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Medicinal plants of Côte d'Ivoire and viral infections: Diagnosis of Begomovirus

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Abstract

Medicinal plants have many active ingredients used in traditional and modern medicine. However, viral diseases pose a real threat to their culture and development. It is useful to determine the health status of medicinal plants in Côte d'Ivoire. Surveys and collection of samples were made at sites identified in Abidjan, Alépé and Yamoussoukro. The symptoms observed were mentioned. Herbal hosts of *Begomovirus*, incidence and severity of these symptoms have been identified. A variety of symptoms of viral infections was observed. These include mosaic, chlorosis, leaf-shoe deformation, leaf curl and plant dwarfism. The mosaic symptoms were observed on the Abidjan and Yamoussoukro samples with incidences of 71.5% and 66.6% among those who were chlorinated in Alépé with 96.67%. The mosaic was most severe on species of medicinal plants displaying severity index ranging from 32.5 ± 12 % to 56.14 ± 9 %. Two samples out of the 91 tests have a positive effect on primers directed against Begomoviruses. These two samples were taken from *Momordica charantia* and *Moringa oleifera*. Medicinal plants are hosts of Begomovirus and a relationship may exist between the amount of active ingredient secreted by the medicinal plant and the severity of the symptoms of the viral infection.

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Introduction

The use of plants for therapeutic purposes is reported in ancient Arabic, Chinese, Egyptian, Hindu, Greek and Roman literatures. In Africa, the therapeutic power of plants was known to ancestors and parents empirically (Nacoulma, 2001). Medicinal plants provide 80% of the health coverage of sub-Saharan African populations (OMS, 2002). However, the chemical compositions and the active principles of certain plants have yet to be elucidated. Several phytochemical investigations have been made to provide a scientific justification for the traditional use of medicinal plants. These plants have many active ingredients used in both traditional medicine and modern medicine (Handa *et al.,* 2006). In developing countries such as Côte d'Ivoire, medicinal plants are used mostly in rural areas to solve public health problems because of their ease of access and low costs in the treatment of diseases (Dro *et al.*, 2013). However, these plants undergo uncontrolled destruction by man's human actions. In addition to this there is a parasitic aggression including viruses. More and more, we are witnessing the emergence of new viral diseases on crops. This poses a real threat to food security and human health. In African countries, many viral epidemics are affecting essential food crops, such as *Rice yellow mottle virus* (Fargette *et al.*, 2006), corn with *Maize streak virus* (Shepherd *et al.*, 2010) and cassava with *Begomovirus* responsible for cassava mosaic (Pita *et al.,* 2001) and more recently with the viruses that cause brown cassava streak (CBSV) (Legg *et al.*, 2011). To date, very few studies have focused on the identification of viruses infecting medicinal plants in Côte d'Ivoire. However, in the presence of a pathogen, plants secrete more toxins than seemingly healthy plants (Klarzynski et Fritig, 2001). A comparative study of the active ingredients of these substances commonly used in the pharmaceutical industry, opens promising avenues of research for the scientific community. The general objective of this study is to make the diagnosis of viral diseases on medicinal plants. More specifically, it is to make the symptomatology of viral diseases present on the medicinal plants and identify those attacked by viruses of the genus *Begomovirus*.

Prospecting sites

The prospecting sites were the cities of Abidjan, Alépé and Yamoussoukro (Fig. 4). The climate of the city of Abidjan and that of the city of Alépé is that of the south of Côte d'Ivoire. It is a humid tropical climate with rainfall. It is characterized by the alternation of four seasons including two rainy seasons and two dry seasons. The dry seasons are mild because they are tempered by the sea breeze. The Yamoussoukro region also has a four seasons climate: a long dry season from mid-November to mid-March, characterized by the presence, in December and January, of the harmattan, a dry and powerful wind from the Sahara, which considerably lowers the humidity; a long rainy season from mid-March to mid-July; a short dry season from mid-July to mid-September; a short rainy season, from mid-September to mid-October. The average rainfall amounts in the Yamoussoukro region vary from 900 to 1100mm per year with a very variable spatial distribution in the year and from one year to the next. The average temperature of the region is about 26°C. The relative humidity varies between 75 and 85% with falls at 40% during the harmattan period and is between 80 and 85% during the rainy season.

