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Bioactive, antioxidant, and inhibitory potential against amylase and glucosidase of medicinal plants used in the treatment of infants

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Abstract: Medicinal plants have been a significant part of health care since time immemorial. They are used in folk medicine to treat and manage infant health. In this study, bioactive, antioxidant, and inhibitory potential against amylase and glucosidase of nine (*Carica papaya L, Mangifera indica L, Ocimum gratissimum, Vernonia amygdalina Schreb, Azadirachta indica A.juss, Cymbopogon citrate (DC.) Stapf, Psidium guajava L, Jatropha curcas L, and Momordica charantia L*) medicinal plants were sought. The bioactive potential was evaluated using total phenolics and total flavonoid assays. Total antioxidant capacity (TAC), ferric reducing antioxidant potential (FRAP), 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity, and 2-azobis-3-ethylbenzothiazoline-6-sulfonate radical scavenging ability (ABTS) were used to determine antioxidant capacity. In contrast, the inhibitory potential was evaluated against amylase (AMY) and glucosidase (GLU) activities. All assays were conducted using standard procedures. The results showed that *Azadirachta indica A.juss* has the highest total phenolic contents (82.82 ± 1.57 mg GAE/100 g) and total flavonoid (71.95 ± 1.19 mg QUE/100 g), while *Vernonia amygdaline Schreb* (54.08 ± 1.69 mg AAE/100 g) and *Mangifera indica L* (68.83 ± 0.09 mg Fe^{2+}E/100 g) were highest for TAC and FRAP, respectively. All extracts exhibited concentration-dependent inhibition for DPPH, ABTS, AMY, and GLU. Inhibition coefficient (IC_{50}) was calculated; *Mangifera indica L* exhibited the lowest IC_{50} for DPPH (28.50 ± 0.57 µg/mL) and ABTS (34.50 ± 0.88 µg/mL), while *Jatropha curcas L* exhibited the lowest IC_{50} for AMY (140.40 ± 1.08 µg/mL) and GLU (85.50 ± 1.17 µg/mL) inhibitions. The results show that medicinal plants possess bioactive, antioxidant, and inhibitory potentials, justifying their use in infants.

Introduction

Infant is a term used to describe children during the earliest period of their life, before they can walk and talk. Infants are susceptible to infections and deficiency diseases during this period due to the undeveloped immune system [1]. In 2022, about five million infant deaths were recorded globally, while the infant mortality rate is reported as 72 per 1000 live births [2, 3]. In developing countries, infant health is managed by using medicinal plants due to their low cost and availability [4]. Medicinal plants have been the mainstay of health systems since time immemorial due to their ability to modulate physiological functions and inhibit infection. Because of their

phytochemical compounds with diverse biological activities, medicinal plants are used to treat diseases in infants, such as cough, fever, cold, and rashes [5, 6]. Several common medicinal plants are used in Nigeria for infants with various diseases, as described below.

Carica papaya L. (CPL), known as Papaya, is a member of the Caricaceae allied to the Passifloraceae family. The decoction made from fresh Papaya leaves is mixed with tea to treat malaria, high blood glucose, and hypertension in Nigeria [7]. Fresh young leaves are used empirically as food for humans and animals [8, 9].

Mango *Mangifera indica* L. (MIL) is named Mango, belongs to the Anacardiaceae family. Its leaf is used in ethnomedicine to treat diarrhea [10]. Leaves of MIL were used to treat respiratory diseases/infections [11]. It is reported by Ediriweera and others [12] to possess antimicrobial properties over antiplasmodial, antibacterial, antifungal, antiviral, and antimalarial activities.

Ocimum gratissimum L. (OCM), called scent leaf, belongs to the Lamiaceae family and is well-cultivated in Africa, Asia, and South America. It is used to treat diseases such as cough, anemia, diarrhea, pneumonia, pains, and infections [13, 14]. Its leaf has been reported to possess antidiabetic, antimicrobial, and anticancer properties [15]. OCM is considered a prominent source of phytochemicals/secondary metabolites [16]. Over its use in traditional medicine, the leaves are used in various soups [17].

