Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
www.plantsjournal.com
JMPS 2021; 9(4): 92-99

Received: 01-05-2021
Accepted: 03-06-2021

Annekathrin M Keiler
1. Environmental Monitoring & Endocrinology, Faculty of Biology, Technische Universität Dresden, Dresden, Germany
2. Institute of Doping Analysis & Sports Biochemistry Dresden, Kreischa, Germany

Antje Frühauf
Environmental Monitoring & Endocrinology, Faculty of Biology, Technische Universität Dresden, Dresden, Germany

Anika Aderhold
Environmental Monitoring & Endocrinology, Faculty of Biology, Technische Universität Dresden, Dresden, Germany

Friederike Kiene,
Environmental Monitoring & Endocrinology, Faculty of Biology, Technische Universität Dresden, Dresden, Germany

Oliver Zierau
Environmental Monitoring & Endocrinology, Faculty of Biology, Technische Universität Dresden, Dresden, Germany

Maria Chalkiadaki
Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece

Theodora Nikou
Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece

Maria Halabalaki
Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece

Mawunu Monizi
University Kimpa Vita, Province of Uíge, Rua Henrique Freitas No. 1, Bairro Popular, Uíge, Angola

Christoph Neinhuis
Institute of Botany, Faculty of Biology, Technische Universität Dresden, Dresden, Germany

Thea Lautenschläger
Institute of Botany, Faculty of Biology, Technische Universität Dresden, Dresden, Germany

Corresponding Author:
Annekathrin M Keiler
1. Environmental Monitoring & Endocrinology, Faculty of Biology, Technische Universität Dresden, Dresden, Germany
2. Institute of Doping Analysis & Sports Biochemistry Dresden, Kreischa, Germany

Investigation of *in vitro* lactogenic action of selected Angolan plants ethnobotanically described to affect human breast milk

Annekathrin M Keiler, Antje Frühauf, Anika Aderhold, Friederike Kiene, Oliver Zierau, Maria Chalkiadaki, Theodora Nikou, Maria Halabalaki, Mawunu Monizi, Christoph Neinhuis and Thea Lautenschläger

DOI: https://doi.org/10.22271/plants.2021.v9.i4b.1314

Abstract
A recent ethnobotanical study collected traditional knowledge on treatments and plants of tribes in Uíge, Northern Angola, a region suffering from infant malnutrition and mortality. Aqueous extracts of twelve different plant species (leaves or stems) collected were prepared and subject of chemical profiling. The impact of twelve plant extracts on mammary epithelial cell differentiation was tested *in vitro* by dose-dependent treatment of HC11 cells. Leaf extracts of *Carica papaya* and *Morinda lucida* induced HC11 differentiation and elevated the mRNA expression of the milk protein β-casein, while the remaining ten inhibited the HC11 mammary differentiation. The majority of the plant extracts did not induce *in vitro* mammary epithelial cell differentiation assuming that the plants might rather exert their effects on prolactin levels or on other mechanism influencing the mammary gland health. Phenolic acids and flavonoids were identified as the predominant and potentially active chemical classes responsible for the observed lactogenic effects.

Keywords: galactagogue, HC11 cells, Angola, *Morinda lucida*, *Carica papaya*, ^{1}H-NMR

Introduction
The World Health Organization (WHO) recommends breastfeeding as the sole nutrition of infants during the first six months due to the protective effects against infections, on reduction of newborn mortality and as supply of essential micronutrients [1, 2]. The beneficial impact of breastfeeding on infant health has been also shown in a randomized trial [3]. Besides, prolongation supplementary lactation up to two years is advised to fight against malnutrition of children. Quality and quantity of breast milk are significantly affected by the mother's nutritional status. While the portion of group 1 nutrients like thiamine, riboflavin, vitamin B6, vitamin B12, choline, retinol and vitamin A, vitamin D, selenium and iodine depend on their maternal dietary intake, nutrients of the second group (including iron, zinc, calcium, folate and copper) are constantly secreted into breast milk.
A possibility to enhance lactation is the use of so-called galactagogues, that are pharmaceutical drugs or herbs increasing, promoting or maintaining milk secretion [4, 5]. In traditional medicine, the application of galactagogue plants is widespread in different cultures, especially in regions with malnutrition and high infant mortality [6-8]. Application of different galactagogues of natural origin have been described in various African countries for breastfeeding mothers as well as for livestock, e.g. in Morocco, Benin, Nigeria [9-11]. A recent ethnobotanical survey collected information on traditional knowledge of breast milk affecting plants in the Angolan province Uíge [12]. According to UNICEF, the South-Western African country suffers from malnutrition and one of world's highest under-five mortalities [13]. The respondents of the ethnobotanical study made a distinction between plants that increase milk production, plants that reduce milk production, plants that should be avoided during the breastfeeding period and plants that help to 'clean the milk'. According to the interviewees, the breast milk needs to be cleaned when it appears to be watery because this provokes diseases like diarrhea. Hence, we have adopted this unusual term also for the present study.
The murine mammary epithelial HC11 cells derived from the parental COMMA-1D cell line has been shown to differentiate in response to the lactogenic hormones prolactin, dexamethasone and insulin [14].

Moreover, HC11 cells have been used to investigated the impact of extracts of diverse plant species on the *in vitro* mammary cells differentiation [15-17]. Herein, we describe the preparation of aqueous extracts of twelve different Angolan plants described to affect human breast milk quantity or quality by tribes in the province Uíge, Northern Angola. The aqueous extracts were subsequently tested on their influence on the *in vitro* mammary cell differentiation of murine HC11 cells. The most active extracts were characterized with HPLC-DAD and NMR analyses to explore their chemical profile and the existence of major chemical classes in extracts composition.

Materials and Methods

Collection of plant material

Fresh plant material of twelve plant species (Fig. 1), mentioned in Jendras *et al.* [12] was collected by Gesine Jendras in February and March 2018 during field studies in the province Uíge, Angola. Subsequently the samples were cleaned, immediately dried in a drying cabinet (HTD 100 Bench Top, LinTek) at 30 ^{0}C, then vacuum-sealed, stored in a freezer and later sent to Athens, Greece, for extraction. At the same time, herbarium vouchers of the plant species mentioned were prepared, identified and stored in the Herbarium Dresdense (DR, voucher no. see Table 1).