Materials and Methods

Material

The plant material were samples of young leaves and stems of medicinal plants from the following families: Apocynaceae, Asteraceae, Caesalpiniaceae, Caricaceae, Costaceae, Curcubitaceae, Fabaceae, Laminaceae, Malvaceae, Moracae, Moringaceae, Plantaginaceae, Poaceae, Rubiacae, Solanaceae. The plants were collected in three localities of Côte d'Ivoire and sampling of medicinal plants were equally conducted in three localities of the country: one in Abidjan (in the medicinal plant market, a relic Banco forest at Nangui Abrogoua University, and in Port-Bouët); the second in Alépé (at Brofodoumé), and the third one in Yamoussoukro (at Logbakro). In the two localities exclusive of Abidjan, and based on previous studies, two collection sites of medicinal plants worth 1/2ha were selected and prospected. Z sampling was conducted on each site of 1/2ha. Twelve samples of young leaves showing or not symptoms of viral infections were collected per site.

In the medicinal plant market, 49 samples of different species were randomly collected from nine traders. In total, 121 samples were collected. The young leaves collected in each locality were labelled, with the labels carrying the following information: date of collection, sample code and site code. Samples were sent to the Plant Health Unit of the Plant Production Research Center of Nangui Abrogoua University.

Identification of medicinal plants and their therapeutic indications

The samples collected were identified at Nangui Abrogoua University using the keys of identifications of Poilecot (1995), of Hutchinson and Dalziel (1972) and of Hawthorne and Jongking (2006). A survey carried out in the form of an individual interview with traditional healers and specialists from the University Nangui Abrogoua (UNA) made it possible to determine the therapeutic effects of the plant species collected (Appendix 1).

Description of symptoms and identification of impacts

The symptoms developed by the plants in the field have been described taking into account the appearance, coloring, distribution, shape and size of these on the plants. The incidence of each symptom at each site was evaluated according to the formula below:

$$I = \frac{ES}{N} \times 100$$

I : Incidence of symptoms
ES : Number of samples with a symptom type on the site
N : Total number of samples with symptoms at the site

Evaluation of symptoms severity

The severity of each symptom on the samples was evaluated using Mignouna *et al*. (2001) scale ranging from 1 to 5 where: 1: Absence of visible symptoms; 2: 1-25% of leaves show the symptom; 3: 26-50% of leaves show the symptom; 4: 51-75% of leaves show the symptom; 5: more than 75% of leaves show the symptom. The severities obtained were used to calculate the severity index according to the formula of Rempel and Hall (1996).

$$IS = \frac{\Sigma(Xi \times Ni \times 100)}{5 \times Nt}$$

IS: Severity index of the symptom considered.
Xi: Note attributed to a symptom
Ni: Number of plants of the same species presenting the symptom
Nt: Total number of plants with or without symptoms

Characterization of pathogens

Extraction of plant DNA

Total DNA was extracted from the leaves of the plants which showed symptoms according to the modified Doyle and Doyle (1990) technique, by the CTAB method (20g of CTAB, 100ml of 1 M Tris-HCl, pH 8; 40ml of 0.5 MEDTA, pH 8; 81,8 1 of NaCl; 10g of PVP-40; the volume is adjusted to 1 liter with sterile distilled water). Whole leaves (0.1g) were crushed in liquid nitrogen and collected in 1.5ml of CTAB solution preheated in a 65°C water bath for 30min.

The ground material obtained was transferred to 2ml Eppendorf tubes and incubated in a water bath at 65°C for 30min and mixed by inversion at 10min intervals. After incubation, 550µl of chloroform-isoamyl alcohol was added to the ground material and the mixture was vortexed to homogenization and centrifuged at 13000rpm for 10min at 25°C.

The resulting supernatant was transferred to a new 1.5ml Eppendorf tube. This step was repeated twice. To precipitate the DNA, 100µl of cold isopropanol (-20°C) was added to the supernatant and the solution was stored in the freezer at -20°C for at least 2 hours after homogenization. After centrifugation at 13000rpm for 10min, the supernatant was removed from the tubes while preserving the DNA. Then, 500µl of 70% ethanol (-20°C) was added to each tube and the solution was centrifuged at 13000 rpm for 10 min. After centrifugation, the ethanol was removed and the tubes were dried at room temperature (27°C). Finally, the DNA was eluted in 70µl of TE buffer (5ml of 1M Tris-HCl, 1 ml of 0.5M EDTA, pH 8) and kept in the freezer at -20°C.

