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Phylogenetic classification and secondary metabolites confirm non-random selection of commonly used aphrodisiac plants by local people from Benin

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Abstract

Understanding how medicinal plants are selected for local use may contribute to provide valuable insights for their efficacy. This study aimed to document secondary metabolites of commonly used aphrodisiac plants in Benin and determine their distribution in these plants phylogeny. Literature review data were combining with phytochemical screening carried out on cassava tubers to identify secondary metabolites. Phylogenetic analysis were performed using 61rbc*L* gene sequences retrieved from the NCBI web-based site. A total of13 secondary metabolite groups were identified. The most represented are flavonoids (78.13% of plants), alkaloids (62.50% of plants) and tannins (56.25% of plants) while mucillag and coumarins (each 6.25% of plants) are less represented. Phylogenetic classification detected a strong phylogenetic signal of ethnomedicinal plants confirmed by non-randoming distribution of secondary metabolites in the main taxa of aphrodisiac plants. This supports traditional uses of aphrodisiac plants and leads to select potential candidate species for biocreening and toxicity studies regarding new aphrodisiac drugs discovery and conservation priorities.

Keywords: Aphrodisiac plants, secondary metabolites, phylogeny, rbc*L*, Benin.

Introduction

There is no existence of life without plants. People of all cultures always depend on plants in their environment for primary needs mainly food, shelter, warmth and medicines (Pandey & Tripathi, 2017; Gaoue et al., 2021). The use of medicinal plants has been done since ancient times and may even be considered as the origin of modern medicine (Salmerón-Manzano *et al.*, 2020). According to the World Health Organization (WHO), more than 80% of people rely on traditional medicine for their primary health care needs (Ahouansikpo et al., 2016). The number of traditionally used plant species worldwide is estimated to be between 10,000 and 53,000 (McChesney et al., 2007; Saslis-Lagoudakis et al., 2012). Plants constitute a dominant source of pharmacological drugs to treat and cure various disorders and diseases classifications (Johnson-Fulton & Watson, 2018). Ethnobotany, the science of human–plant interactions, has for long focused on documenting the traditional knowledge that humans have developed and accumulated over centuries toward plant uses (Garnatje et al., 2017; Gaoue et al., 2021). Traditional knowledge of medicinal plants has led to discoveries that have helped combat diseases and improve healthcare (Saslis-Lagoudakis et al., 2011). Medicinal plants are more important sources for drug discovery, specifically lead molecules as these offer several advantages over the synthetic molecules. (Singh et al., 2018). Then, ethnobotanical and ethnopharmacology studies are proving to be powerful tools to find new drugs (de Albuquerque, 2010; Aslam & Ahmad, 2016; Lei et al., 2018). Aspirin (from *Filipendula ulmaria* (L.) Maxim.), codeine and papaverine (from *Papaver somniferum* L.), colchicine (from *Colchicum autumnale* L.), digoxin and digitoxin (from *Digitalis purpurea* L.), tetrahydrocannabinol and cannabidiol (from *Cannabis sativa* L.), and vinblastine and vincristine (from *Catharanthus roseus* (L.) G.Don) are among the most famous classical drugs developed from ethnobotanical records (Prance et al., 1994; Garnatje et al., 2017). Findings also led to the isolation and development of artemisinin (from *Artemisia annua* L.) as a powerful antimalarial drug whose relevance was recognized with the 2015 Nobel Prize in Physiology or Medicine (Su & Miller, 2015).

However, selecting the most biologically active plant species for further screening is still challenging (Johnson-Fulton & Watson, 2018). Given the immense societal benefits of medicinal plants, there has been a renewed interest in the search for new plants that might be medicinally useful in local ethnopharmacopoeias and perhaps for new drug discovery. According to Zaman et al.,(2021), the search for new medicinal plants is crucial because of the global increasing demand for phytomedicines. Further, with the alarming rate of biodiversity loss, systematic methodologies to find new plant-derived drugs are urgently needed (Ernst et al., 2016). Because medicinal plants are not distributed randomly across lineages (Moerman, 1979; Ford & Gaoue, 2017), it has been suggested that phylogeny along with traditional knowledge of plant uses can guide the identification of new medicinally useful plants (Yessoufou et al., 2014). So, determining the phylogenetic relationships amongst plant species could be an appropriate tool for discovering new drugs based on recorded plant medicinal uses and analysing ethnobotanical data (Garnatje et al., 2017). Phylogenetic clustering of traditional medicinal plants can be used to interpret ethnomedicinal uses of plants and to search for new medicines (Gaoue et al., 2021a; Saslis-Lagoudakis et al., 2011).

Beyond its potential to lead to lineages that are more prominent in local ethnopharmacopoeias, the phylogenetic perspective of ethnobotany has been proposed as a way at discovering new plants that have medicinal properties (Saslis-Lagoudakis et al., 2011, 2012; Rønsted et al., 2012; Zaman et al., 2020). Plants evolving in the same lineage have more medicinal uses than evolutionarily isolated species, and the diversity of medicinal uses is correlated with the evolutionary history of the species (Garnatje et al., 2017). Based on the phylogenetic hypothesis, alternative species with specific medicinal properties have been identified through bioscreening, helping meet increasing demands and reducing harvest pressure on wild populations of traditionally used species (Bindu et al., 2014; Pellicer et al., 2018).