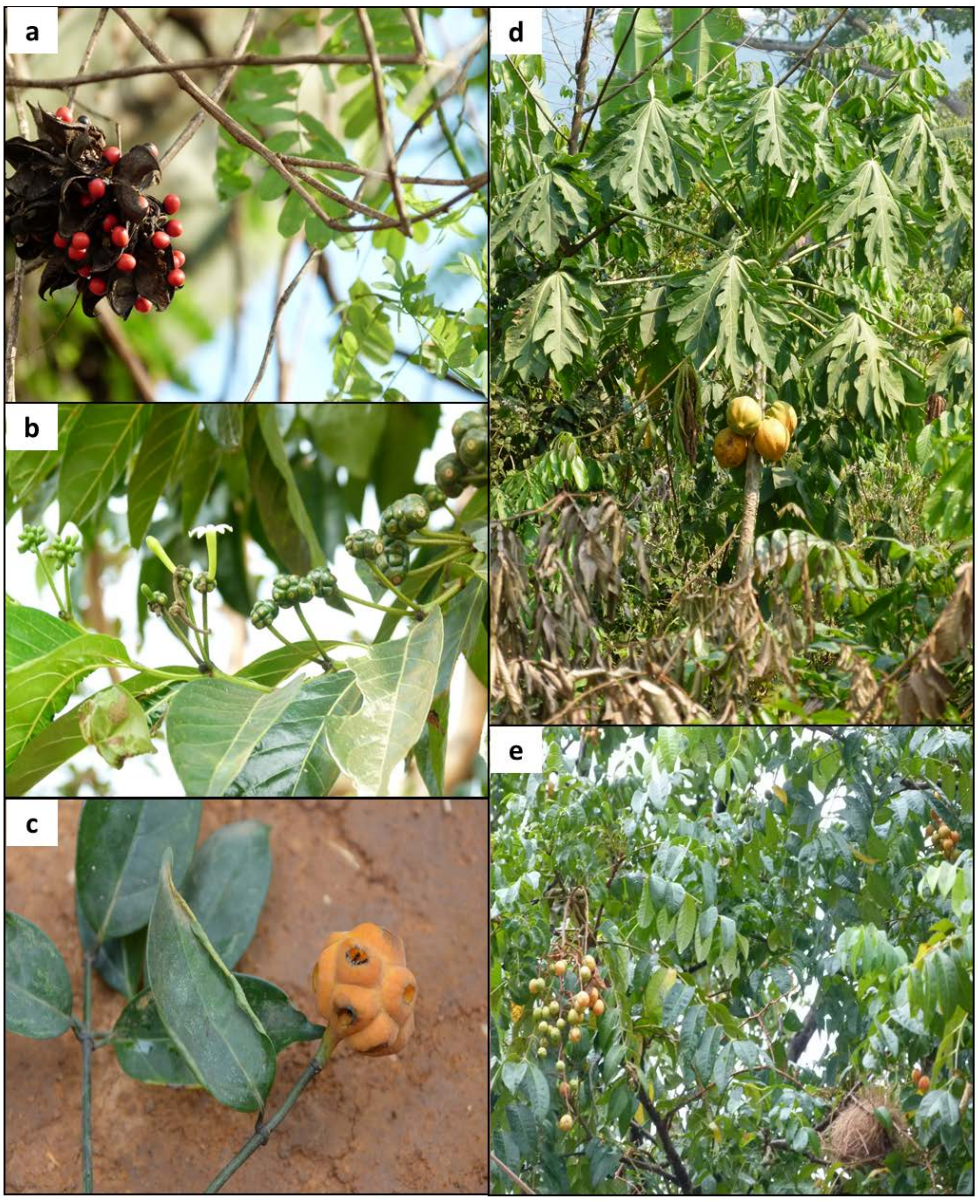


Fig 1: Plant material was collected from the wild in Uíge, Northern Angola. (a) *Abrus precatorius*, (b) *Morinda lucida*, (c) *Morinda morindoides*, (d) *Carica papaya* and (e) *Spondias mombin*.

Plant extraction

Extractions were carried out for each plant material employing ultrasound assisted extraction (UAE) methodology. More specifically, 2.0 g of the dried and pulverized plant materials were mixed with 20 mL of water (H_2O). Samples were set into the sonication bath for 2h, while temperature was adjusted at 30 ^{0}C. Finally, twelve extraction solutions were produced, which were then centrifuged for 20 min at 3500 rpm, for separation of the initial plant precipitates and powders (sediments) with the supernatants. After recovery of the supernatants, they were evaporated under vacuum conditions at 40 ^{0}C. Final extracts were weighted and stored in dark glassy vials at 4 ^{0}C. Table 1 illustrates in detail the initial plant materials and the final weight of the corresponding extracts.

Table 1: Representation of the used plants with their scientific name, the used part of the plant in the study and the final weight of the aqueous extracts. Local names are listed in Kikongo language. Herbarium vouchers are stored at the Herbarium Dresdense (DR), Institute of Botany, TU Dresden; photo vouchers are archived with the author TL. Naming of the plants related to the effects refer to the ethnobotanical survey in Uíge, + lactation: mentioned to increase lactation; - lactation: mentioned to decrease lactation; *total number of interviews.