Vernonia amygdalina (VA), referred to as the bitter leaf, is a member of the Compositae family. VA is consumed as a vegetable or used as an herb to treat diabetes and malaria in traditional medicine [18]. In ethnomedicine, the leaves and roots are used for fever, hiccups, kidney problems, and stomach discomfort [19]. The antihelmitic, antimalarial, antitumourigenic, hypoglycemic, and hypolipidaemic properties of VA have been reported [20]. VA is also well used in insect control as an insect repellent against bean weevil [21].

Azadirachta indica A.juss (AI), referred to as Neem, belongs to the family of Meliaceae. AI is used in malaria, diabetes mellitus, and as an insect repellent [22]. Various parts of Neem have been widely used to treat human illnesses for centuries. Neem twigs are used for cleaning teeth and are regarded as an efficient kind of dental care in traditional medicine [23]. The flower of the Neem is used for the prevention and treatment of bile diseases [19]. The antidiabetic, antimalarial, anticancer, and antimicrobial properties have been reported [24, 25].

Cymbopogon citrate straph (CC), known as lemon grass, is a member of Poacae family. CC is well used for gastrointestinal problems and antispasmodic effects in folk medicine [26]. The antiviral, antibacterial, antifungal, and anticancer activities of CC have been documented [27]. CC is used as a seasoning or tea substitute for food. Its tea is frequently used in some Saharan countries, such as India and Cuba, to lower blood glucose concentration [28]. In Ayurveda, CC essential oil is used to treat hypertension, fever, stomach disease, and inflammation related to rheumatism, colds, and flu [28]. The CC leaf extract and decoction have various therapeutic effects, including anti-inflammatory, cough-relieving, digestive, anti-influenza, antipyretic, anti-diabetes, and antimalarial [28].

Psidium guajava L. (PG) is referred to as guava. PG belongs to the Myrtaceae family, and the extract from its leaves is used for diarrhea, rheumatism, digestive problems, malaria, and bacterial infections [29]. Guava leaves are used to manage health complications, including blood sugar levels, healthy bowel movements, and constipation [30]. It is documented that PG possesses pharmacological potentials such as anti-inflammatory, analgesic, antibacterial, antioxidant, and antitumor activities [31].

Jatropha curcas (JC), known as the purging nut, belongs to the family of Euphorbiaceae. JC is used in ethnomedicine for diarrhea, wounds, and diabetes mellitus [32, 33]. It is well used in the treatment of malaria infection [34]. Studies of JC roots, stems, bark, leaves, seeds, and fruits have shown numerous biological effects,

such as coagulative, antioxidant, antimalarial, anticancer, wound-healing, antimicrobial, and anti-inflammatory activities [35-37].

Momordica charantia L. (MC) belongs to the family of Cucurbitaceae and is referred to as either bitter melon or bitter gourd. In ethnomedicine, its leaves and fruit are used to treat diseases such as rheumatism, gout, worms, colic, spleen, and liver disorders [38]. MC is documented for the treatment of malaria, microbial and parasitic infections, diabetes, inflammation, hypertension, and strengthening of immunity in the Benin Republic [39, 40]. Numerous studies have stated its ability to lower blood glucose levels in induced diabetic acid [32, 41, 42].

Therefore, despite the use of these medicinal leaves in treating infant diseases. More information is needed on their mechanism of action. Hence, this study was designed to evaluate the polyphenol, antioxidant, and inhibitory potentials against amylase and glucosidase of commonly used medicinal plants in the treatment of infants.

Materials and methods

Sourcing of medicinal plants: The plants were sourced from the Ilaje Local Government Area (LGA), Ondo State, Nigeria, in 2025. The plants were identified and authenticated at the herbarium unit of the Department of Biological Sciences (**Table 1**). The leaves were dried at room temperature for 42 days and milled into a powder form.