Amplification of the DNA using PCR technique

DNA was dissolved in a mixture consisting of sterile pure water (11, 8 µl x n), 5x buffer (5µl x n), air of primers Cluster 4 F342 (5'-TATMATCATTTCCA CBCCVG-3'; 1 µl x n) and Cluster 4 R1032 (5'-GCATGAGTACATGCCATATAC-3; 1µl x n'), des dNTPs à 2mM (2, 5µl x n), of $MgCl_2$ (1.5µl x n) and GoTaq (0.2µl x n). Denaturation of the target DNA was carried out at high temperature (94°C), for 5min, hybridization of the specific primers was at a temperature (50-94°C) for 30 seconds to 1 min and in 30 cycles. Finally, the elongation of the DNA strands was carried out in 5 min at 72°C

Agarose gel electrophoresis

The migration was made on a 1% agarose gel (sigma grade molecular biology RNase free) (p v) was prepared in TBE buffer (100mM Trizma base, 100mM boric acid, 2mM EDTA) and then poured onto the gel carrier for electrophoresis.

The electrophoresis tank was filled with the TBE buffer (1%) until the gel was completely immersed. A quantity of 10µl of PCR amplicon was deposited in the wells of the 1% agarose gel (Fig. 6). Electrophoretic migration was done at 110 volts for 35 min. Finally, the visualization was done on a transilluminator.

Statistical analysis

The Statistica 7.1 software was used for statistical analysis. One factor was taken into account, the plant species with the variation of the severity index of each observed symptom. The Kruskall Wallis variant test was used to evaluate the effect of this factor on the studied variants.

Results

Description of symptoms observed

Two main symptoms were observed on the sampled plants. This is about changing the color and the deformation of organs. The modification of the color of the organs was materialized by the mosaic, the lightening of the veins and sometimes the discolorations occur. The deformation of organs, meanwhile, was materialized by a reduction in the length of the inter-node of the stems of the infected plants and a very pronounced reduction of the limb of the leaves in the form of laces of shoes-shoestring. On the all sites, 15 families of medicinal plants have been identified including 21 species. The main use has been the fight against malaria. In Abidjan, 11 families including 13 species of medicinal plants have been identified. Regarding Alépé, eight families of medicinal plants were collected including 12 species. Finally, in Yamoussoukro, six families of medicinal plants were identified including 8 species (Table 1).

Table 1. Plants collected and therapeutic indications.

Families	Plants Species	Collection site	Number of samples collected	Therapeutic effects
Apocynaceae	*Rauvolfia vomitoria*	Abidjan Alépé	3 2	Epilepsy, edema of the feet, malaria
Poaceae	*Cymbopogon citratus*	Abidjan Yakro	5 2	Tired
Asteraceae	*Ageratum conyzoides*	Abidjan Alépé Yakro	4 2 2	Migraine, malaria
	Aspilia africana	Abidjan Alépé Yakro	5 2 5	Cough, stop bleeding
	Chromolaena odorata	Alépé Yakro	2 4	Diabetes, Diarrhea,malaria, healing agent
	Synedralla nodiflora	Alépé	2	Antioxydant
	Vernonia colorata	Alépé	2	Belly ache, easy childbirth
Caesalpiniaceae	*Cassia occidentalis*	Abidjan	7	Malaria
Caricaceae	*Carica papaya*	Alépé	2	Malaria, gastrointestinal disorders
Costaceae	*Costus afer*	Abidjan	7	Snake bite
Curcubitaceae	*Momordica charantia*	Abidjan Yakro	6 2	Malaria, fever

