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RESEARCH ARTICLE

IN VIVO EVALUATION OF ANTIDIARRHEAL ACTIVITY AND FORMULATION OF A FLOUR BASED ON EXTRACTS FROM THE LEAVES OF COMBRETUM GRANDIFLORUM G. DON (COMBRETACEAE)

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Abstract

Althoughmany conventional antidiarrheal agents are available, the inherenttoxicityof thesedrugsprovides an avenue to explore for safe and effective alternative medications. The objective of thisstudyis to evaluate in vivo the antidiarrhealactivity of extractsfrom the leaves of Combretumgrandiflorum and to formulate an antidiarrhealflour.The antidiarrhealactivity of Combretumgrandiflorumextractswascarried out usingmodels of castor oil-induceddiarrhea, castor oil-induced anti-enteropolling and intestinal transit. The formulation of the antidiarrhealflourwas made following the classic CCC calculationmethod. The results show a significantreduction ($p<0.01$) in the frequency of wetstools and water content of diarrhea, intestinal motility, intestinal fluid accumulation and a delay in the onset of diarrhea by compared to controls, wereobserved in micetreatedwith the aqueousextract of Combretumgrandiflorum. Furthermore, the test batchestreatedwith the hydroethanolicextract and the referencemolecule (loperamide), no emission of diarrhealstools, no mass of freshdiarrhealstools, wasobserved. However, thiseffect of loperamideisstatisticallyidentical to that of the hydroethanolicextract. The formulation of antidiarrhealflourwith the hydroethanolicextract of Combretumgrandiflorumwillprovide an antidiarrhealfoodwith good nutritional value and acceptable rheologicalproperties. Additionalresearchisthereforenecessary to findflours to resolvediarrheaproblems.

Introduction:-

Diarrhealdiseases have long been a serious public health problem in low-income countries, with high morbidity and mortality rates. Globally, diarrheais the second leading cause of death in childrenunder five years of age, accounting for 9% of all childdeaths [1]. In childrenunder 5 yearsold, it causes approximately 15%, or more than 1,600

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childdeathsdaily out of all childdeaths [2]. Approximately 80% of all deathsfromdiarrheaamongchildrenoccur in countries in Africa and Southeast Asia [3]. About 25% and 31% of the overalldiarrhealburdenwasattributed to diarrhea in childrenunder five years of age in Africa and Asia, respectively, with the highestmortality rate in Africa [4].

According to the WHO report, more than 80% of the world's population depends ontraditionalmedicine for theirprimary health needs [5]. It isoftennotedthat 25% of all medicationsprescribedtoday come from plants [6]. This estimatesuggeststhat plant-basedmedicinesconstitute an important segment of naturalproduct-basedpharmaceuticals [7]. Herbalproducts are preferred due to their shorter testing time, safety, effectiveness, cultural acceptability, and fewersideeffects [8].

Chemical compounds present in herbalproducts are part of the physiologicalfunctions of living organisms and are thereforebelieved to have better compatibility with the human body [9]. Additionally, the use of investigationaldrugs for the treatment of diarrheaisemerging [10, 11]. For example, new antidiarrhealdrugs, Eluxadoline and rifaximin, bothapproved by the Food and Drug Administration (FDA) in 2015, have been shown to relievediarrheaassociatedwithinflammatorybowel syndrome [11,12].

Plant extracts can have variouspharmacologicaleffectssuch as decreasingspasms, increasing water absorption, inhibiting intestinal motility, and delaying gastrointestinal transit [13]. These actions could help explainwhy certain plants are useful in treatingdiarrhealdiseases.

Plant species of the samegenusCombretum are commonlyusedthroughoutnortheastern Brazil as a natural expectorant, treatment of hemorrhages and influenza [14]. Combretumleprosumbarkextract has substantialantidiarrhealproperties [15]. Therefore, Combretumgrandiflorum, whichbelongs to the samegenus, could have a similareffect. Antidiarrheal action has also been reported in herbalproductscontainingalkaloids, tannins, saponins, flavonoids and terpenoids [16]. Previousphytochemicalstudies have shownthatCombretumgrandiflorumpossessessuchsecondarymetabolites, implyingthat the plant may have antidiarrhealactivity [17]. Therefore, thisstudyaimed to evaluate the antidiarrhealactivity of Combretumgrandiflorumextractagainst castor oil-induceddiarrhea in mice. Then a formulation of antidiarrhealflourwithCombretumgrandiflorumextract.