Benin Republic is 2807 plant species richness (Akoègninou et al., 2006), including nearly 507 medicinal species used to treat human diseases (Ajanonhoun et al.,1989). Recent studies have reported 148 medicinal plants traditionally used for sexual dysfunctions (Batcho et al., 2022). Thirty-three (33) plant were the most commonly used and eight (8) of them were threatened. Roots are plant parts most used. In addition, several aphrodisiac plant species are less available in their occurrence zone.

In this study, we investigate the phylogenetic patterns in aphrodisiac plants traditionally used in Benin. This study aimed to explore the contribution of phylogenetic approaches in understanding the interactions between humans and floristic environments and in the biodiscovery of new aphrodisiac plant. The screening of the new species was also done to know more about aphrodisiac secondary metabolites.

Materials and Methods

Phytochemical analyses

Some of medicinal plant are fully chemical screening due to their importance. So firstly, the main aphrodisiac plant species of which secondary metabolites are highlight, were identified from the literature through Google scholar, PubMed and Sciencedirect databases using keys words such as “phytochemicals compounds”, “secondary metabolites“, “phytochemical screening” and “phytochemical properties” for each concerned species. Apart from the cassava, thirty two of the 33 main plant species were fully described (Table 1) so that *Manihot esculenta* Crantz was less studied biochemically and it was selected for analyses.

Plant material

Manihot esculenta roots were used as plant material due to their high implication in aphrodisiac recipes (Batcho et al., 2022). Thus, cassava tubers of local varieties “sowé” were harvested from the field of one farmer during the month of March, 2022 at Dassa-Zoumé district in central Benin. Authentication were carried out with the help of several farmers. The tubers were washed thoroughly 2-3 times with running tap water then peeled and cut before air dried under shade at room temperature (25°C). After complete shade drying, the plant material was grinded to a uniform powder and stored in a dry container.

Phytochemical screening

Phytochemical profiling of the cassava roots was carried out at the Laboratory of Study and Research in Applied Chemistry (LERCA/EPAC/UAC)" according to the classic procedure described by Houghton and Raman (1998). It concerned the major families of secondary metabolites and consisted of a qualitative chemical test based on coloring and/or precipitation reactions. Results of screening were treated with those obtained from phytochemical review of 32 others commonly used aphrodisiac plants (Table 1) in order to find out their main bioactive compounds.

Table 1: Phytochemical composition of commonly used aphrodisiac plant species (Batcho et al., 2022)

Species	Families	Parts used	Main secondary metabolites	Authors
Abrus precatorius L.	Fabaceae	Leafy stem	Flavonoids, triterpens, glycosides, alkaloids	Garaniya & Bapodra, 2014
Acridocarpus smeathmannii (DC.) Guill. & Perr.[@]	Malpighiaceae	Roots	Flavonoids	Kale et al., 2019
Aframomum melegueta (Roscoe) K. Schum.	Zingiberaceae	Fruits, Seeds	Flavonoids, alkaloids, Tannins, terpens, saponins, glycosides	Osuntokun, 2020
Allium cepa L.	Alliaceae	Bulbs	Glycosides, phenolic acids	Bystrická et al., 2013
Annona senegalensis Pers.	Annonaceae	Leaves, Roots	flavonoid, tannis, cardiac glycoside, saponins, alkaloid, steroid, volatile oils	Ijaiya et al.,2014
Borassus aethiopum Mart.	Arecaceae	Hypocotyles, Roots	Tannins, anthocyans, leucoanthocyans, mucillag, saponosids	Gbesso et al.,2016
Caesalpinia bonduc (L.) Roxb.	Fabaceae	Roots	Tannins, flavonoids, anthocyans, leucoanthocyans, mucillag, saponosids, alkaloids, quinones	Gbankoto et al.,2015
Carica papaya L.	Caricaceae	Roots	Alkaloids, saponins, tannins, glycosides, phenol	Doughari et al.,2007
Carissa spinarum L.	Apocynaceae	Roots, Barks, Leaves	Alkaloids, tannins, flavonoids, anthocyanins, saponosides, reducing compounds	Afanyibo et al.,2019
Carpolobia lutea G. Don	Polygalaceae	Roots, Stem	Saponins, anthraquinones, flavonoids, glycosides, terpens	Nwidu et al., 2015
Cissus populnea Guill. & Perr.	Vitaceae	Stems, Roots	Alkaloids, flavonoids, Saponins, Tannins	Soladoye & Chukwuma 2012
Cocos nucifera L.	Arecaceae	Fruits, Roots	tannins, flavonoids, polyphenols, phlobatannins	Okon et al., 2012