Table 1: Common medicinal plants identifications

Botanical	Code	Herbarium number
Carica papaya L.	**CPL**	OAUSTECH/H/00674
Mangifera indica L.	**MIL**	OAUSTECH/H/00690
Ocimum gratissimum	**OCM**	OAUSTECH/H/00333
Vernonia amygdalina Schreb	**VA**	OAUSTECH/H/00680
Azadirachta indica A.juss	**AI**	OAUSTECH/H/00278
Cymbopogon citrata (DC.) straph	**CC**	OAUSTECH/H/00691
Psidium guajava L.	**PG**	OAUSTECH/H/00692
Jatropha curcas L.	**JC**	OAUSTECH/H/00671
Momordica charantia L.	**MC**	OAUSTECH/H/00657

Preparation of the extracts: The extracts were prepared according to the previous method [43]. 1.0 g of pulverized leaves was solvated in 10 ml of distilled water for 6 hrs. They were centrifuged at 5000 rpm, and the supernatant was used for analysis immediately.

Determination of Total Phenolic Content (TPC): A method of Kim et al. [44], as modified [43], was used to assay TPC. 0.5 ml was added to 0.5 ml of 10.0% of Folin-Ciocalteu phenol reagent. After 5 min, 2.5 ml of 7.0% Na_2CO_3 and 2.5 ml of distilled water were mixed thoroughly. The solution was kept in the dark at ambient temperature for 1 hr. and 30 min and a spectrophotometric reading was taken at 750 nm. TPC was calculated from the Gallic acid standard curve and expressed as Gallic acid equivalent (mg GAE/100 g).

Determination of Total Flavonoid Content (TFC): The method of Park et al. [45] was used to assay TFC. Thus, 0.3 ml of the sample, 3.4 ml of 30.0% of methanol, 0.15 ml of 0.5 M $NaNO_2$, and 0.15 ml of 0.3 M $AlCl_36H_20$ were added successively. After 5 min, 1.0 ml of 1.0M NaOH was added, and the absorbance was measured at 506 nm. TFC was calculated from the standard curve and presented as quercetin equivalent (mg QUE/100 g).

Total Antioxidant Capacity phosphomolybdate assay (TAC): The method of Prieto et al. [46] was used. 0.2 ml of the sample and 2.0 ml of phosphomolybdate reagent were added and shaken to mix. The solution was capped and put in a boiling water bath at 95 °C for 1 hr. and 30 min. The solution was then cooled, and a spectrophotometric

reading was taken at 695 nm. The total antioxidant capacity was calculated from the ascorbic acid calibration curve and expressed as ascorbic acid equivalent (mg AAE/100 g).

Determination of Ferric-Reducing Antioxidant Potential (FRAP): The method by Benzie and Strain [47] was used. 0.2 ml of the sample and 2.80 ml of the FRAP working reagent were added, shaken, and incubated at ambient temperature for 30 min. Spectrophotometric readings were taken at 593 nm, and the FRAP equivalent was calculated from the ferrous sulphate standard curve as (mg Fe^{2+}E/100 g).

Determination of 1, 1-diphenyl-2-picrylhydrazyl scavenging activity(DPPH): The procedure of Gyamfi et al. [48] was used. Appropriate dilutions of 1.0 ml (0.05 - 0.20 mg/ml) were mixed with (40 mg/l) DPPH solution (4.0 ml) solvated in methanol. The solution was mixed, left in the dark for 30 min, and a spectrophotometric measurement was taken at 520 nm.

Determination of 2-azobis-3-ethylbenzothiazoline-6-sulfonate radical scavenging ability (ABTS): The method of Re et al. [49] was used. Appropriate dilutions of 1.0 ml (0.05-0.20 mg/ml) were added to 2.0 ml of ABTS reagent and shaken. Absorbance spectrophotometric measurements were taken at 734 nm, and the percentage inhibition was calculated.

Determination of amylase inhibition assay (AI): The method of Worthington et al. [50] was used. Appropriate dilutions of 1.0 ml (0.05-0.20 mg/ml) and 500 µl (0.02 M) of sodium phosphate buffer (pH 6.9 with 0.006M NaCl) containing 0.5 mg/ml α-amylase solution were added together and permitted to stay at ambient temperature for 10 min. Then, 500 µl (1.0%) of starch solution prepared with 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) was added. The solution was then incubated at 25 °C for 10 min. The reaction was halted with 1.0 ml (96 mM) of dinitrosalicylic acid reagent. The test tubes were then incubated in a boiling water bath for 5 min. The spectrophotometric assessments were taken at 540 nm, and the inhibition percentage was calculated.