Materials and Methods:-

Materials:-

- The plant materialwas the leaves of Combretumgrandiflorum (figure 1).

Figure 1:- Leaves of Combretumgrandiflorum.

-The antidiarrhealactivitystudywascarried out on three-month-old albino male Wistar rats weighing between 150 and 180 gand comingfrom the animal facility of the AppliedMicrobiology and Pharmacology of Natural Substances Research Unit (URMAPha). Rats werekeeped, with free access to standard food and water, under standard conditions (12 h light/12 h dark at 22 ± 2°C).

Methods:-

Preparation of CrudesExtracts

According to the methoddescribed by Deguenonet al. (2023) [17], 50 g of powderedleaves of Combretumgrandiflorum G. Don weremaceratedeach one for 72 h at room temperaturein 500 ml of each solvent (distilled water for the aqueousextract and distilled water/ethanol in a proportion 40:60 v/v for the hydro-ethanolextract). The homogenateobtainedwasfilteredthree times throughhydrophiliccotton and once through Wattman N 1 paper. This filtratewasthenconcentrated in a rotavapor at 40°C. The powderobtainedcorresponded to the extracts. The obtainedextractwasweighed in order to evaluate the extraction yield and thenstored in the refrigerator at 4°C. The yield (Y) of the crudeextractdefined as the ratio between the mass of the dry extractobtained and the mass of the treated plant materialwascalculated by the following formula :

R = {weight of extractafterevaporation/ weight of plant powderused for extration} × 100

Assayof antidiarrhealactivity

The methodology adopted in this study was that used by Assiki et al, [18]. Forty Wistar rats divided into eight lots of 5 rats each were used (one negative control lot, one reference control lot and six lots tested with the extracts) (Table I).

Table I:- Distribution of the differentlotsaccording to the treatmentsadministered.

Lots	Types of lots	Treatment of lots
1	Negative control	Administration of castor oilwithouttreatment
2	Reference witness	Administration of castor oilfollowed by loperamidetreatmentafter 20 minutes
3	Aqueousextract 300 mg/kg	Administration of castor oilfollowed by extracttreatmentafter 20 minutes for three doses
4	Aqueousextract500 mg/kg	
5	Aqueousextract1000 mg/kg	
6	Hydroethanolicextract300 mg/kg	
7	Hydroethanolicextract500 mg/kg	
8	Hydroethanolicextract1000 mg/kg	

The animalsusedwerefasted for 18 hoursbefore the experiment, but with free access to distilled water. Theywerethendeprived of water 2 hoursafter the experiment. Diarrheawasinducedusing castor oil (10 mL/kg) via administration by esophageal gavage. Twenty minutes after the administration of castor oil, the different substances (plant extracts and loperamide) wereadministered by esophageal gavage in a volume of 1 mL per 100 g of weight of the Wistar rats. The negative control batch wastreatedwithdistilled water, the reference control batch wastreatedwithloperamide at a dose of 3 mg/kg/bw and the test batchesweretreatedwithextracts. Three test batchesweretreatedwith the aqueousextract of Combretumgrandiflorum at doses of 300 mg/kg/pc, 500 mg/kg/pc and 1000 mg/kg/pc. The threeremaining test batchesweretreatedwith the hydroethanolicextract at doses of 300 mg/kg/pc, 500 mg/kg/pc and 1000 mg/kg/pc. The rats wereplaced in individual cages underwhich absorbent paperwas spread for observation and collection of diarrhealstools. These cages weremonitoredindividually for four hourswith the absorbent paperrenewedeveryhour. The diarrhealstoolscollectedaccording to the rats and batcheswereweighed and dried on the bench. The differentdiarrhealparametersexplored are as follows [18]:

- The latencyperiod (time between the administration of castor oil and the appearance of the first diarrhealstools).
- The duration of diarrhealstoolemission corresponds to the duration between the first appearance of diarrhealstool and the last appearance of diarrhealstool

- The average mass of diarrhealstools,

$$\mathbf{M} = \frac{\text{Sum of the masses of fresh stools from each rat in a lot}}{\text{Total number of rats with diarrheal stools in a lot}}$$