Cola acuminata (P. Beauv.) Schott & Endl.	Malvaceae	Seeds	Alkaloids, tannins, saponins, flavonoids, steroids, terpenoids, glycosides, volatile oil.	Omwirhiren et al.,2017
Cola nitida (Vent.) Schott. & Endl.	Malvaceae	Seeds	Alkaloids, tannins, saponins, flavonoids, steroids, terpenoids, glycosides, volatile oil.	Omwirhiren et al.,2017
Cyperus esculentus L.	Cyperaceae	Stem (tubercule)	alkaloid, saponin, tannin, glycoside, flavonoid, steroid	Imam et al., 2013
Elaeis guineensis Jacq.	Arecaceae	Roots, Seeds	alkaloids, tannins, flavonoids, terpenoids, and steroids	Owolabi et al.,2013
Garcinia kola Heckel	Clusiaceae	Seeds	Alkaloids, tannins, saponins, flavonoids, steroids, terpenoids, glycosides, volatile oil.	Omwirhiren et al.,2017
Gardenia erubescens Stapf & Hutch.	Rubiaceae	Roots	Alkaloids, tannins, flavonoids, steroids	Omoniwa et al.,2022
Imperata cylindrica (L.) P. Beauv.	Poaceae	Rhizomes	Carbohydrates, flavonoids	Nwokike et al.,2020
Khaya senegalensis (Desr.) A. Juss.	Meliaceae	Barks	Tannins, saponins, flavonoids, alkaloids	Abdallah et al.,2016
Manihot esculenta Crantz[@]	Euphorbiaceae	Roots	alkaloids, catechic tannins, leucoanthocyans, reducing compounds, sterols and terpens	This study
Monodora myristica (Gaertn.) Dunal	Annonaceae	Seeds	alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins, and phenolic compounds	Enabulele et al.,2014
Moringa oleifera Lam.	Moringaceae	Seeds, Roots, Leaves	Alkaloids, saponins, flavonoids, tannin, steroids, glycosides	Paikra, 2017
Musa sapientum auct. div.	Musaceae	Unriped fruits	Tannins, flavonoids, glycosides, terpenoids, alkaloids	Onojah et al., 2017

Pachycarpus lineolatus (Decne.) Bullock[@]	Asclepiadaceae	Rhizomes		
Phoenix dactylifera L.	Arecaceae	Fruits	Flavonoids, glycosides, phenolic acids, Steroids, anthocyanines, carbohydrates	Deshpande et al.,2017
Prosopis africana (Guill. & Perr.) Taub.	Fabaceae	Roots, Leaves, Stem	Flavonoids, alkaloids, tannins, saponins, phenolic compounds	Prabha et al.,2014
Sarcocephaluslatifolius (Sm.) E. A. Bruce	Rubiaceae	Roots	Coumarins, glycoside, tannins, Saponins, flavonoids, alkaloids, triterpenoids, quinonics derived, saponosides	Ahoyo et al., 2019
Syzygium aromaticum (L.) Merr. & L. M. Perry	Myrtaceae	Flowers	saponins, alkaloids, flavonoids, glycosides, tannins, steroids	Kaur & Kaushal, 2019
Tamarindus indica L.	Fabaceae	Roots, branches, Barks	Flavonoids, tannins, alkaloids, saponins, phenolic compounds, glycosides	Idu & Osadolor, 2020
Xylopia aethiopica (Dunal) A. Rich.	Annonaceae	Fruits	Alkaloids, glycosides, Saponins, tannins, flavonoids, polyphenols, reducing sugars	Erhirhie & Moke, 2014
Zea mays L.	Poaceae	Fruits	Flavonoids, saponins, Tannins, phenols, alkaloids, glycosides	Solihah et al., 2012
Zingiber officinale Roscoe	Zingiberaceae	Rhizomes	Flavonoids, carbohydrates, proteins, alkaloids, glycosides, saponins, steroids, terpenoids and tannin	Kumar et al., 2011

[@]Species less studied biochemically

Phylogenetic analyses

Phylogenetic Tree Reconstruction were performed used fifty-five (55) rbc*L* gene sequences of Benin aphrodisiac plant species retrieved from the NCBI web-based site (https://www.ncbi.nlm.nih.gov/) under "FASTA" format. This sampling represents 37.16% of all aphrodisiac plants documented in Benin republic. Additional sequences were used include two aphrodisiac plant species from Asia (*Panax ginseng* C.A. Mey., *Punica granatum* L.) and one from America (*Lepidium meyenii* Walp.) largely documented (Nair et al., 2012; Abdel-Wahhab et al., 2014; Katana et al., 2020; Borkar et al. 2021). Gymnosperm species *Cycas taitungensis* C.F.Shen et al., *Encephalartos lehmannii* Lehm. and *Pinus densiflora* Siebold & Zucc. were used as outgroup. Taxa and accession numbers of all DNA sequences are listed in Table 2. Alignments were performed using MAFFT v 7.504 (Katoh et al., 2019) at interface https://mafft.cbrc.jp/alignment/server/. For Bayesian phylogenetic analysis, general molecular substitution model GTR (Tavaré, 1986) implemented in BEAST was determined to be the best for submitted data. Sequences aligned were analyzed in BEAST 1.6.0 using the Uncorrelated Lognormal relaxed clock model parameters (Drummond et al., 2006) and a Birth-Death speciation process (Gernhard, 2008).