Determination of the glucosidase inhibition assay (GI): The method of Apostolidis et al. [51] was used. Appropriate dilutions of 1.0 ml (0.05-0.20 mg/ml) were added to 1000 µl α-glucosidase solution (1.0 U/l) prepared in 0.1M phosphate buffer (pH 6.9) and pre-incubated for 10 min at 25° C. After pre-incubation, 500 µl of 5.0 mM nitrophenyl-glucopyranoside solution prepared in 0.1M phosphate buffer (pH 6.9) was added. The reaction mixture was incubated at 25°C for 5 min. The absorbance of the reaction mixture was measured at 405 nm. The percentage inhibition was calculated.

Statistical analysis: Data were presented as the mean ± S.D. of triplicate readings. Differences among the groups were tested by a one-way analysis of variance (ANOVA). Statistical differences among the means were considered significant at $p<0.05$.

Results

In this study, the results of the medicinal plants of TPC, TFC, TAC, and FRAP in Nigeria are presented in **Table 2**. Thus, the TPC was statistically significantly higher in AI and MIL than in other medicinal plants ($p<0.05$). In addition, TFC was significantly higher in AI ($p<0.05$), while TAC was significantly higher ($p<0.05$) in VA in the medicinal plants. The FRAP is significantly higher in MIL ($p<0.05$) than in the other medicinal plants. **Figure 1** shows that the ABTS and DPPH scavenging capacities were concentration-dependent, while **Figure 2** shows the medicinal plants' potential to inhibit AMY and GLU. The IC_{50} of the medicinal plants for ABTS, DPPH, AMY, and GLU are presented in **Table 3**. The MIL has the lowest IC_{50} for ABTS (28.50±0.57 µg/ml) and DPPH (34.50± 0.88 µg/ml). The lowest IC_{50} for AMY (140.40±1.08 µg/ml) and GLU (85.50±1.17 µg/ml) was recorded in JC.

Table 2: TPC, TFC, TAC, and FRAP of the medicinal plants

Medicinal plant	*Total Phenolic content* (mg GAE/100 g)	*Total Flavonoid content* (mg QUE/100 g)	*Total antioxidant capacity* (mg AAE/100 g)	*Ferric-reducing antioxidant potential* (mg Fe^{2+}E/100 g)
CPL	75.17±1.58 [b]	15.92±2.28 [f]	19.46±1.10 [d]	67.56±0.24 [b]
MIL	81.22±1.05 [a]	36.25±1.25 [c]	22.43±0.76 [c]	68.83±0.09 [a]
OCM	76.49±1.44 [b]	58.99±2.34 [b]	26.12±1.63 [b]	65.50±0.30 [c]
VA	71.33±0.54 [c]	11.30±0.91 [g]	54.08±1.69 [a]	63.16±1.55 [d]
AI	82.82±1.57 [a]	71.95±1.19 [a]	25.86±1.79 [b]	67.93±0.36 [b]
CC	52.19±0.49 [f]	12.34±0.48 [g]	19.59±1.85 [d]	47.16±1.15 [f]
PG	64.12±0.87 [e]	17.47±0.09 [e]	20.39±1.07 [d]	58.64±0.36 [e]
JC	68.77±0.34 [d]	24.26±0.77 [d]	21.10±0.63 [d]	63.88±0.34 [d]
MC	53.33±0.44 [f]	11.82±1.33 [g]	19.14±1.77 [d]	47.60±0.18 [f]

Data are expressed as Mean ± SD. Figures with different superscripts are significantly different ($p<0.05$)
Carica papaya L (CPL), *Mangifera indica L* (MIL), *Ocimum gratissimum* (OCM), *Vernonia amygdalina Schreb* (VA), *Azadirachta indica A. juss* (AI), *Cymbopogon citrata (DC) straph* (CC), *Psidium guajava L* (PG), *Jatropha curcas L* (JC), *and Momordica charantia L* (MC)