- Defecationfrequency (DF)

$$\mathbf{DF} = \frac{\text{Sum of defecations of rats in a lot}}{\text{Total number of rats having defecated in a batch}} * 100$$

- The percentage of inhibition of diarrhea (PI):

$$\mathbf{PI} = \frac{\text{Average number of stools in the negative control group} - \text{Average number of stools in the test group}}{\text{Average number of stools in the negative control group}} * 100$$

- **The water content of diarrheal stools (TES)**

$$\mathbf{TES} = \frac{\text{mass of fresh stools} - \text{mass of dry stools}}{\text{mass of fresh stools}} * 100$$

- **Purge Index (PI)**

$$\mathbf{PI} = \frac{\%\text{ of rats having diarrhea} * \text{average number of diarrheal stools}}{\text{average latency period}} * 100$$

Flour formulation

Pre-formulation of granules

A literaturereviewwascarried out on the physical and chemicalcharacteristics of the active ingredient and the excipients. Specifically, the followingparameterswеredetermined: the effective dose of the extract applicable to humansbased on thatrecordedduring animal testing and the choice of excipients.

Description of the active ingredient and excipients

The active ingredientconsists of dry extract (hydroethanolicextract) presented in the form of crystals. The effective dose recordedduring in vivo antidiarrhealefficacy tests is 300 mg/kg/bw for Wistar rats for thisextract. Sorbitol wasused as an excipient. This is a diluent suitable for pediatric uses. Pregelatinized corn starchisused as a binder. This binder wasused to producewet granulation. Purified water wasused as a solvent in the preparation. The amount of water usedwasadjustedaccording to the needs of the formulation.

Table II:- Summary of the description of the components of the granules.

role of the Components	Names of components	Properties	References
Principe actif	Dry extract (hydroethanolic)	-antidiarrhealeffect	[18].
Diluent	Sorbitol	tocomplete the volume of the active ingredient to produce the desired dosage form	[19].
Binder	Pregelatinizedmaizestarch	To performgranulation of the powder	
Solvent	Purified water	to carry out wet granulation	[19].

Formulation and quality control of granules (flour)

The preparation of the granules (flour) wascarried out hot according to the methodproposed by Ouedraogo et al, [20]. Different tests wereused for quality control.

Organolepticexamination:
The organolepticcharacteristics of the granules wereevaluatedfollowing the methodproposed by Ouedraogo et al, [20].

Particlesize:
The particle size analysis of the granules wascarried out usingdifferentsievecolumnswithstirring for a few minutes Ouedraogo et al, [20].

Wettabilitytest:
The methodproposed by Alghunaim et al, [21] wasused to assess the wettability of the granules developed.

Determination of solubility:
Solubilitywasdetermined by the methoddescribed by OECD (1995) and used by Ouedraogo et al, [20].

Determination of pH:
The hydrogenpotential (pH) of the redissolved granules wasdeterminedfollowing the methoddescribed by Chougouo-Nkuitchou et al, [22].

Determination of the adhesivenessrate:
The adhesiveness rate wasdeterminedfollowing the methoddescribed by Chougouo-Nkuitchou et al, [22].

Statisticalanalysis of data
The data generatedfrom the tests performedweresubjected to statisticalanalysisusing SPSS 26.0 software. Quantitative variables are presented as mean and standard deviation. ANOVA analysis of variance wasused to compare the data of the differentparameters of the efficacy tests of the antidiarrhealeffect of plant extracts. The significancethresholdwas set at 5%.

Results:-

Antidiarrheal activity of Combretum grandiflorum extracts

Effect of treatments on the latency period of diarrhea

Figure 1 shows the effect of the different treatments administered to rats on the latency period of diarrhea. This figure tells us that for the negative control lot which did not receive any treatment after administration of castor oil, diarrhea appeared after 34 minutes. For the reference control batch (loperamide, administered at a dose of 3 mg/kg), no diarrheal stool emission was noted. The same observation was made for the batches treated with the hydroethanolic extract of Combretum grandiflorum at the different doses tested (300 mg/kg, 500 mg/kg and 1000 mg/kg). For the batches having received the aqueous extract of Combretum grandiflorum, a significant delay in the emission of diarrheal stools was observed compared to the negative control batch. Diarrheal stools appeared 1 hour after the induction of diarrhea for the batches treated at doses of 300 mg/kg/bw and 500 mg/kg/bw and 2 hours for the batch treated at a dose of 1000 mg/kg /pc ($P<0.05$).