Families	Plants Species	Collection site	Number of samples collected	Therapeutic effects
Fabaceae	*Baphia nitida*	Alépé	2	Inflamed and infected umbilical cords
		Alépé	2	
	Desmodium adscendens	Abidjan	8	Cutaneous buttons, diarrhea
		Yakro	2	
	Hoslundia opposite	Abidjan	4	Child care
Laminaceae	*Mentha* sp.	Abidjan	7	Vertues, spasmolytics, carminatives, antiseptic, tonic and stimulating
Malvaceae	*Abelmoschusesculentus*	Alépé	2	Increases blood volume
Plantaginaceae	*Scoparia dulcis*	Alépé	2	Hemorrhoid, aphrodisiac, diabetes, hypertension, sickle cell disease
Rubiacae	*Morinda morindoides*	Abidjan	10	
Moracae	*Ficus exasperata*	Abidjan	4	Easy delivery, enteralgia, typhoid fever, Malaria
		Yakro	3	
Moringaceae	*Moringa oleifera*	Abidjan	2	antidiabetic, vermifuge, skin care, digestion and antiseptic
Solanaceae	*Capsicum frutescens*	Abidjan	3	Rheumatism, laxative stimulant
		Yakro	4	
		Total	121	

Yakro: Yamoussoukro.

Incidence of symptoms

In Abidjan, leaf mosaic was the most common symptom with 71.5% incidence, while dwarfism was the least observed symptom with an incidence of 15.38% (Table 2). Regarding Alépé, leaf chlorosis was the most prominent symptom with an incidence of 96.67% against 16.33% for winding and 'Shoestring' (Table 2). In addition, samples from Yamoussoukro showed mostly mosaic symptoms with an incidence of 66.6%. Chlorosis was the least prominent symptom with an incidence of 8.33% (Table 2).

Severity of symptoms

Leaf chlorosis had the highest severity with *Rauvolfia vomitoria* (50.6 ± 7%). Its lowest rate of severity was obtained with the species *Chromolaena odorata* (17.5 ± 3%). With regard to leaf curl, the highest severity index was observed with the species *Capsicum frutescens* (49 ± 9%). Its lowest degree of severity index was obtained with the species *Abelmoschus esculentus* (30 ± 10%). As for the embossing of the leaves, its index of highest severity was observed with the species *Chromolaena odorata* (59.5 ± 10%).

Its lowest rate of severity was obtained with the species *Rauvolfia vomitoria* (24 ± 5%). Leaf mosaic had the highest index of severity with *Cassia occidentalis* (56.4 ± 9%). Its lowest degree of severity index was obtained with *Vernonia colorata* (32.5 ± 12%). Finally, the 'shoestring' had the highest index of severity with the species *Hoslundia opposite* (33.8 ± 5%). Its lowest rate of severity was obtained with the species *Capsicum frutescens* (19.42 ± 3%). However, a significant difference ($p < 0.05$) in severity indices of each symptom was noted between plant species with the exception of the symptom (Table 3).

Table 2. Incidence of symptoms by area.

Symptoms	Incidence (%)		
	Abidjan	Alépé	Yamoussoukro
Leaf chlorosis	51.6	96.67	8.33
Leaf curl	11.7	16.66	33.33
Embossing leaves	40.4	51.28	33.33
Mosaic of leaves	71.5	33.33	66.6
Dwarfism	04.0	0	0
Shoestring leaves	0	16.66	0

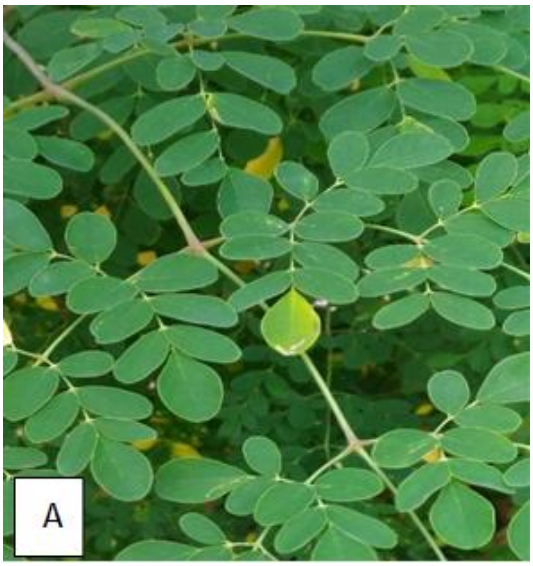
Table 3. Severity index of various symptoms depending on plant species