Plant	Local name	Herbarium code	Code	Part of the plant	extract [mg]	Beneficial effects (12)		Adverse effects (12)	
						+ Lactation 259*	Clean milk 220*	Avoid 236*	- Lactation 124*
Abrus precatorius L.	Maique, Kiambiembie	DR 051649	Ap	leaves and tendrils	221.50	2 (3)	9 (9)		1 (2)
Carica papaya L.	Papayi	F_01	Cp	leaves	651.90	0 (1)	15 (15)		
Costus afer Ker Gawl.	Nsangalavua	DR 053482	Ca	fleshy stems	131.10	1 (1)			
Crassocephalum rubens (Juss. ex Jacq.) S.Moore	Bungudia	DR 051645	Cr	leaves	587.50			2 (4)	0 (1)
Maprounea africana Müll.Arg.	Nsiele nsiele	DR 051640	Ma	leaves	497.5	2 (2)	3 (3)	0 (1)	1 (1)
Momordica charantia L.	Lumbuzua, Mambuzu	DR 051646	Mc	leaves and tendrils	319.90	1 (2)	3 (5)		
Morinda lucida Benth.	Nsiki, masiki	DR 051642	Ml	leaves	378.00		4 (7)		
Morinda morindoides (Baker) Milne-Redh.	Meso-nkama, Nkongobololo	DR 051623	Mm	leaves	203.10		7 (10)	0 (1)	
Salacia erecta (G.Don) Walp.	Mbonda, Kanzangu	DR 053480	Sp	leaves	453.00			6 (12)	2 (2)
Solanum macrocarpon L.	Couve preta	F_02	Som	leaves	513.15	2 (2)			
Spondias mombin L.	Mungiengie	DR 051647	Spm	leaves	483.50	5 (8)	46 (80)	1 (1)	
Vernonia amygdalina Delile	Malulua	DR 051610	Va	leaves	188.4	3 (3)	11 (24)	3 (4)	1 (1)

Extracts chemical profiling

After extraction, the twelve aqueous extracts were analyzed with three different methodologies for investigation of their chemical profile. Initially, HPTLC and then HPLC-DAD and ^{1}H-NMR analyses were performed towards this purpose.

HPTLC analysis

For the High Performance Thin Layer Chromatography profiling, 20 µL of extracts were applied onto 20 × 10 cm HPTLC plates (silica gel 60 F_{254}, 0.20 mm layer thickness; Merck) using the Automatic TLC sampler (ATS4, CAMAG) under the control of the software platform VisionCats 2.5 (CAMAG). Samples were applied using a syringe of 25 µL (Hamilton) and a nitrogen aspirator. The plates were developed with an automatic development chamber (ADC2, CAMAG). The development system used as mobile phase dichlomethane (DCM)/MeOH (95:5 v/v). Plate images were recorded at 254 nm and 366 nm on a Visualizer 2 Documentation System (CAMAG). For visualization of the spots, the HPTLC plates were sprayed with sulphuric vanillin derivatization reagent (i.e. 5% w/v vanillin in MeOH/5% v/v H_2SO_4 in MeOH 1:1 v/v)

HPLC-DAD analysis

For the High Performance Liquid Chromatography-Diode Array Detector analysis of extracts, a Thermo Finnigan® HPLC-PDA System (P4000 Pump, AS3000 Autosampler, PDA Detector UV8000, Chromquest™ 4.1 Software) and a Supelco® RP18 Discovery HS-C18 (250 mm, 4.6 mm, 5 µm) column were employed. The mobile phase consisted of water with 0.1% acetic acid (Solvent A) and acetonitrile (Solvent B). Elution started with 2% of B and maintained at this proportion for 5 min. In the next 40 min B reached 100% and stayed for 5 min. At the 55th min system returned to the initial conditions and stayed for 5 min for system equilibration. The total analysis time was 60 min at a flow rate of 1 mL/min. Column temperature was set at 40 °C, while the injection volume was 20 µL. Chromatograms were recorded at 254 nm, 280 nm and 366 nm.

^{1}H-NMR experiments

^{1}H-NMR experiments were performed on a 600MHz Bruker Avance AVIII-600 spectrometer equipped with a TXI cryoprobe. 5mg of each extract were diluted in 600 mL of MeOD. Chemical shifts (δ) are expressed in ppm and coupling constants in Hz.

Cell culture

HC11 cells were cultivated in RPMI-1640 with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin (P/S), 10 µg/mL epidermal growth factor (EGF) and 5 µg/mL insulin (all components purchased from Sigma-Aldrich) (14). HC11 cells seeded at a density of 200.000 cells per 25 cm² flask were put on RPMI-1640 containing 10% FBS, 1% P/S and 10 µg/mL EGF for four days after reaching confluence. Thereafter, the cells were incubated for four days in differentiation medium consisting of RPMI-1640, 10% FBS, 1% P/S, 5 µg/mL insulin, 10^{-6} M dexamethasone (Sigma-Aldrich) and 0.5 µg/mL prolactin (Sigma-Aldrich) in the presence of either 1‰ DMSO or the aqueous extracts dissolved in DMSO. The aqueous extracts were tested in a dose-dependent manner (0.1, 1 and 10 µg/mL) in three independent cell culture experiments.

β-Casein gene expression analysis

Cells were lysed by the addition of peqGOLD TriFast™ and total RNA was isolated according to the manufacturer's instructions. After digestion of genomic DNA contaminations by RQ DNase, 3 µg RNA were incubated with MMLV reverse transcriptase and oligo $(dT)_{12-18}$ primers for first-strand cDNA synthesis.

The iCycler iQ™ Real-Time PCR Detection System (Bio-Rad Laboratories GmbH) and SybrGreen I as detection dye were applied for quantitative real-time PCR. The primer sequences used for amplification of mouse β-casein: forward 5'-AGGTGAATCTCATGGGACAGC-3', reverse 5'-CACAGGGGGTTGAGCAATAG-3', and for the mouse ribosomal protein S18 (*Rps*18): forward 5'-AGGATGTGAAGGATGGGAAG-3', reverse

5'-TTGGATACACCCACAGTTCG-3'. The expression was normalized to the housekeeping gene *Rps*18 and the ΔΔcT method was applied to evaluate the β-casein expression level relative to the solvent control cells treated with DMSO solely (set to 1) [18]

Statistical Analysis

Gene expression data are shown as arithmetic mean ± standard deviation from three independent cell culture experiments respectively. Statistical analysis comprised one-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc test. Results were considered as statistically significant at $p \leq 0.05$.