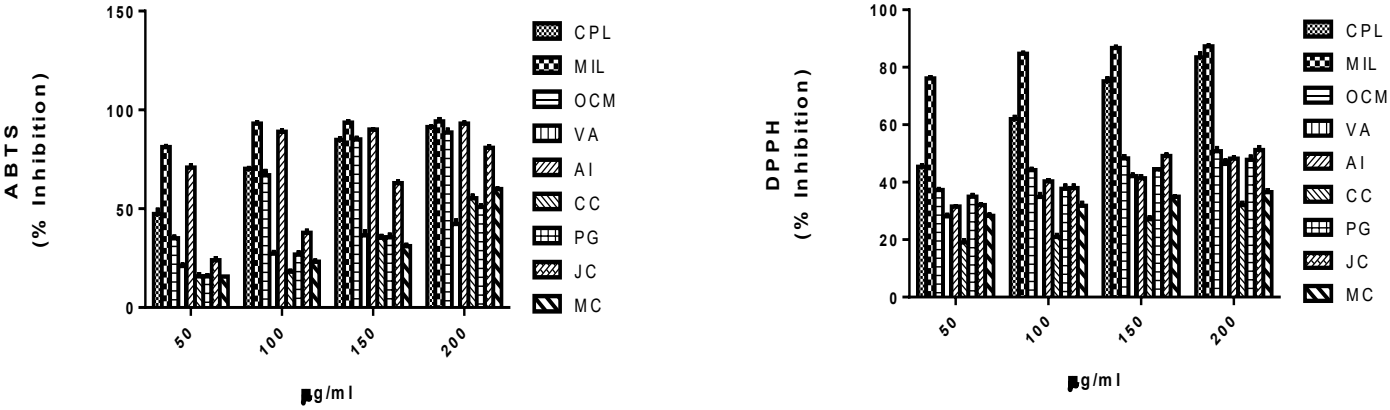

Figure 1: ABTS and DPPH scavenging activities of the medicinal plants

Carica papaya L (CPL), *Mangifera indica L* (MIL), *Ocimum gratissimum* (OCM), *Vernonia amygdalina Schreb* (VA), *Azadirachta indica A. juss* (AI), *Cymbopogon citrata (DC) straph* (CC), *Psidium guajava L* (PG), *Jatropha curcas L* (JC), *and Momordica charantia L* (MC)

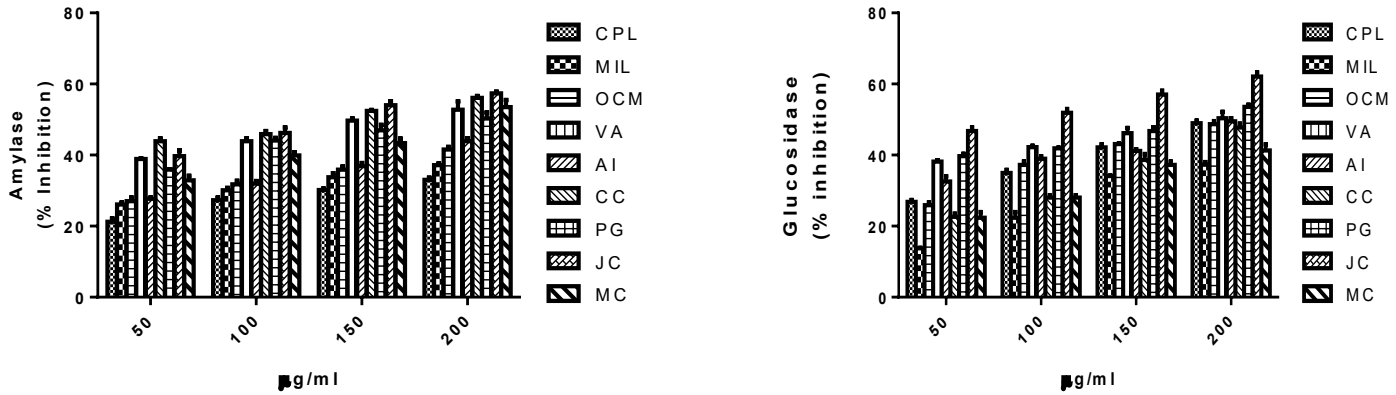

Figure 2: Amylase and glucosidase inhibitory activities of the medicinal plants

Carica papaya L (CPL), *Mangifera indica L* (MIL), *Ocimum gratissimum* (OCM), *Vernonia amygdalina Schreb* (VA), *Azadirachta indica A. juss* (AI), *Cymbopogon citrata (DC) straph* (CC), *Psidium guajava L* (PG), *Jatropha curcas L* (JC), *and Momordica charantia L* (MC)

