INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES
(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijcrcps Coden: IJCROO(USA) Volume 13, Issue 6- 2026

Research Article

DOI: http://dx.doi.org/10.22192/ijcrcps.2026.13.06.002

Phytochemical Profile and Galactogenic Activity of *Carica papaya* L. and *Momordica charantia* L. Extracts

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Abstract

Hypolactation remains a significant barrier to optimal breastfeeding practices in developing countries. Medicinal plants represent a promising therapeutic alternative to existing pharmacological interventions. This studyaimed to evaluate and compare the galactogenic potential of ethanolic extracts of *Carica papaya* L. (CP) and *Momordica charantia* L. (MC) in lactating Wistar rats. Both extracts were subjected to qualitative and quantitative phytochemical characterization. Galactogenic activity was assessed *in vivo* via serum prolactin quantification by enzyme-linked immunosorbent assay (ELISA) and milk yield estimation using the weigh-suckle-weigh technique. Qualitative phytochemical screening identified the common presence of alkaloids, flavonoids, and tannins in both extracts, with coumarins and leucoanthocyanins detected exclusively in CP. The *C. papaya* extract exhibited an extraction yield of 15.00 ± 1.10% and a total polyphenol content of 922.66 ± 0.0001 mg GAE/g. At day 6 (D6), CP administered at 250 mg/kg induced a serum prolactin level of 24.15 ng/ml compared to 22.45 ng/ml for MC and 20.60 ng/ml for the negative control. Milk production in CP-treated animals (5 ± 0.30 ml) markedly exceeded that observed in MC-treated animals (1 ± 0.30 ml). The ethanolic extract of *Carica papaya* L. demonstrated superior galactogenic efficacy and warrants further pharmacological investigation.

Keywords: *Carica papaya*, *Momordica charantia*, ethanolic extract, galactogogue, prolactin, phytochemistry.

1. Introduction

Breastfeeding constitutes an essential physiological process for the growth, development, and survival of the neonate. It is regulated by a complex network of hormonal interactions primarily involving prolactin, acting in synergy with glucocorticoids, progesterone, estrogens, and oxytocin (Sevrin, 2020; Ennouini, 2023). According to the World Health Organization (WHO, 2023), insufficient or inadequate lactation may lead to severe consequences, including increased risk of malnutrition, infections, growth retardation, and infant mortality. The WHO estimates that inappropriate infant feeding practices are associated with approximately 45% of deaths among children under five years of age, whereas optimal breastfeeding could prevent more than 820,000 deaths annually (WHO, 2023).

In Africa, the use of galactogenic plants constitutes a widespread practice for enhancing milk production (Boko et al., 2021; Agabi et al., 2021). These plants contain diverse phytochemical constituents, including polyphenols, flavonoids, saponins, alkaloids, and tannins (Ben Moussa, 2022; Konan et al., 2021), which are capable of modulating the hormonal mechanisms underlying lactation, particularly prolactin secretion (Larry et al., 2018). Among these, *Carica papaya* L. and *Momordica charantia* L. are extensively employed in traditional medicine for their anti-inflammatory, antimicrobial, antifungal, and antioxidant properties (Chokki et al., 2023; Kassi et al., 2020; Cherry et al., 2021).

Despite their widespread traditional use, scientific data regarding their galactogenic efficacy remain limited. Therefore, the present study aimed to evaluate the galactogenic activity of ethanolic leaf extracts of *Carica papaya* L. and *Momordic acharantia* L. by examining their capacity to stimulate prolactin secretion in lactating female rats.

2. Materials and Methods

2.1. Phytochemical analysis

2.1.1. Plant Material

Plant material (figure 1) was collected in the early morning hours from natural forest habitats surrounding the municipalities of Natitingou, Za-Kpota, Covè, Zagnanado, Houinhi, and Zogbodomè.

***Carica papaya* L.** ***Momordica charantia* L.**

Figure 1 : leaves of *carica papaya* L. and *Momordica charantia*L.