The analysis was performed with 10 million generations on two Monte Carlo Markov chains (MCMC) with sampling parameters every 1,000 steps.

TreeAnnotator was used to obtain the estimated phylogenetic tree ("Maximum clade credibility tree" option). The phylogenetic trees were visualized with FigTree v1.4.2 (Rambaut, 2014).

Table 2: Taxa of aphrodisiac plant species and outgroup included in molecular phylogenetic analyses

Species	Plants families	GenBank Accession No.	Locus No.	Aligned sequence	Associated publication
Abelmoschus esculentus (L.) Moench*	Malvaceae	PRJNA394355	NC_035234	1434 bp	Rabah et al., 2017 (Unpublished)
Abrus precatorius L.*	Fabaceae	PRJNA628333	NC_047402	1428 bp	Zhang et al.,2020
Allium cepa L.*	Alliaceae	PRJNA261041	NC_024813	1440 bp	von Kohn et al.,2014 (Unpublished)
Allium sativum L.	Alliaceae	PRJNA353416	NC_031829	1440 bp	Direct Submission
Aloe vera (L.) Burm. f.	Asphodelaceae	PRJNA399916	NC_035506	1455 bp	Lee & Yang, 2017 (Unpublished)
Ananas comosus (L.) Merr.	Bromeliaceae	PRJNA273206	NC_026220	1440 bp	Nashima et al., 2015 (Unpublished)
Arachis hypogea L.	Fabaceae	PRJNA453365	NC_037358	1428 bp	Prabhudas & Natarajan, 2018 (Unpublished)

Azadirachta indica A.Juss.*	Meliaceae	PRJNA241235	NC_023792	1428 bp	Square & Kane, 2014 (Unpublished)
Bambusa vulgaris Schrad. ex Wendel	Poaceae	PRJNA672379	NC_050780	1434 bp	Liu et al.,2020
Boerhavia diffusa L.	Nyctaginaceae	PRJNA628456	NC_047478	1443 bp	Direct Submission
Calotropis procera (Aiton) W.T. Aiton	Asclepiadaceae	PRJNA532054	NC_041440	1428 bp	Islam et al., 2019 (Unpublished)
Capsicum annuum L.	Solanaceae	PRJNA174499	NC_018552	1434 bp	Jo et al.,2012
Carica papaya L.*	Caricaceae	PRJNA28721	NC_010323	1428 bp	Rice et al.,2008
Cassytha filiformis L.	Lauraceae	PRJNA413205	NC_036001	1428 bp	Wu et al.,2017
Citrus aurantifolia (Christm. & Panzer) Swingle	Rutaceae	PRJNA261939	NC_024929	1428 bp	Su et al.,2014
Citrus sinensis Osbeck	Rutaceae	PRJNA17655	NC_008334	1428 bp	Bausher et al.,2006
Cocos nucifera L.	Arecaceae	PRJNA221173	NC_022417	1455 bp	Huang et al.,2013
Colocasia esculenta (L.) Schott	Araceae	PRJNA81917	NC_016753.1	1443 bp	Ahmed et al.,2012
Corchorus olitorius L.	Malvaceae	PRJNA563377	NC_044468	1449 bp	Zhang et al.,2019 (Unpublished)
Cymbopogon citratus (DC.) Stapf	Poaceae	PRJNA541620	NC_042144	1431 bp	Bhatt & Thaker,2019 (Unpublished)
Cyperus esculentus L.*	Cyperaceae	PRJNA775539	NC_058698	1440 bp	Ren et al.,2021
Daucus carota L. ssp. sativus (Hoffm.) Arcang.	Apiaceae	PRJNA17617	NC_008325	1428 bp	Ruhlman et al.,2006
Dioscorea dumetorum (Kunth) Pax	Dioscoreaceae	PRJNA504254	NC_039691	1434 bp	Magwe-Tindo et al.,2018 (Unpublished)
Dioscorea praehensilis Benth.	Dioscoreaceae	PRJNA504093	NC_039837	1434 bp	Magwe-Tindo et al.,2018 (Unpublished)
Dioscorea rotundata Poir.	Dioscoreaceae	PRJNA251641	NC_024170	1434 bp	Mariac et al.,2014
Elaeis guineensis Jacq.*	Arecaceae	PRJNA158449	NC_017602	1455 bp	Uthaipaisanwong et al., 2012