Table 3: IC_{50} (µg/ml) of the medicinal plants against ABTS, DPPH, AMY, and GLU inhibitions

Plant	ABTS	DPPH	AMY	GLU
CPL	52.25±0.45 ^{c}	78.56±0.56 ^{b}	357.28±2.11 ^{g}	201.96±1.09 ^{c}
MIL	28.50±0.57 ^{a}	34.50±0.88 ^{a}	252.50±2.01 ^{f}	240.05±2.26 ^{e}
OCM	84.40±1.02 ^{d}	200.05±2.68 ^{c}	228.40±1.57 ^{e}	200.50±1.08 ^{c}
VA	215.40±1.21 ^{h}	208.57±1.99 ^{e}	180.40±2.84 ^{b}	199.40±2.10 ^{c}
AI	34.38±0.24 ^{b}	204.55±1.57 ^{d}	218.50±1.44 ^{d}	200.15±1.89 ^{c}
CC	169.78±1.08 ^{f}	333.50±2.54 ^{g}	141.80±1.98 ^{a}	206.50±2.08 ^{d}
PG	197.34±1.11 ^{g}	203.28±1.08 ^{d}	202.10±2.57 ^{c}	181.23±1.68 ^{b}
JC	124.32±1.28 ^{e}	199.04±1.45 ^{c}	140.40±1.08 ^{a}	85.50±1.17 ^{a}
MC	188.87±2.45 ^{g}	274.20±1.25 ^{f}	185.40±1.50 ^{b}	212.50±2.88 ^{d}

Results are expressed as Mean ± SD. Figures with different superscripts are significantly different ($p<0.05$)
Carica papaya L (CPL), *Mangifera indica L* (MIL), *Ocimum gratissimum* (OCM), *Vernonia amygdalina Schreb* (VA), *Azadirachta indica A. juss* (AI), *Cymbopogon citrata (DC) straph* (CC), *Psidium guajava L* (PG), *Jatropha curcas L* (JC), *and Momordica charantia L* (MC)

Discussion

Since ancient times, medicinal plants have been used in infants to treat diseases and improve children's health in traditional medicine [52]. Despite advances in the development of drugs, they are still used to date, especially in developing countries, because of their availability and marginal costs. Thus, this study investigated the bioactive, antioxidant, and inhibitory potentials of nine medicinal plants used in folk medicine to treat children. Medical plants are sources of many bioactive compounds, such as phenolics and flavonoids [53-55]. These compounds have been reported to exhibit biological activities: antiviral, antibacterial, anticancer, antidiabetic, and anti-inflammatory [5, 56]. These secondary metabolites are believed to be safer when derived from medicinal plants than when synthesized in the laboratory [57]. This study has confirmed the presence and abundance of phenolics and flavonoids in the medicinal plants studied. These phytochemicals conferred many therapeutic functions on the medicinal plants. The values obtained for TPC and TFC were comparable to those of other medicinal plants [58].

Fetal growth restriction has been reported to affect up to 25.0% of live births in low- and middle-income countries, and this has been linked to oxidative stress, which might lead to neurodegenerative diseases in the future [59]. Antioxidants are chemical entities that halt the activities of free radicals, thereby inhibiting oxidative stress that can lead to chronic and neurodegenerative diseases [60, 61]. The current study has confirmed that these medicinal plants have potent antioxidant power, which can mitigate oxidative stress and prevent the development of chronic and neurodegenerative diseases in infants. This enormous antioxidant potential might serve as the basis for using medicinal plants in infants. The antioxidant value of these medicinal plants ranked well compared with values reported for other medicinal plants by other researchers [62]. Diabetes mellitus is an autoimmune disease that affects children around the globe and has continued to be on the rise [63, 64]. Genetics alone cannot explain this incidence; therefore, the lifestyle and diet of expecting and nursing mothers might also be implicated [65]. One of the treatment mechanisms for this disease is the inhibition of amylase and glucosidase. These break large polysaccharides, such as starch, into smaller monosaccharide units such as glucose. The plants could inhibit these enzymes, thereby conferring certain antidiabetic properties. This could be the reason for its use in children's health. The IC_{50} values of these medicinal plants ranked well compared with other plants [66].