The collected specimens were identified at the National Herbarium of Benin by comparison with reference herbaria using the Analytical Flora of Benin, following drying procedures (Table 1).

Table 1: Herbarium identification numbers of the plant specimens

Identification No.	Scientific Name	Family
YH 1119/HNB	*Carica papaya L.*	Caricaceae
YH 1121/HNB	*Momordica charantia L.*	Cucurbitaceae

2.1.2. Preparation of Plant Powders

Leaves of both galactogenic plants were dried in the shade under ambient laboratory conditions at the Kaba Research Laboratory in Chemistry and Applications (LaKReCA), University of Technical Sciences, Engineering and Mathematics (UNSTIM), for a period of two weeks (Balogoun et al., 2025). The dried material was subsequently ground into a fine powder using an electric mill and stored in amber glass jars protected from light until further use.

2.1.3. Preparation of Extracts

Extraction was performed by maceration (solid-liquid extraction), a method that involves immersing the plant material in a solvent to extract bioactive compounds (Lehout and Laib, 2015). This approach allows solvent volume conservation and the recovery of bioactive constituents at low temperatures. Briefly, 60 g of plant powder was suspended in 600 mL of 96% ethanol (Balogoun et al., 2025). Following 72 hours of maceration in the dark, the mixture was filtered and the filtrate evaporated at 35°C under reduced pressure (Balogoun et al., 2025). The extraction yield was calculated using the following formula:

R = 100 × (Mass of dry powder obtained / Mass of plant material)

2.1.4. Qualitative Analysis of Phytochemical Groups

Qualitative phytochemical screening was conducted to identify the major secondary metabolite classes present in each extract through solubility tests, colorimetric reactions, precipitation assays, and ultraviolet light examination, using the method of Houghton and Raman (1998).

2.1.5. Quantitative Analysis of Total Polyphenols

Total polyphenol content was determined using the Folin-Ciocalteu reagent. This reagent under goes reduction upon oxidation of phenolic compounds to yield a mixture of blue tungsten and molybdenum oxides, exhibiting maximum absorbance at approximately 750 nm. Absorbance values, measured against a calibration curve constructed with gallic acid as a reference phenolic compound, allowed quantification of total phenolic content expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g) (Fagbohoun, 2014).

2.1.6. Quantitative Analysis of Flavonoids

Total flavonoid content was estimated using the aluminum trichloride ($AlCl_3$) colorimetric method, with quercetin as the reference compound for calibration curve construction (Fagbohoun, 2014). Total polyphenol and flavonoid contents were calculated using the following formula:

$$T = (C \times V_r) / (V_p \times C_p)$$

Where: T = compound content; C = concentration derived from the calibration curve; V_r = reaction volume; V_p = volume of extract sampled; C_p = concentration of the extract solution.

2.1.7. Quantitative Analysis of Tannins

Total condensed tannin content was determined by the vanillin-hydrochloric acid colorimetric method. This method is based on the reaction of condensed tannins with vanillin in an acidic medium (hydrochloric acid) to produce a red-colored complex, the intensity of which is measured spectrophotometrically at 500 nm (Hagerman et al., 2002). Quantification was performed using a calibration curve with quercetin or catechin as a reference standard, with results expressed as standard equivalents per gram of extract.

2.2. Assessment of galactogenic activity

2.2.1. Animal Material and Experimental Groups (Prolactin Assay)

The study was conducted on non-pregnant, lactating female Wistar rats with a mean body weight of 188 g. Animals were randomly assigned to six homogeneous groups of four rats each and housed in standardized cages. A two-week acclimatization period preceded all experimental procedures, during which animals had *ad libitum* access to food and distilled water, in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). Housing conditions were maintained constant at 22 ± 2°C with a 12-hour light/dark cycle, consistent with international ethical principles governing animal experimentation (Ogbuewu, 2011). All procedures were conducted at the animal facility of the Para-University Institute for Research and Development in Health and Industry (APIPHARMA), located in Cana/Zogbodomey, Benin.

