it falls in the pink area of the polygon (Figure 2), indicating their good bioavailability[22]. For a molecule to be drug-like the optimal range for each property is depicted as a pink region in the radar; within this pink region, a radar plot of the molecule must completely fall in order to be classified as drug-like; although Dibutyl phthalate is slightly flexible, other pharmacokinetics parameters suggest it as having drug-like property (Table 4).

4. Conclusion

The present study emphasized the scientific validation of the indigenous utilization of this plant extract (*M. balbisiana*) as an abortifacient along with their physicochemical, phytochemical, and *in silico* ADME features. It revealed that the rhizome extract is enriched with potential bioactive compounds and the analysis of physicochemical properties of traditional medicines aids in checking the quality control of the extract formulationsof medicinal extract utilized by

the indigenous tribe. As the identified compounds shows potential reproductive toxicity and having drug-likeness property, one can conclude that this plant can be considered as pharmaceutically important medicinal plant whose active extract from the rhizome can be processed as orally active plant based drugs.

5. Acknowledgement

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6. Author Contribution

First author, Reena Terangpicontributed to concept mapping, experimental design, plant identification, collection of data, biochemical analysis, interpretation, drafting and writing of the manuscript; other co-authors, Sangeeta Agarwal, Kolomsing Engti and Partha Chakdar assisted in interpretation of FTIR and GC-MS data; Dwimu Basumatary assisted in processing of the plant specimen and interpretation of *in silico* SwissADME parameters. The last author, Farishta Yasmin involved in the proofreading and crosschecked of the manuscript. All authors have read and agreed to the published version of the manuscript.

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