Results and Discussion

Extracts isolated from twelve different Angolan plant species selected on the basis of their mention in an ethnobotanical survey on effects on breast milk production in the province Uíge (Table 1, [12]) were tested for their impact on the mammary differentiation of murine HC11 cells. Amongst them, nine plants were recorded from interviewees asked about plants with beneficial impact on breast milk, that the respondents further separated in either increasing lactation or cleaning breast milk [12]. Four plant species have been already mentioned in a previous large-scale ethnobotanical study, that investigated general traditional medicinal plant use in Uíge [19]. Two of the plant species investigated herein were mentioned by the respondents when asked about a lactation-decreasing impact or about plants that should be avoided in general during breastfeeding (*Salacia erecta* and *Crassocephalum rubens*). Regarding *Vernonia amygdalina*, the survey recorded more or less balanced mentions for beneficial and adverse effects on breastfeeding.

Treatment of the HC11 cells with differentiation medium solely, significantly increased the β-casein mRNA-expression proving that the HC11 mammary cell differentiation can be induced and monitored by β-casein mRNA-levels (4000-fold compared to the proliferating cells, data not shown). The β-casein mRNA expression was significantly increased by treatment with 1 µg/mL *Carica papaya* extract (Fig. 2a, **Cp**), while the one-tenth lower as well as the tenfold higher doses had no impact on the mRNA expression. The interviewees described the usage of *Carica papaya* leaves to "clean breast milk", that was defined to be necessary if the milk seems too watery provoking diseases, e.g. diarrhea [12]. Traditional usage of *Carica papaya* is also described in Thailand, where the ripe fruit or a soup of unripe fruit is used to promote lactation [7, 20]. In lactating rats fed with a root extract, an increase of the mammary gland size, protein and prolactin content was shown [21]. A recent Indonesian study observed a significant increase of prolactin blood levels in breast-feeding mothers after a seven-day intake of a *Carica papaya* leaf juice (prepared from leaves with boiled water) [22]. The constraint of this study is the lack of a control group. Another study investigating the impact of an aqueous *C. papaya* fruit extract on dairy cows revealed a regulatory effect on immune- and antioxidant related genes and proteins in the milk, but no impact on the milk yield [23]. The authors conclude that *C. papaya* might exert beneficial effects on mammary gland health, e.g. decreasing the mastitis risk, and thereby affecting milk quality. This might be one of the reasons for the frequently mentioned 'clean the breast milk' as type of application in the ethnobotanical survey in Uíge, Angola [12].

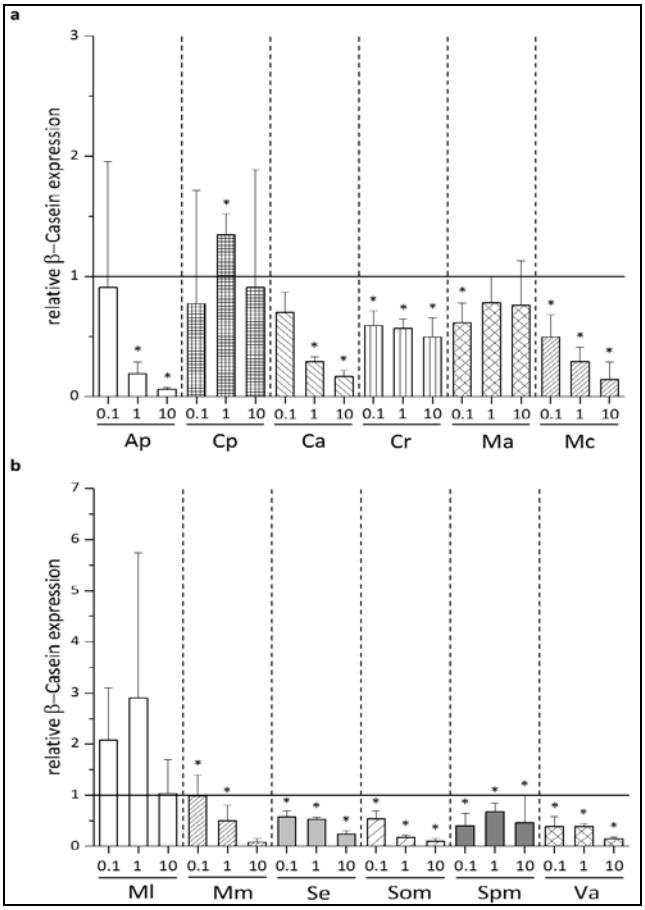


Fig 2: Relative β-casein expression in murine HC11 cells. HC11 cells were treated with differentiation medium containing 0.5 µg/mL prolactin and 0.1, 1 or 10 µg/mL of the respective plant extract. As solvent control, cells were treated with the differentiation medium and 1 ‰ DMSO (set to 1). * Denotes statistically significant differences in comparison to DMSO-treated cells ($p \leq 0.05$).

In the present study, the *Morinda lucida* extract also increased the β-casein mRNA expression in HC11 cells, though statistically not significant (Fig 2B, Ml). Just like for *C. papaya*, *Morinda lucida* leaves were mentioned to be used by breast-feeding women in Angola for the so-called cleaning the breast milk. Traditional usages of *Morinda lucida* are also described for Nigeria, while there are no references for its utilization regarding lactation [24].

Among the other seven extracts referred to as beneficial for human breast milk production or quality, the β-casein mRNA-expression was rather reduced compared to the DMSO-treated cells in a dose-dependent manner (Fig. 2). This down-regulation of the β-casein gene expression supposes an inhibitory effect on the induced mammary cell differentiation of HC11 cells. The highest inhibitory effects were observed for the highest concentrations of *Abrus precatorius*, *Momordica charantia*, *Morinda morindoides*, and *Solanum macrocarpon* (Ap 0.06±0.01; Mc 0.14±0.14; Mm 0.08±0.06; Som 0.09±0.04; $p \leq 0.05$).

Different traditional usages of *Abrus precatorius*, amongst others effect on anti-fertility and abortifacient effects supported by data from rat studies, have been described for African and Indian countries, while description of effects on breast milk production or quality to our knowledge are missing [25–27].