2.2.2. Extract Administration and Experimental Groups

Six experimental groups were constituted as follows: Group 1 (negative control) received distilled water at 2 mL/100 g body weight per day; Group 2 (positive control) received domperidone (Nauselium® 10 mg) at 0.75 mg/kg/day, a reference compound recognized for its galactogenic properties through stimulation of prolactin secretion (Forinash et al., 2012). Groups 3 and 4 received 250 mg/kg/day and 500 mg/kg/day, respectively, of the ethanolic extract of *Carica papaya* L. (CP); Groups 5 and 6 received identical doses of the ethanolic extract of *Momordica charantia* L. (MC). All treatments were administered by gastric gavage from day 0 (D0) to day 6 (D6), according to the technique described by Turner et al. (2011).

2.2.3. Serum Prolactin Quantification

Blood samples were collected at two time points: at D0, prior to the onset of treatment, and at D6, uponcompletion of the administration period. Animals were anesthetized by ether or chloroform inhalation, and blood was collected by retro-orbital sinus puncture using capillary tubes, following a technique routinely employed in rodent studies (Hoff, 2000). Samples were collected in dry tubes and centrifuged promptly to isolate serum and preven themolysis. Serum prolactin concentration was determined by enzyme-linked immunosorbent assay (ELISA), performed in accordance with the manufacturer's kit instructions (Freeman et al., 2000).

2.3. Effect of extracts on milk production

2.3.1. Animal Material and Experimental Groups (Milk Yield Assay)

This component of the study involved non-pregnant, lactating female Wistar rats with a mean body weight of 150 g, each accompanied by two pups. Animals were randomly assigned to four groups, each comprising four dams with their respective pups. Unlike the hormonal assay protocol, animals were denied free access to food and water during the experimental phase in order to avoid interference with milk yield measurements (Ogbuewu et al., 2011).

2.3.2. Extract Administration and Experimental Groups (Milk Yield)

To evaluate milk production before and after extract administration, Group 1 (negative control, four dams with two pups each) received distilled water at 2 mL/100 g body weight; Group 2 (positive control) received domperidone (Nauselium® 10 mg) at 0.75 mg/kg/day; Groups 3 and 4 received CP and MC extracts, respectively, at a single dose of 250 mg/kg/day by gastric gavage (Turner et al., 2011; Akintunde et al., 2012).

2.3.3. Milk Yield Estimation Protocol

Milk production was estimated in directly by measuring pup weight gain, using the weigh-suckle-weigh method described by Taga et al. (2009) and widely applied in the evaluation of plant-derived galactogogues. Each day, pups were individually weighed at 08:00 h (S1), then separated from their dams for a four-hour period to allow milk accumulation in the mammary glands. At 12:00 h, pups were reweighed (S2) before being returned to their dams for a 22-hour suckling period. A third weighing (S3) was performed the following morning at 08:00 h. Milk yield following maternal gavage was calculated as S3 − S2, representing the quantity of milk ingested by pups during the suckling period. Dams were also weighed daily to correct for body weight losses attributable to metabolic processes occurring during the measurement period (Sevrin et al. 2017).

3. Results

3.1. Phytochemical analysis

3.1.1. Extraction Yields and Physical Characteristics

The ethanolic extract of *Carica papaya* L. (CP) presented as a firm paste with an extraction yield of 15.00 ± 1.10%, while the ethanolic extract of *Momordica charantia* L. (MC) appeared as a granular paste with a lower yield of 10.83 ± 1.96%.

3.1.2. Qualitative Phytochemical Analysis

Table 2 reveals a high abundance of bioactive secondary metabolites in both extracts. Qualitative analysis identified the common presence of alkaloids, polyphenols, catechic and gallic tannins, flavonoids, anthocyanins, triterpenoids, steroids, reducing compounds, and O-heterosides with reduced genins. Leucoanthocyanins, coumarins, and quinonic derivatives were detected exclusively in CP, whereas mucilages and free anthraquinone derivatives were identified solely in MC.