Conclusion: The nine medicinal plants included in this study used for the treatment of infants possess bioactive, antioxidant, and inhibitory properties against amylase and glucosidase. These properties justify their use in infant health cases.

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الفعالية البيولوجية، ومضادات الأكسدة، والقدرة التثبيطية للنباتات الطبية المستخدمة في علاج الرضع ضد إنزيمي الأميليز والجلوكوزيداز

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الملخص: لطالما شكلت النباتات الطبية جزءًا هامًا من الرعاية الصحية منذ القدم. وتُستخدم في الطب الشعبي لعلاج صحة الرضع والحفاظ عليها. في هذه الدراسة، تم البحث عن النشاط الحيوي، ومضادات الأكسدة، والقدرة التثبيطية لإنزيمي الأميليز والجلوكوزيداز في تسعة نباتات طبية (كاريكا بابايا، مانجيفيرا إنديكا، أوسيموم غراتيسيموم، فيرنونيا أميغدالينا، أزاديراختا إنديكا، سيمبوبوجون سيترات، بسيديوم جوافة، جاتروفا كركاس، وموموردیکا شارانتیا). تم تقييم النشاط الحيوي باستخدام فحوصات الفينولات الكلية والفلافونويدات الكلية. استُخدمت السعة الكلية لمضادات الأكسدة (TAC)، وقدرة اختزال الحديد المضادة للأكسدة (FRAP)، ونشاط إزالة جذور 1-ثنائي فينيل-2-بيكرايل هيدرازيل (DPPH)، وقدرة إزالة جذور 2-أزوبيس-3-إيثيل بنزوثيازولين-6-سلفونات (ABTS) لتحديد القدرة المضادة للأكسدة. في المقابل، قُيِّمَت القدرة التثبيطية ضد نشاطي الأميليز (AMY) والجلوكوزيداز (GLU). أُجريت جميع الاختبارات وفقًا للإجراءات القياسية. أظهرت النتائج أن مستخلص النيم (Azadirachta indica A.juss) يحتوي على أعلى نسبة من المركبات الفينولية الكلية (82.82 ± 1.57 ملغ مكافئ حمض الغاليك/100 غرام) وأعلى نسبة من الفلافونويدات الكلية (71.95 ± 1.19 ملغ كيرسيتين/100 غرام)، بينما كان مستخلص نبات فيرنونيا أميغدالين (Vernonia amygdaline Schreb) (54.08 ± 1.69 ملغ مكافئ حمض الأسكوربيك/100 غرام) ومستخلص المانجو (Mangifera indica L) (68.83 ± 0.09 ملغ مكافئ الحديد الثنائي/100 غرام) الأعلى من حيث القدرة الكلية المضادة للأكسدة (TAC) وقدرة اختزال الحديد (FRAP)، على التوالي. وأظهرت جميع المستخلصات تثبيطًا يعتمد على التركيز لكل من DPPH وABTS وAMY وGLU. وتم حساب معامل التثبيط (IC50). أظهر نبات المانجو (Mangifera indica L) أقل قيمة IC50 لتثبيط DPPH (28.50 ± 0.57 ميكروغرام/مل) وABTS (34.50 ± 0.88 ميكروغرام/مل)، بينما أظهر نبات الجاتروفا (Jatropha curcas L) أقل قيمة IC50 لتثبيط AMY (140.40 ± 1.08 ميكروغرام/مل) وGLU (85.50 ± 1.17 ميكروغرام/مل). تشير هذه النتائج إلى أن النباتات الطبية تمتلك خصائص حيوية ومضادة للأكسدة ومثبطة، مما يبرر استخدامها لدى الرضع.