In contrast, one of *Momordica charantia*'s traditional applications described is as galactagogue [28, 29]. Hence, preclinical studies observed an impact of *M. charantia* leaf extract on murine mammary gland development as well as an increase of alveoli diameter and blood prolactin in lactating mice in response to fruit juice [30, 31]. *Spondias mombin* was the most frequently mentioned plant species with 36% of the respondents assigning breast milk cleaning properties (Table 1) [12]. So far, there is no other description of its traditional usage as galactagogue in humans. However, several studies investigated *S. mombin* leaves impact on milk production for improvement of breeding in the West African Dwarf sheep. These investigations from Benin and Nigeria observed lactogenic effects of fresh or dried *S. mombin* leaves fed to ewes as an increase of the milk yield, the serum prolactin levels as wells as the lamb's body weight gains [10, 11, 32, 33]. In the present study, the aqueous of *S. mombin* leaf extract inhibited the mammary differentiation of HC11 cells, as the β-casein mRNA expression was half of that induced in the DMSO-treated control cells (Fig. 2b, Spm).

With regard to the three species mentioned to have adverse effects on breastfeeding, all three extracts repressed the β-casein mRNA expression significantly in a dose-dependent manner (Fig. 2, Cr, Se, Va).

Our observations diverging from those of *in vivo* studies might be based on different molecular actions or on the metabolisation of the compounds. While the *in vitro* HC11 mammary cell differentiation is based on a prolactin receptor agonistic activity of the investigated compounds, a compound can be shown to act as a galactagogue in *in vivo* studies by an indirect effect, e.g. influencing prolactin blood levels. Furthermore, the often ascribed breast milk cleaning effect might be based on other indirect mechanisms, for example an impact on mammary gland health by immune regulation as described for *Carica papaya* [23]. Those mechanisms could only be addressed applying *in vivo* assays. In response to the biological results, the chemical profiles of the two most active extracts, *Morinda lucida* and *Carica papaya*, were further investigated. Initially, HPTLC was used as a fast screening method, however, the high abundance of sugars as expected in aqueous extracts didn't reveal meaningful information (Fig. 3).

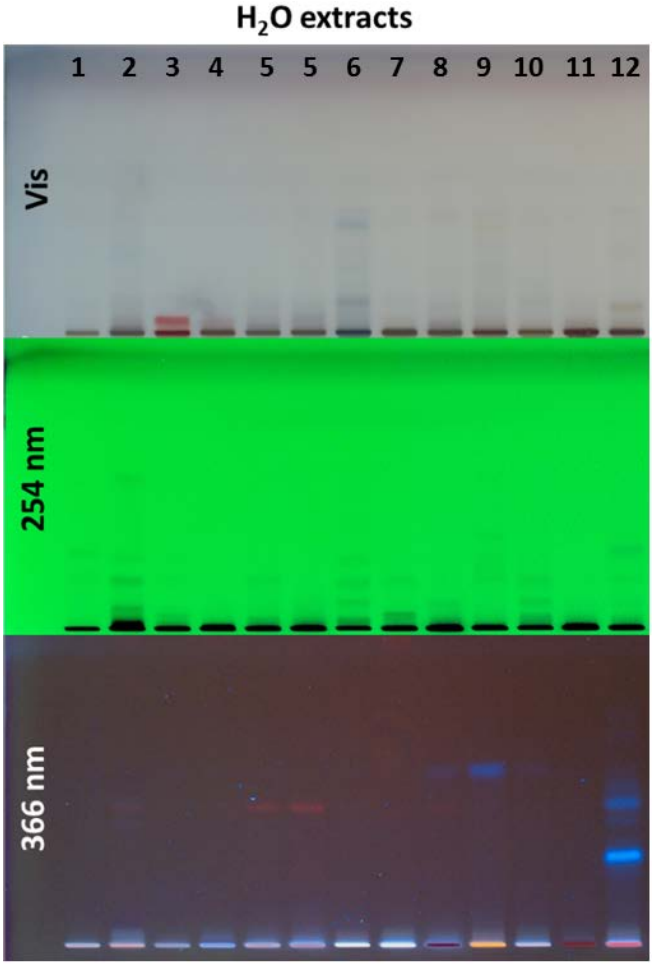


Fig 3: HPTLC analysis of the aqueous extracts developed in DCM/MeOH 95:5. Plates were recorded at vis after spraying with sulfuric vanillin, at 254 nm and at 366 nm. 1: Ca, 2: Ap, 3: Sp, 4: Spm, 5: Va, 6: Mc, 7: Cp, 8: Cr, 9: Mm, 10: Som, 11: Ma, 12: Ml.

Therefore, HPLC-DAD and ^{1}H-NMR analysis were used in order to identify at least the major chemical classes. Beginning with HPLC-DAD analysis, *Morinda lucida* showed richer profile and more intense chromatographic peaks in comparison to *Carica papaya* (Fig. 4). It is worth noting that both extracts revealed similar UV spectra in several peaks (λmax at 254 and 320 nm) characteristic for cinnamic acid derivatives and flavonoids [34, 35].