Table 2: Qualitative composition of *Carica papaya* L. (CP) and *Momordica charantia* L. (MC) Extracts

Phytochemical Compound	CP	MC	Phytochemical Compound	CP	MC
Alkaloids	+	+	Saponins	−	−
Polyphenols	+	+	Triterpenoids	+	+
Tannins	+	+	Steroids	+	+
Catechic Tannins	+	+	Cyanogenic Derivatives	−	−
Gallic Tannins	+	+	Mucilages	−	+
Flavonoids	+	+	Coumarins	+	−
Anthocyanins	+	+	Reducing Compounds	+	+
Leucoanthocyanins	+	−	Free Anthraquinone Derivatives	−	+
O-heterosides with reduced genins	+	+	Quinonic Derivatives	+	−
C-heterosides with reduced genins	+	+			

+ = Present; − = Absent

3.1.3. Quantitative PhytochemicalA nalysis

Table 3 presents the quantitative phytochemical profile of both extracts. CP exhibited a markedly higher total polyphenol content (922.66 ± 0.0001 mg GAE/g) and a total flavonoid content of 259.97 ± 0.0001 mg QE/g, with total tannins at 210 ± 0.0001 mg CE/g. MC displayed higher total tannin (350 ± 0.0001 mg CE/g) and flavonoid (345.02 ± 0.0001 mg QE/g) contents, but a considerably lower total polyphenol content of 188.10 ± 0.0001 mg GAE/g.

Table 3: Quantitative analysis of *Carica papaya* L. (CP) and *Momordica charantia* L. (MC) extracts

Extract	Total Tannin Content (mg CE/g extract)	Flavonoid Content (mg QE/g extract)	Polyphenol Content (mg GAE/g extract)
CP	210 ± 0.0001	259.97 ± 0.0001	922.66 ± 0.0001
MC	350 ± 0.0001	345.02 ± 0.0001	188.10 ± 0.0001

3.2. Galactogenic Activity

3.2.1. Serum Prolactin Levels

Figure 2 illustrates the serum prolactin concentrations between D0 and D6 across all treatment groups. Baseline prolactin levels at D0 were homogeneous, ranging from 19.51 to 21.33 ng/mL. At D6, the highest values were recorded in the domperidone group (Nauselium, 25.19 ng/mL; Δ = +5.25), followed by CP 250 mg/kg (24.15 ng/mL; Δ = +2.82), CP 500 mg/kg (22.85 ng/mL; Δ = +2.54), MC 250 mg/kg (22.45 ng/mL; Δ = +2.94), MC 500 mg/kg (21.61 ng/mL; Δ = +1.05), and distilled water (20.60 ng/mL; Δ = +0.65).