Euphorbia hirta L.	Euphorbiaceae	PRJNA772492	NC_058203	1428 bp	Direct Submission
Evolvulus alsinoides (L.) L.	Convolvulaceae	PRJNA775504	NC_058590	1455 bp	Shidhi et al.,2021. (Unpublished)
Flacourtia indica (Burm. f.) Merr.	Flacourtiaceae	PRJNA453260	NC_037410	1437 bp	Zhang et al., 2018 (Unpublished)
Flueggea virosa (Roxb.ex Willd) Voigt.	Euphorbiaceae	PRJNA680145	NC_051502	1449 bp	Wang et al., 2020
*Guilandina bonduc**	Fabaceae	PRJNA628209	NC_047380	1428 bp	Zhang et al., 2020
Imperata cylindrica (L.) P. Beauv.	Poaceae	PRJNA328829	NC_030487	1431 bp	Burke et al.,2016
Ipomoea batatas (L.) Lam.	Convolvulaceae	PRJNA278593	NC_026703	1464 bp	Yan et al.,2015
Jatropha curcas L.	Euphorbiaceae	PRJNA34925	NC_012224	1428 bp	Asif et al.,2009 (Unpublished)
Khaya senegalensis (Desr.) A. Juss.*	Meliaceae	PRJNA453339	NC_037362	1428 bp	Mader et al.,2018
Lagenaria siceraria (Molina) Standl.	Cucurbitaceae	PRJNA433138	NC_036808	1449 bp	Zhu et al.,2018 (Unpublished)
Lawsonia inermis L.	Lythraceae	PRJNA545799	NC_042369	1428 bp	Gu et al.,2019 (Unpublished)
Manihot esculenta Crantz*	Euphorbiaceae	PRJNA28953	NC_010433	1434 bp	Daniell et al.,2008
Momordica charantia L.	Cucurbitaceae	PRJNA433137	NC_036807	1428 bp	Zhu et al.,2018
Moringa oleifera Lam.*	Moringaceae	PRJNA532154	NC_041432	1449 bp	Dai & Zhou, 2019 (Unpublished)
Ocimum gratissimum L.	Lamiaceae	PRJNA754975	NC_057196	1434 bp	Balaji et al., 2021
Persea americana Mill.	Lauraceae	PRJNA344273	NC_031189	1428 bp	Song et al., 2016
Phoenix dactylifera L.*	Arecaceae	PRJNA46761	NC_013991	1455 bp	Yang et al., 2010
Phyllanthus amarus Schumach. & Thonn.	Euphorbiaceae	PRJNA628303	NC_047474	1428 bp	Direct Submission
Piper nigrum L.	Fabaceae	PRJNA387960	NC_034692	1428 bp	Zhang et al., 2017 (Unpublished)

Psidium guajava L.	Myrtaceae	PRJNA362375	NC_033355	1428 bp	Jo et al.,2017 (Unpublished)
Saccharum officinarum L.	Poaceae	PRJNA394356	NC_035224	1431 bp	Lloyd et al., 2017 (Unpublished)
Solanum nigrum L.	Solanaceae	PRJNA298794	NC_028070	1434 bp	Kim et al., 2015 (Unpublished)
Syzygium aromaticum (L.) Merr. & L. M. Perry*	Myrtaceae	PRJNA628191	NC_047249	1428 bp	Direct Submission
Tamarindus indica L.*	Fabaceae	PRJNA278588	NC_026685	1428 bp	Sabir et al., 2015 (Unpublished)
Trema orientalis (L.) Blume syn Trema guineensis (Schumach. & Thonn.) Ficalho	Celtidiaceae	PRJNA504085	NC_039734	1428 bp	Zhang et al., 2018
Tribulus terrestris L.	Zygophyllaceae	PRJNA624406	NC_046758	1428 bp	Yan et al., 2019
Vitellaria paradoxa C.F. Gaertn. ssp. paradoxa	Sapotaceae	PRJNA754891	NC_057076	1428 bp	Wang et al., 2021
Zea mays L.*	Poaceae	PRJNA124	NC_001666	1431 bp	Maier et al., 1995
Zingiber officinale Roscoe*	Zingiberaceae	PRJNA573337	NC_044775	1464 bp	Cui et al., 2019
Panax ginseng C.A. Mey.*	Araliaceae	PRJNA13047	NC_006290	1437 bp	Kim & Lee, 2004
Lepidium meyenii Walp.*	Brassicaceae	PRJNA383493	NC_034363	1437 bp	Guo et al., 2017
Punica granatum L.*	Lythraceae	PRJNA394497	NC_035240	1428 bp	Rabah et al., 2017 (Unpublished)
Cycas taitungensis C.F.Shen & al.	Cycadaceae	PRJNA19987	NC_009618	1428 bp	Wu et al., 2007
Pinus densiflora Siebold & Zucc.	Pinaceae	PRJNA546323	NC_042394	1428 bp	Shim et al., 2019 (Unpublished)
Encephalartos lehmannii Lehm.	Zamiaceae	PRJNA289826	NC_027514	1428 bp	Wu & Chaw, 2015

*Species commonly used

Results

Phytochemical Screening

Phytochemical screening of *Manihot esculenta* roots powder (Table 3) revealed the presence of alkaloids, catechic tannins, leucoanthocyans, reducing compounds, sterols and terpens. However, several compounds such as flavonoids, gallic tannins, anthocyans, anthraquinones, mucillag, saponosides, coumarins and cyanogenic derivatives were absent (Table 3). Considering a total of 32 commonly used aphrodisiac plant species (Table 1), 13 secondary metabolite groups were identified. The most represented are flavonoids (78.13% of plants), alkaloids (62.50% of plants) and tannins (56.25% of plants) while mucillag and coumarins (each 6.25% of plants) are less represented.