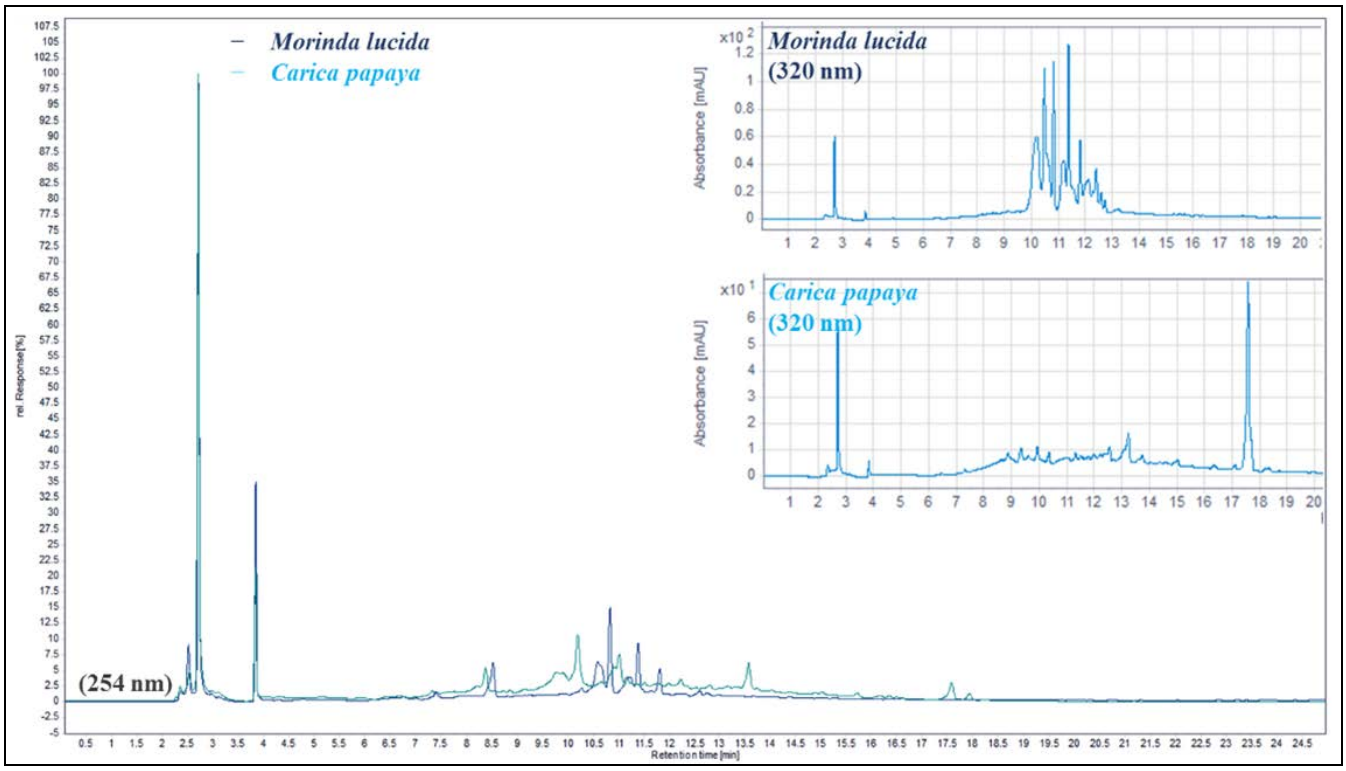
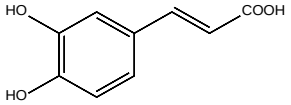
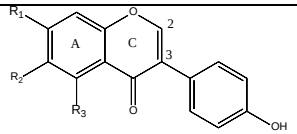
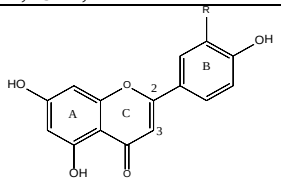
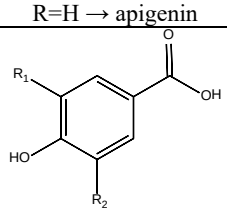


Fig 4: HPLC-DAD chromatograms of aqueous extracts of *Morinda lucida* (dark blue line) and *Carica papaya* (light blue line) at 254 nm. The respective chromatograms at 320 nm are illustrated in the inserted chromatograms respectively.

Continuously, ^{1}H-NMR experiments were performed to investigate characteristic resonances indicating certain chemical classes (Table 2). Like HPLC-DAD analysis, samples presented similar spectra, with *Morinda lucida* being richer verifying HPLC-DAD observations. The majority of peaks was detected in the area between 6-9 ppm, indicating the presence of phenolic compounds. Both spectra were also characterized by resonances (at 3-5 ppm), indicating the presence of sugars, while the characteristic double peaks of anomeric protons witness the presence of both free and conjugated sugars.

Table 2: NMR spectroscopic data (600 MHz, MeOD) of Morinda lucida and Carica papaya extracts. Chemical shifts (δ) are expressed in ppm and coupling constants in Hz. The respective chemical structures are also illustrated.

δ_H, multiplicity (*J* inHz)	**Chemical structures**
7.59, d (15.9) 6.34, d (15.9) 6.96, dd (8.1/2.1) 7.07, d (2.1) 6.78, d (8.1)	 Caffeic acid
8.2, s 8.04, d (8.9) 6.91, d (8.9)	 R_1,R_3=OH, R_2=H →genistein R_1,R_3=H, R_2=OH → daidzein
6.69, s 7.72, d (8.6) 7.10, d (8.6) 7.58, dd, (8.1/2.0) 6.92, d (8.1) 7.71, d (2.0)	 R=OH →luteolin R=H → apigenin
7.73, d (1.7) 7.58, dd (8.1/1.7) 6.92, d (8.1) 3.41, d (2.1) 7.45, d (2.1) 7.41, d (2.1)	 R_1=H, R_2=OCH_3 → vanillic acid R_1,R_2=OH → gallic acid

The ^{1}H-NMR spectrum of *Morinda lucida* was characterized by *trans*-hydroxycinnamic derivatives as indicated by the characteristic resonances of a *trans*-double bond and a substituted aromatic ring. For instance, it is evident the clear presence of caffeic acid also previously reported in *M. lucida* leaves extract [36]. Moreover, dishielded signals at approx. δ_H 8.20 (2H, s), characteristic of isoflavones were detected. In combination with additional spin systems in the aromatic region, the presence of daidzein and genistein derivatives, the well-known phytoestrogens is evident [37, 38]. Concerning *Carica papaya*, only signals corresponding to flavonoids were detected and most specifically flavones e.g. luteolin and flavonols signals [39]. Additionally, phenolic acids such as vanillic and gallic acid derivatives were identified in the same extract [40, 41]. According to literature, the two plants are mainly characterized by phenolic compounds and flavonoid derivatives [37, 39], compounds which have been already reported for their galactagogue properties [42, 43]. Table 1 shows the characteristic peaks demonstrating the existence of specific chemical categories with the respective general structure.