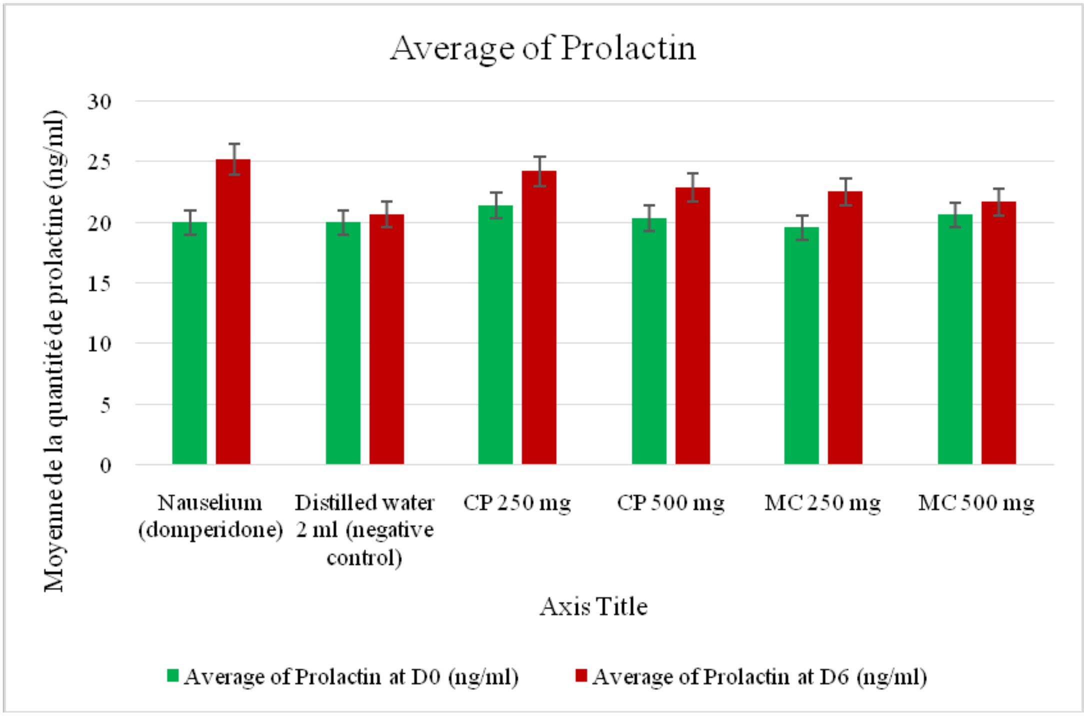

Figure 2: Variation in serum prolactin levels induced by plant extracts

3.2.2. Body weigh tbefore and after lactation

Figure 3 illustrates differences in dam body weight before and after lactation as a function of treatment. The distilled water group exhibited the greatest weight difference (3 ± 0.30 g), followed by domperidone and CP 250 mg/kg (2 ± 0.30 g each). The MC 250 mg/kg group recorded a negative weight difference (−1 ± 0.30 g), suggesting the absence of functional galactogenic activity at this dose.

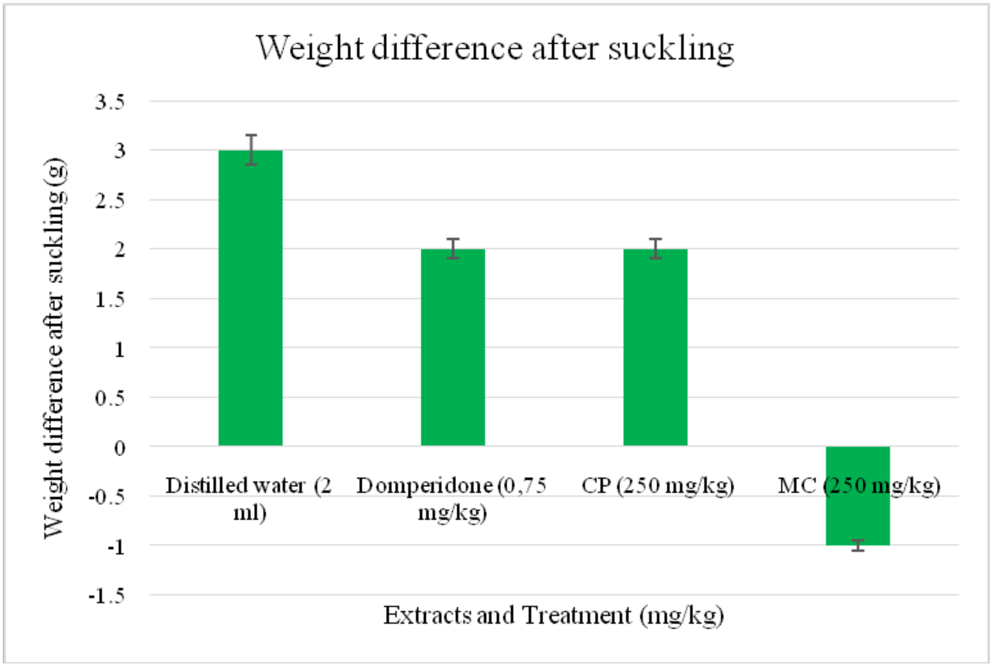

Figure 3: Dam body weight difference before and after lactation following treatment

3.2.3. Milk Yield Measurements

Figure 4 presents markedly different milk production volumes depending on treatment. Domperidone (0.75 mg/kg) induced the highest milk yield (8 ± 0.30 mL), followed by CP 250 mg/kg (5 ± 0.30 mL), distilled water (3 ± 0.30 mL), and MC 250 mg/kg (1 ± 0.30 mL).