Table 3: Result of phytochemical screening of cassava tubers

Major secondary metabolites families	Type of reaction	Results
Alkaloids	Mayer test	+
Flavonoids	Cyanidin reaction	-
Catechic tannins	Stiasny's reagent	+
Gallic tannins	FeCl3 reaction	-
Anthocyans	Hydrochloric acid reaction	-
Leucoanthocyans	Hydrochloric alcohol reaction	+
Anthraquinones	Bornträger test	-
Mucillag	Alcohol reaction	-
Saponosides	Lather test	-
Coumarins	Ether and ammonia reactions	-
Reducing compounds	Fehling's solution	+
Cyanogenic derivatives	Picric test of acid test	-
Sterols and terpens	Liebermann-Burchard test	+

+= Presence of compound; - =Absence of compound

Phylogeny

A total of sixty-one (61) rbc*L* sequences were used for phylogeny relationship assessed. It contained 1479 total characters. Sequences length ranges from 1428 to 1464 base pairs (bp). The results of the phylogenetic analysis are presented in Fig. 1. The phylogenetic tree based on rbcL gene classified all species into four (4) major groups (Outgroup, Paleodicotyledons, Monocotyledons and Eudicotyledons). All available commonly used aphrodisiac plants were grouped into four groups including less used species. The first group had 2 sub-groups. Secondary metabolites, particularly flavonoids, alkaloids, tanins, leucoanthocyans and glycosides were concentered in this group. The first sub-group displaying five different species from Fabaceae plant family included *Abrus precatorius*, *Guilandina bonduc, Tamarindus indica*(the most used species) and *Arachis hypogea*, *Trema orientalis* (less used species). The second subgroup included the commonly used species *Manihot esculenta* which is sister species to *Euphorbia hirta* and *Jatropha curcas belonging to* the family Euphorbiaceae.

The group II contained three sub-groups in which subgroup I had three commonly used species including *Carica papaya*, *Moringa oleifera* and the American species *Lepidium meyenii*. These species belong to Brassicales order.

The subgroup II was constituted by two species from Meliaceae, *Khaya senegalensis* and *Azadirachta indica*. The sub-group III included three other species belonging to Myrtales order such as *Psidium guajava*, *Syzygium aromaticum* and *Punica granatum* (Asia species). These group II species were rich in secondary metabolites mainly flavonoids, alkaloids, tannins and saponins.

The group III had two species from order Apiales including *Daucus carota* and *Panax ginseng*. They contained major secondary metabolites such as Flavonoids, Alkaloids, Saponins and Triterpens. The group IV contained four sub-groups. The subgroup I had eight species from the Poalesorder. They are sisters to sub-group II that contained three species from Arecaceae (Fig 1). The subgroup III with a unique species (*Zingiber officinale*) and this sub-group was highly diverged from the other prior two sub-groups. The sub-group IV included three species belong to two plant families and one order (Asparagales). Species of group IV were rich in phytochemical compounds such as flavonoids, alkaloids, tannins, carbohydrates, steroids and glycosides.