In conclusion, extracts from twelve different plant species either recommended or disadvised for improving human breast milk in an ethnobotanical survey in Uíge, Angola were screened on mammary cell differentiation of murine HC11 cells. Leaf extracts of *Carica papaya* and *Morinda lucida* induced HC11 differentiation elevated the mRNA expression of the milk protein β-casein, which is used as a marker of mammary cell differentiation. The observation that the majority of the plant extracts did not induce mammary epithelial cell differentiation, assumes that the plants might rather exert their effects on prolactin levels or on other mechanism influencing the mammary gland health. Phenolic compounds and flavonoids were identified as the major chemical classes in *Morinda lucida* and *Carica papaya* aqueous extracts, demonstrating them as potentially chemical classes responsible for the observed activity.

Acknowledgements

We thank Gesine Jendras for collecting the plant material during her studies in Uíge. Furthermore, the article would not have been possible without the contribution of knowledge from the villagers of the Province Uíge. The University Kimpa Vita provided logistical support. The fieldwork in Angola was supported by the program “Strategic Partnerships” of the TU Dresden. These published results were obtained in collaboration with the Instituto Nacional da Biodiversidade e Áreas de Conservação (INBAC) of the Ministério do Ambiente da República de Angola. The authors would like to thank the European Union (ERDF) and Greek national funds through the Operational Program “Competitiveness, Entrepreneurship and Innovation”, under the call “STRENGTHENING RESEARCH AND INNOVATION INFRASTRUCTURES” (project code: 5002803 - PlantUP).

References

1. World Health Organization. Infant and young child feeding. https://www.who.int/news-room/fact-sheets/detail/infant-and-young-child-feeding. 9 may 2021
2. Pretorius CE, Asare H, Genuneit J, Kruger HS, Ricci C. Impact of breastfeeding on mortality in sub-Saharan Africa: a systematic review, meta-analysis, and cost-evaluation. Eur J Pediatr 2020;179(8):1213–25.
3. Kramer MS, Chalmers B, Hodnett ED, Sevkovskaya Z, Dzikovich I, Shapiro S *et al*. Promotion of Breastfeeding Intervention Trial (PROBIT): A Randomized Trial in the Republic of Belarus. J Am Med Assoc 2001;285(4):413.
4. Bazzano AN, Hofer R, Thibeau S, Gillispie V, Jacobs M, Theall KP. A Review of Herbal and Pharmaceutical Galactagogues for Breast-Feeding. Ochsner J. 2016;16(4):511-24.
5. Neuwinger H. African Traditional Medicine. A Dictionary of Plant Use and Applications. Edn 1. Medpharm Scientific Publishers, Stuttgart, 2000, 589.
6. Dandotiya H, Singh G, Kashaw S. The Galactagogues Use by Indian Tribal Communities to Over Come Poor Lactation. Int J Biotechnol Bioeng Res. 2013;4(3):243-8.
7. Luecha P, Umehara K. Thai medicinal plants for promoting lactation in breastfeeding women. In: Handbook of dietary and nutritional aspects of human breast milk. Wageningen Academic Publishers; (Human Health Handbooks; 2013;(5):645-54.
8. Bekoe EO, Kitcher C, Gyima NAM, Schwinger G, Frempong M. Medicinal Plants Used as Galactagogues. In: Pharmacognosy - Medicinal Plants. Intech Open 2018.
9. Bnouham M. Medicinal Plants with Potential Galactagogue Activity Used in the Moroccan Pharmacopoeia. J Complement Integr Med [Internet]. 2010 [cited 2021 Feb 9];7(1). Available from: https://www.degruyter.com/document/doi/10.2202/1553-3840.1268/html
10. Akouèdégni C, Tossa I, Ahoussi E, Hounzangbé-Adoté M. Effects of the fresh leaves of *Spondias mombin* L. on milk production of West African Dwarf (WAD) ewes and their lamb’s growth performance. Glob J Res Med Plants Indig Med 2013;2(3):126-34.
11. Oguike M, Udeh N. Influence of the Ethnoveterinary Plant *Spondias mombin* L. on Partial Daily Milk Yield (PDM), Haematology and Serum Biochemistry of Lactating West African Dwarf (WAD) Ewes. J Anim Vet Adv 2008;7(5):584-8.
12. Jendras G, Monizi M, Neinhuis C, Lautenschläger T. Plants, food and treatments used by BaKongo tribes in Uíge (northern Angola) to affect the quality and quantity of human breast milk. Int Breastfeed J 2020;15(1):88.
13. UNICEF. Angola Appeal - Humanitarian Action for Children. https://www.unicef.org/appeals/angola. 9 may 2021
14. Ball RK, Friis RR, Schoenenberger CA, Doppler W, Groner B. Prolactin regulation of beta-casein gene expression and of a cytosolic 120-kd protein in a cloned mouse mammary epithelial cell line. EMBO J 1988;7(7):2089-95.
15. Starvaggi Cucuzza L, Motta M, Miretti S, Accornero P, Baratta M. Curcuminoid-phospholipid complex induces apoptosis in mammary epithelial cells by STAT-3 signaling. Exp Mol Med 2008;40(6):647–57.
16. Starvaggi Cucuzza L, Motta M, Accornero P, Baratta M. Effect of *Echinacea augustifolia* extract on cell viability and differentiation in mammary epithelial cells. Phytomedicine Int J Phytother Phytopharm 2008;15(8):555-62.
17. Tchoumtchoua J, Makropoulou M, Ateba SB, Boulaka A, Halabalaki M, Lambrinidis G, *et al.* Estrogenic activity of isoflavonoids from the stem bark of the tropical tree *Amphimas pterocarpoides*, a source of traditional medicines. J Steroid Biochem Mol Biol 2016;158:138-48.

18. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 2001;29(9):e45.
19. Lautenschläger T, Monizi M, Pedro M, Mandombe JL, Bránquima MF, Heinze C *et al*. First large-scale ethnobotanical survey in the province of Uíge, northern Angola. J Ethnobiol Ethnomedicine 2018;14(1):51.
20. Elgadir MA, Salama M, Adam A. *Carica papaya* as a source of natural medicine and its utilizationin selected pharmacetical applications. Int J Pharm Pharm Sci. 2014;6(1):880-4.
21. Tossawanchuntra G, Aritajat S. Effect of Aqueous Extract of *Carica papaya* Dry Root Powder on Lactation of Albino Rats. Acta Hortic 2005;(678):85-90.
22. Ikhlasiah M, Winarni LM, Poddar S, Bhaumik A. The effects of papaya leaf juice for breastfeeding and working mothers on increasing prolactin hormone levels and infant's weight in Tangerang. Enferm Clínica 2020;30:202-5.
23. Abouzed TK, Sadek KM, Ayoub MM, Saleh EA, Nasr SM, El-Sayed YS *et al*. Papaya extract upregulates the immune and antioxidants-related genes, and proteins expression in milk somatic cells of Friesian dairy cows. J Anim Physiol Anim Nutr 2019;103(2):407-15.
24. Adeleye OO, Ayeni OJ, Ajamu MA. Traditional and medicinal uses of Morinda lucida. J Med Plants Stud. 2018;6(2):249-54.
25. Solanki A, Zaveri M. Pharmacognosy, Phytochemistry and Pharmacology of *Abrus precatorius* Leaf: A review. Nternational J Pharm Sci Rev Res 2012;13(2):71-6.
26. Ogbuehi IH, Ebong OO, Obianime AW. A Preliminary Study on the Effect of *Abrus precatorius* Linn. on Reproductive Parameters in Female *Rattus norvegicus*, Wistar Strain. Eur J Med Plants 2015;156-66.
27. Bhakta S, Das SK. The medicinal values of *Abrus precatorius*: a review study. J Adv Biotechnol Exp Ther 2020;3(2):84-91.
28. Grover JK, Yadav SP. Pharmacological actions and potential uses of *Momordica charantia*: a review. J Ethnopharmacol 2004;93(1):123–32.
29. Sathis Kumar D, Vamshi Sharathnath K, Yogeswaran P, Harani A, Sudhakar K, Sudha P et al. A Medical Potency of *Momordica charantia*. Int J Pharm Sci Rev Res 2010;1(2):95-100.
30. Hayati N, Aini AN, Nafisatuzzamrudah N, Lestari U. The Effects of Water Fraction of Bitter Melon (*Momordica charantia*) Leaf Extract in Mammary Gland Development of Balb/c Mice (*Mus musculus*) with Histological and Molecular Biological Analysis of Protein Approaches. UNEJ E-Proceeding 2017, 27-9.
31. Luangpirom A, Kourchampa W, Somsapt P. Effect of bitter melon (*Momordica charantia* Linn.) fruit juice on blood prolactin level and histological change of mammary gland in lactating mice 2013;5(2):249-54.
32. Akouèdégni C, Houndonougbo P, Adenile A, Allowanou O, Hounzangbé-Adoté M. Comparative Effectiveness of Fresh Leaves and Leaves Powder of *Spondias mombin* on Milk Production of Djallonke Ewes and Weight Growth of Theirs Lambs in Southern Benin. Int J Adv Res 2019;7(10):114-20.
33. Akouèdégni C, Adenile AD, Olounlade PA, Ahoussi E, Hamidou HT, Hounzangbe-Adote MS. Lactogenic effects of the leaf's powder of *Spondias mombin* L. on West African Dwarf (WAD) sheep performance and serum prolactin level in Republic of Benin. Indian J Anim Res 2020;54(2):254-8.
34. Bengoechea L, Hernández T, Quesada C, Bartolomé B, Estrella I, Gómez-Cordovés C. Structure of hydroxycinnamic acid derivatives established by high-perfomance liquid chromatography with photodiode-array detection. Chromatographia 1995;41(1):94-8.
35. Sisa M, Bonnet SL, Ferreira D, Van der Westhuizen JH. Photochemistry of Flavonoids. Molecules 2010;15(8):5196-245.
36. Adefegha SA, Molehin O. Inhibitory Activities of Brimstone (*Morinda lucida*) Roots Extract on α-Amylase and α-Glucosidase-*In vitro*. Vegetos. 2017;30:105-9.
37. Chokki M, Cudălbeanu M, Zongo C, Dah-Nouvlessounon D, Ghinea IO, Furdui B *et al*. Exploring Antioxidant and Enzymes (A-Amylase and B-Glucosidase) Inhibitory Activity of Morinda lucida and *Momordica charantia* Leaves from Benin. Foods. 2020, . 9.
38. Tchoumtchoua J, Mathiron D, Pontarin N, Gagneul D, van Bohemen A-I, Otogo N'nang E *et al*. Phenolic Profiling of Flax Highlights Contrasting Patterns in Winter and Spring Varieties. Molecules 2019;24(23).
39. Nugroho A, Heryani H, Choi JS, Park H-J. Identification and quantification of flavonoids in *Carica papaya* leaf and peroxynitrite-scavenging activity. Asian Pac J Trop Biomed 2017;7(3):208-13.
40. Sankarganesh P, Joseph B, Kumar G, Illanjiam S, Srinivasan T. Phytomedicinal Chemistry and Pharmacognostic Value of *Carica papaya* L., Leaf. J Pure Appl Microbiol 2018;12:751-6.
41. López-Martínez LM, Santacruz-Ortega H, Navarro R-E, Sotelo-Mundo RR, González-Aguilar GA. A 1H NMR Investigation of the Interaction between Phenolic Acids Found in Mango (*Manguifera indica cv* Ataulfo) and Papaya (*Carica papaya cv* Maradol) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) Free Radicals. PLOS ONE2015;10(11):e0140242.
42. Setyono FS, Adi AC, Ismawati R. Galactogogue Instant Powder Combination of Papaya Leaves and Red Ginger for Breastfeeding Mother. Int J Prev Public Health Sci. 2016;2(4):32-6.
43. Koko BK, Konan AB, Kouacou FKA, Djétouan JMK, Amonkan AK. Galactagogue Effect of *Euphorbia hirta* (Euphorbiaceae) Aqueous Leaf Extract on Milk Production in Female Wistar Rats. J Biosci Med 2019;07(09):51.