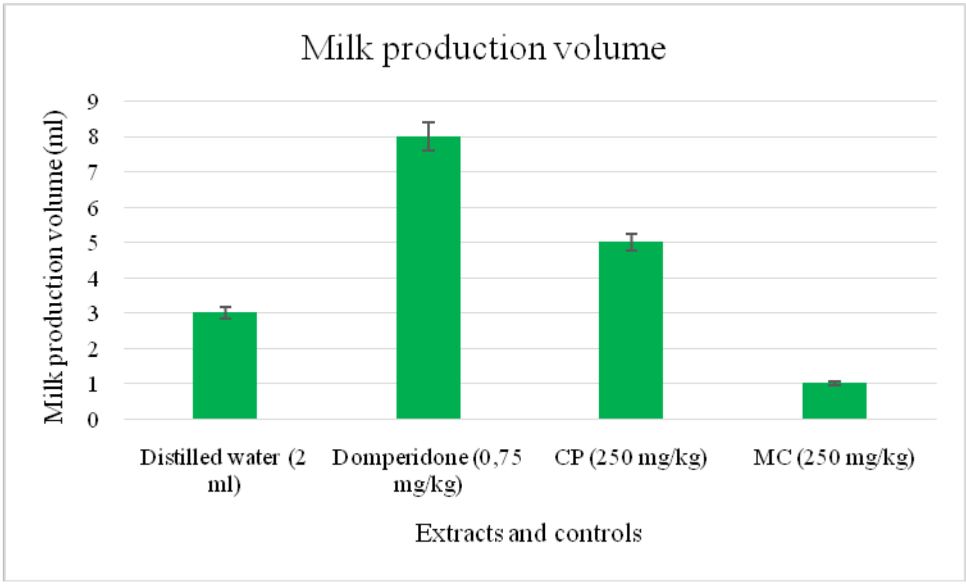

Figure 4: Milk yield following treatment

4. Discussion

The present study evaluated and compared the galactogenic potential of ethanolic extracts of *Carica papaya* L. (CP) and *Momordica charantia* L. (MC) through physicochemical characterization and *in vivo* biological assessment. Cross-analysis of the data revealed strong internal consistency between phytochemical composition and observed biological activity.

The extraction yield of CP (15.00 ± 1.10%) exceeded that of MC (10.83 ± 1.96%), reflecting superior solubilization of *C. papaya* constituents in ethanol. Such yield differences are commonly reported in the literature and are primarily attributed to solvent polarity, which conditions both the nature and quantity of extracted molecules (Bruneton, 2009). Although a higher yield offers practical advantages for subsequent therapeutic applications, it is ultimately the qualitative and quantitative composition that determines the pharmacological potential of an extract.

Qualitative phytochemical screening revealed that both extracts share a globally rich profile, including alkaloids, flavonoids, tannins, polyphenols, triterpenoids, steroids, and reducing compounds compound families extensively documented for their interactions with endocrine systems (Adewale et al., 2019). Nevertheless, CP was distinguished by the exclusive presence of coumarins and leucoanthocyanins, while MC contained only mucilages and free anthraquinone compounds. This qualitative differentiation carries significant pharmacological relevance: coumarins are recognized phytoestrogenic compounds known to modulate hormonal pathways involved in the regulation of lactation (Bruneton, 2009). Their exclusive presence in CP thus provides a plausible biochemical basis for its superior galactogenic activity.