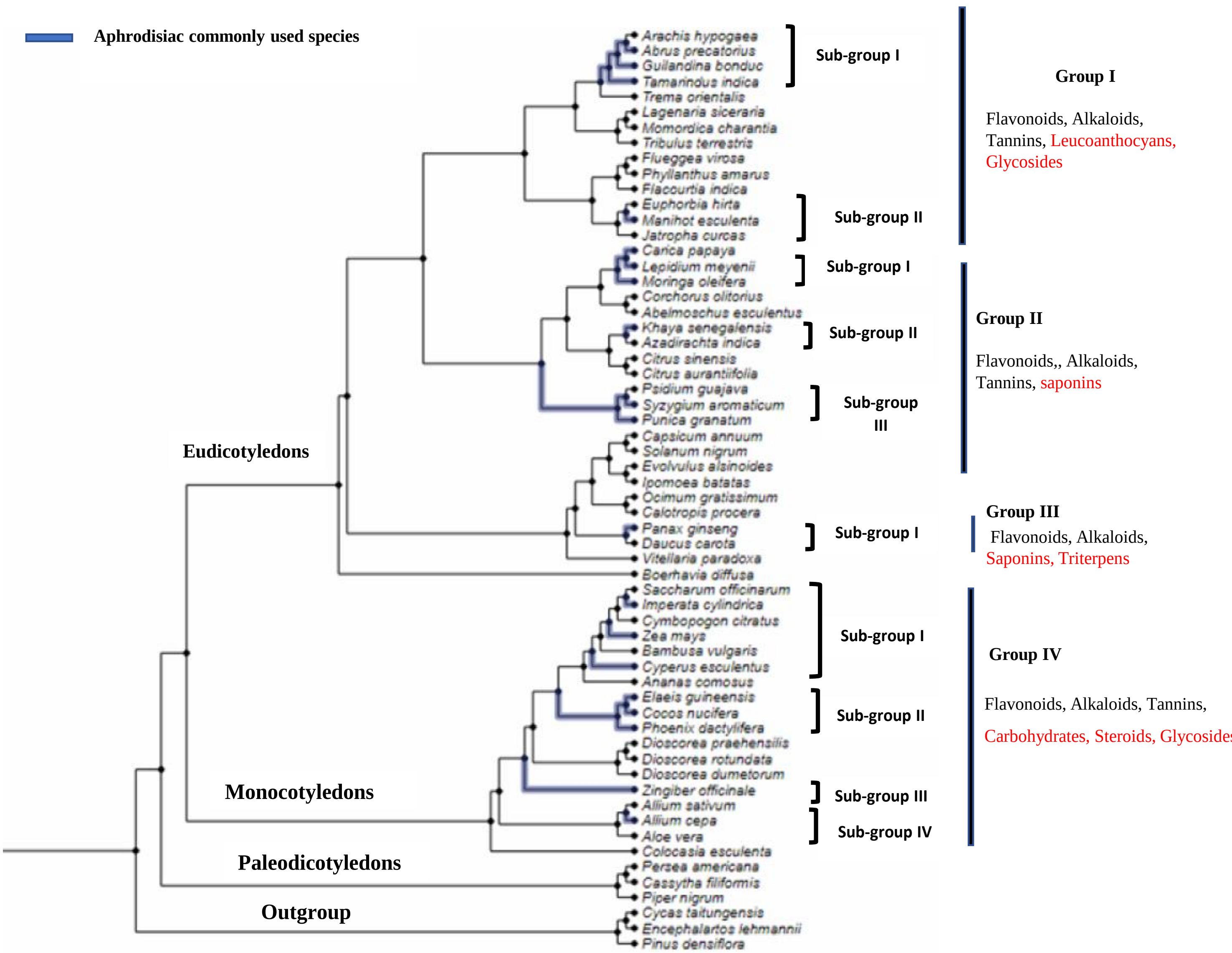

Figure 1: Phylogenetic tree of aphrodisiac plants inferred from rbc*L*sequences dataset by using BEAST and TreeAnnotator. Obtained after 10,000,000 replicates of Bayesian inference.

Discussion

Aphrodisiac phytochemical compounds

The results of the phytochemical screening revealed that medicinal properties of *M. esculenta* roots are due to the presence of phytochemical groups such as alkaloids, catechic tannins, leucoanthocyans, reducing compounds, sterols and terpens. Alkaloids, tannins and leucoanthocyans were also discovered in the roots of *C. bonduc* (Gbankoto et al., 2015) and in Hypocotyls extracts of *B. aethiopum* (Gbesso et al., 2016). Besides, steroids and terpens were presents in seeds of *C. acuminata*, *C. nitida* (Omwirhiren et al., 2017) and in *Z. officinale* rhizomes (Kumar et al., 2011). All these species are well known as aphrodisiac plants (Alhowiriny et al., 2013; Ademiluyi et al., 2018). Furthermore, steroids (sterols) include testosterone (Tauchen et al., 2021), a sexual hormone related to sexual desire especially in men. However, biological studies like in vivo evaluation are necessary to evaluate the effectiveness of *M. esculenta* roots. Considering all commonly used aphrodisiac plants species, results showed that flavonoids, alkaloids and tannins were the most represented phytochemicals compounds.These results suggest that these active compounds could be the main that confer aphrodisiac plants properties. Alkaloids have several medicinal properties related to sexual dysfunctions treatment such as stimulant (caffeine, nicotine, yohimbine), muscle relaxant (tubocurarine), vasodilating (vincamine) and aphrodisiac (yohimbine) (Babbar, 2015). Yohimbine is the principal alkaloid extracted from the bark stem of *Pausinystalia yohimbe*, the most commonly used aphrodisiac plant in the world (Dabo, 2022). Physiologically, this alkaloid enhance the release of nitric oxide to stimulate soluble guanylate cyclase that increase intracellular cyclic guanosine monophosphate (cGMP) levels, leading to relaxation of the corpus cavernosal smooth muscles (Tam et al., 2001). This end up by inducing penile erection. As far as flavonoids are concerned, they have widely been known for their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function (Panche et al., 2016). For sexual dysfunction treatment several studies had showed the importance of flavonoids (Sohn & Sikora, 1991; Benson et al., 2008; Oboh et al., 2017; Gu *et al.,* 2021). Moreover, five pyrano-isoflavones with erectile-dysfunction activity have been isolated from the rootstock of *Eriosema kraussianum* N. E. Br (Drewes et al., 2002). And the most active of the compounds had an activity of 75% of that found in viagra (Sildenafil) in the erectile dysfunction test on rabbit penile smooth muscle (Drewes et al., 2002). Regarding to tannins, they hold several medicinal applications included anticancer, antioxidant, antimicrobial, anti-inflammatory, antidiabetic and cardiovascular (Pizzi, 2021). Like that, tannins could be effective for chronic diseases treatment such as hemorrhoids, diabetes, ulcers, hypertension and cancer which are main causes of sexual dysfunctions (Batcho et al., 2022). Collectively, the presence of flavonoids, alkaloids and tannins in a plant part could well explain it aphrodisiac properties.