Vegetables species	Severity index (%)				
	Chlorosis	Curling	Embossing	Mosaic	Shoestring
Abelmoschus esculentus	-	30 ± 10 a	30 ± 5 ab	50 ± 7 a	-
Ageratum conyzoïdes	-	37.5 ± 6 a	-	37.5 ± 8 ab	-
Aspilia africana	36.25 ± 5 ab	-	-	44.44 ± 3 a	-
Baphia nitida	25 ± 5 ab	-	-	-	-
Capsicum frutescens	-	49 ± 9 a	53 ± 6 a	-	19.42 ± 3 b
Carica papaya	-	-	47.5 ± 8 ab	47.5 ± 3 a	-
Cassia occidentalis	-	-	-	56.14 ± 9 a	-
Chromolaena odorata	17.5 ± 3 b	-	59.5 ± 10 a	-	-
Costus afer	-	-	-	46.28 ± 9 a	-
Cymbopogon citratus	-	-	-	46.02 ± 6 a	-
Desmodium adscendens	18 ± 3 b	-	-	36 ± 5 ab	-
Ficus exasperata	-	-	-	34.71 ± 6 ab	-
Hoslundia opposite	41.25 ± 11 ab	-	-	-	33.75 ± 5 a
Mentha sp.	34.7 ± 6 ab	-	-	-	-
Momordica charantia	-	-	-	49.6 ± 7 a	-
Morinda morindoides	46.4 ± 6 a	-	-	-	-
Moringa oleifera	-	-	-	35 ± 4 ab	-
Rauvolfia vomitoria	50.6 ± 7 a	-	24 ± 5 b	-	18 ± 2 b
Scoparia dulcis	25 ± 8 ab	-	-	-	-
Synedralla nodiflora	45 ± 10 ab	-	-	-	-
Vernonia colorata	-	-	-	32.5 ± 12 b	-
H	24.8	2.71	10.05	91.8	9.73
P	0.003	0.257	0.048	0.03	0.043

In the columns the values bearing the same letters are not significantly different according to the Kruskall wallis ANOVA test at the threshold of 5%.

Begomovirus hosting medicinal plants identified by PCR

The DNA of two samples on a total of 91 samples tested revealed a fragment of expected size (690 bp) after amplification by the PCR method (Fig. 2). It is the species *Moringa oleifera* belonging to the family *Moringaceae* and *Momordica charantia* (Curcubitaceae) collected in Port-Bouët - Abidjan. Symptoms of infection include marginal discoloration of inward leaf blades and discoloration of the veins (Fig. 1).



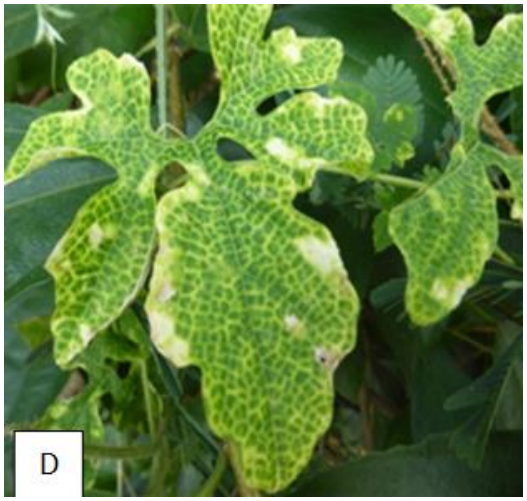


Fig. 1. Symptoms of color change observed on medicinal plants.
A: Leaves of *Moringa oleifera* apparently healthy. B: Marginal yellowing of the limb of *Moringa oleifera* (*Moringaceae*). C: Leaves of *Momordica charantia* apparently healthy.D: Vein clearing of *Momordica charantia* (Curcubitaceae).

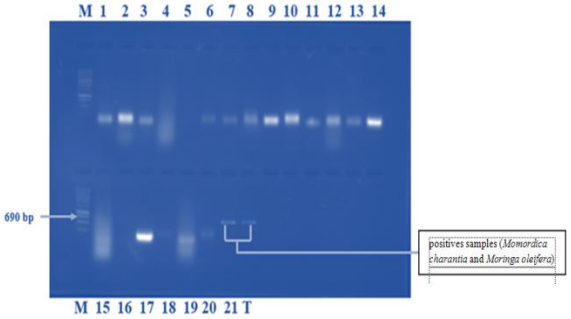


Fig. 2. Agarose electrophoresis gel 1% of PCR products for the detection of Begomovirus in leaf samples of medicinal plants; M: label = 1 Kb; 1-21 and T: samples.