Quantitatively, CP exhibited a considerably higher total polyphenol content (922.66 ± 0.0001 mg GAE/g) relative to MC (188.10 ± 0.0001 mg GAE/g), while MC predominated in total tannins and flavonoids. High polyphenol concentrations are known to interact with estrogen receptors, thereby modulating prolactin synthesis and release by the pituitary gland (Raman et al., 2015). This polyphenolic richness of CP provides strong biochemical evidence for its superior galactogenic effects, establishing a direct mechanistic link between the data in Table 3 and the three figures. Furthermore, the flavonoids detected in notable quantities in both extracts are documented phytoestrogens capable of stimulatin glactogenesis through partial agonist activity at estrogen receptors (Diallo et al., 2021).

Serum prolactin quantification confirmed the differential galactogenic activity of the two extracts. At D6, CP 250 mg/kg induced a prolactin level of 24.15 ng/mL, exceeding both MC 250 mg/kg (22.45 ng/mL) and the negative control (20.60 ng/mL), while remaining below that of domperidone (25.19 ng/mL). Since prolactinis the central hormone of lactogenesis, its elevation constitutes a direct biological marker of galactogenic activity (Freeman et al., 2000). It is note worthy that both extracts exhibited reduced effects at 500 mg/kg compared to 250 mg/kg, suggesting a non-linear dose-response relationship.

The convergence between hormonal data and functional lactation measurements strengthens the robustness of these findings. CP 250 mg/kg produced 5 ± 0.30 mL of milk compared to 1 ± 0.30 mL for MC 250 mg/kg and 3 ± 0.30 mL for distilled water, while domperidone remained the reference with 8 ± 0.30 mL. The fact that CP surpassed the negative control by 67% in milk yield, while also significantly elevating serum prolactin, suggests a partially similar mechanism of action, possibly mediated by its phytoestrogenic constituents. Conversely, the negative body weight difference observed for MC following lactation (−1 ± 0.30 g) confirms the absence of significant functional galactogenic activity at 250 mg/kg, consistent with its low milk yield and moderate prolactin levels (Figures 2, 3, and 4). These results are in agreement with the findings of Zava et al. (1998), who demonstrated that the estrogenic activity of plant extracts is closely correlated with their polyphenol and flavonoid content, and that extracts with low concentrations of these compounds exhibit limited hormonal effects.

Taken together, the findings demonstrate that the superior polyphenolic richness of the ethanolic extract of *Carica papaya* L., as confirmed by quantitative analysis, underlies a more pronounced hormonal stimulation of prolactin, which is functionally translated into greater milk production.

5. Conclusion

This study characterized two plant ethanolic extracts, *Carica papaya* L. (CP) and *Momordica charantia* L. (MC), from chemical analysis and biological perspectives, revealing a notable phytochemical richness. In CP, this richness is dominated by an exceptional total polyphenol content, directly associated with its superior galactogenic effects as evidenced by elevated serum prolactin levels and enhanced milk production. The ethanolic extract of *Carica papaya* L. (CP) thus emerges as the most promising fraction, displaying galactogenic activity intermediate between the negative control and domperidone, there by suggesting genuine therapeutic potential in the management of lactation insufficiency. However, complementary studies focusing on the isolation of active fractions, elucidation of the molecular mechanism of action, and toxicological evaluation of *Carica papaya* L. are warranted to support its valorization in both traditional and conventional medicine.

Conflicts of interest

The authors declare no conflicts of interest.

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DOI: 10.22192/ijcrcps.2026.13.06.002

How to cite this article:
Narcisse Aïzan, Amoussatou Sakirigui, Kossivi Issa Saloufou, Franck Yovo, Nourou-dine Balogoun, Nicaise Tohossi Kinnoudo, Eléonore Yayi Ladékan, Joachim Djimon Gbénou. (2026). Phytochemical Profile and Galactogenic Activity of *Carica papaya* L. and *Momordica charantia* L. Extracts. Int. J. Curr. Res. Chem. Pharm. Sci. 13(6): 11-21.DOI: http://dx.doi.org/10.22192/ijcrcps.2026.13.06.002