Phylogeny reconstruction

The phylogenetic tree obtained in the present study is strongly support and reveals different taxonomic groups (associated outgroup for the analysis, Paleodicotyledons, Monocotyledons and Eudicotyledons) thus showing the effectiveness of the plastid DNA marker rbc*L* used to test the phylogenetic signal between aphrodisiac plants. rbc*L* gene has been used also by similar studies to assess phylogenetic relationships (Saslis-Lagoudakis et al., 2012; Rønsted et al., 2012; Pahlavan et al., 2021). According to Pahlavan et al., (2021), Chloroplast DNA sequences are suitable barcodes for phylogenetic studies due to the involvement of a number of traits including presence in high copy number in the cell, occurrence of conserved genes and having general primers and high reproducibility. Also, the plastid DNA marker rbc*L* was selected for this study because of its large sequences availability for concerned species.

Distribution of secondary metabolites

We found in this work that commonly used aphrodisiac plants species were grouped into four groups and share three common bioactive compounds (flavonoids, alkaloids and tannins). So medicinal plants used are closely related, with a strong phylogenetic signal for aphrodisiac properties. Based on this result, it is apparent that aphrodisiac properties are not randomly distributed in commonly used plant species. Similarly, Ford & Gaoue, (2017) found that medicinal and food plants are not selected randomly but according to their chemical traits and also indirectly for their physical traits. Even, medicinal plant parts used are not randomly selected (Gaoue et al., 2021). Endogenous knowledge of aphrodisiac plants are then supported by phylogeny. Local people don't choose randomly plants in aphrodisiac recipes formulation. Understanding how medicinal plants are selected for local use or incorporated into written pharmacopoeias may contribute to understanding of plant efficacy (Lei et al., 2018). But, further phylogenetic studies using specific markers related to aphrodisiac plant properties are needed to confirm our findings.

Based on the results of this study, phylogenetic analysis showed that commonly used aphrodisiac plants are related closely with those species less used. These species are *Arachis hypogaea*, *Euphorbia hirta*, *Azadirachta indica*, *Psidium guajava*, *Saccharum officinarum*, *cymbopogon citratus*, *Bambuba vulgaris* and *Allium sativum.*Since these species are closely related with commonly used plants, they likely share common aphrodisiac secondary compounds mainly flavonoids, alkaloids and tannins (Saslis-Lagoudakis et al., 2012; Johnson-Fulton & Linda E. Watson, 2018; Zaman et al., 2022). Among them, aphrodisiac properties of species *Psidium guajava*, *Saccharum officinarum*, *cymbopogon citratus* and *Allium sativum* have already been proved scientifically (Batcho et al., 2022). They could be used at the same level like commonly used aphrodisiac species. The remain species less study such as *Arachis hypogaea*, *Euphorbia hirta*, *Azadirachta indica* and *Bambuba vulgaris* (Batcho et al., 2022) are the potential candidates for further bioscreening and toxicity studies. So, it appear clearly that phylogenetic approach could be used as a potential guiding tool for choosing new species to be investigated concerning aphrodisiac properties.

Besides, our study has shown that some species are related closely with commonly worldwide used aphrodisiac plants such *Lepidium meyenii*, *Punica granatum* and *Panax ginseng* (Dabo, 2022). Species are *Carica papaya*, *Moringa oleifera*, *Psidium guajava*, *Syzygium aromaticum* and *Daucus carota*. Studies are needed to compare aphrodisiac effects of these species with the others already well known in the world. Maybe it can lead to discovery new aphrodisiac drugs (Ronsted et al., 2012; Singh et al., 2018).

Also, based on our results species such as *Tamarindus indica*, *Abrus precatorius*, *Arachis hypogaea* and *Azadirachta indica* are related closely respectively with *Caesalpinia bonduc* (*Guilandinan bonduc*) and *Khaya senegalensis* which were threatened species in Benin (Adomou et al., 2011). This result can be taken account in order to reduce pressure on these threatened species because they share evolutionary traits such as secondary chemistry with the other species.

Conclusion

In this study we showed that flavonoids, alkaloids and tannins are the main secondary metabolites that confer aphrodisiac plants properties. Phylogenetic analyses revealed that these bioactive compounds are not randomly distributed in the commonly used plants. This has implication to select potential candidate species for biocreening and toxicity studies regarding new aphrodisiac drugs discovery and conservation priorities. However, further phylogenetic methods are needed to confirm our findings.

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Supplementary data

rbc*L* gene sequences used in this work can be found in the file Supplementary data_Batcho et al (2022).
Link: https://ijarbs.com/pdfcopy/2022/nov2022/ijarbs014.pdf

Conflict of interest

The authors declare that they have no conflict of interest for this study.

Informed Consent

Not applicable

Human and Animal Rights

Not applicable

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