Discussion

Fifteen plants families were across the sites including 20 species. Most of the collected species were grasses/herbaceous plants and small trees, being mostly catalogued as medicinal plants (N'guessan *et al.*, 2009 ; Dro *et al.*, 2013). The authors conducted a phytochemical screening of some medicinal plants in Côte d'Ivoire and observed various symptoms of viral diseases on the different species of medicinal plants on the collection sites. These symptoms are leaf curl, thinning of the veins and discoloration of leaves, mosaic, chlorosis, shoestring and plant dwarfism. The various symptoms may be caused by the infections of one or several viral strains. The results are consistent with the findings of Séka *et al.* (2016 and 2017), Tiendrébéogo *et al.* (2010 and 2012) and Alassane *et al.* (2016) for detection of Begomoviruses on market gardening showing leaf curl, thinning of the veins and mosaic. The mosaic symptoms were mostly observed in the samples of Abidjan and Yamoussoukro. Then, chlorosis was the most observed signs on the samples of Alépé. In addition, some symptoms may be present on some sites and absent on other sites. This variation in occurrence may be due to several factors such as environmental conditions on the collection sites, plant susceptibility to viruses, phenological stages of sampled plants, and mode of transmission of the viruses. Indeed, studies conducted by Eni *et al.* (2008), showed that the incidence of YMV varied according to the species and the phenological stage of a plant. The same variations in the incidence of viral diseases depending on the area were highlighted by Séka *et al.* (2009), demonstrating that the incidence of YMV was decreasing from the forest/savannah transition zone (Toumodi) to the savannah zone (Bouaké). The same authors further demonstrated that the absence of a type of symptom (embossing) on varieties of *Dioscorea alata* is likely due to their thick cuticle (varietal resistance) and the narrow form of *D. cayenensis-rotundata* leaves announce "shoestring" and leaf curling.

In addition, the assumption incriminating the mode of transmission of the viruses associated with these symptoms was confirmed by Olivera *et al.* (2001), who showed that the polyphagous character of a phytovirus vector increases the host range and the ability of the viruses to disseminate. Molecular analyzes revealed the presence of Begomovirus in two of the medicinal plants, namely *Moringa olifera* of the family of Moringaceae with marginal discoloration and *Momordica charantia* - Curcubitaceae. Such results are alike with those of Leke *et al.* (2012 and 2016). Both medicinal plants are part of the potential hosts of *Begomoviruses* already identified in the West Africa subregion. The infection of those medicinal plants could be due to their cohabitation with food crops and market gardening. Indeed, studies conducted in Côte d'Ivoire by Pita *et al.* (2001); Séka *et al.* (2016 and 2017) and in Burkina

Faso by Tiendrébéogo *et al.* (2010 and 2012) and Alassane *et al.* (2016) indicated the presence of a range of Begomoviruses population causing disease in food crop and market gardening in the West Africa sub-region. However, despite noting symptoms that are alike those caused by viruses, the other samples tested negative in *Begomovirus*. These plants could be infected with viruses of a genus other than *Begomovirus*. Given that these plants are used for therapeutic purposes, changes in leaf color due to the presence of viral particles could have an impact on active ingredients.

Conclusion

It emerges from this study that Côte d'Ivoire is full of diversity of medicinal plants. The plants sampled exhibited various leaf symptoms including chlorosis, leaf curl, mosaic and 'shoestring'. Symptoms of plant dwarfism have also been observed. The symptoms observed varied according to the medicinal plant species in each collection area. Mosaic was the predominant symptom on all plants sampled. There is a relationship between the severity of viral symptoms and the infected plant species. Medicinal plants are also susceptible to infections caused by Begomoviruses in Côte d'Ivoire. But, it could also have a relationship between the amount of active ingredient secreted by the plant and the severity of the symptoms of the viral infection. The results of this study open new avenues of research on *Begomoviruses* of medicinal plants; it would be appropriate for further work to be undertaken. It involves: identifying the *Begomovirus* species attacking medical plants in Côte d'Ivoire; identifying other virus species present on medicinal plants; studying the effect of viral diseases on the effectiveness of treatments based on these plants.

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