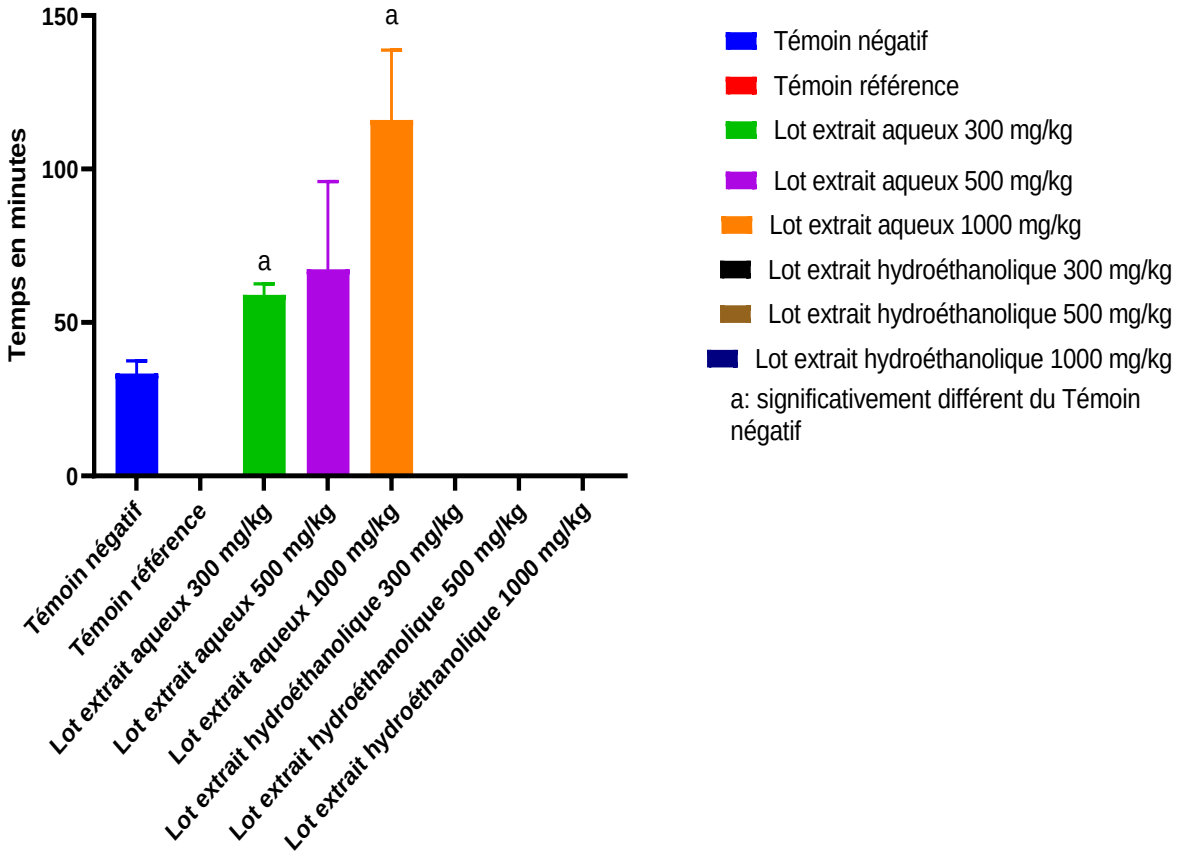

Figure 2:- Effect of treatments on the latencyperiod of diarrheal.

Effect of treatments on the duration of diarrhealstools

Figure 2 illustrates the duration of diarrhealstools. For the negative control batch, the emission of diarrhealstoolslasted on average 3 hours 40 minutes. For the test batches, the duration of diarrhealstoolswas 3 hours 30 minutes on average for the batch treatedwith the aqueousextract of Combretumgrandiflorum at a dose of 300 mg/kg/bw. At the dose of 500 mg/kg/bw and 1000 mg/kg/bw, thisemission duration wassignificantlyreduced to 1h30 on averagecompared to the negative control batch. For the test batchestreatedwith the hydroethanolicextract and the referencemolecule (loperamide), no emission of diarrhealstoolswasobserved.

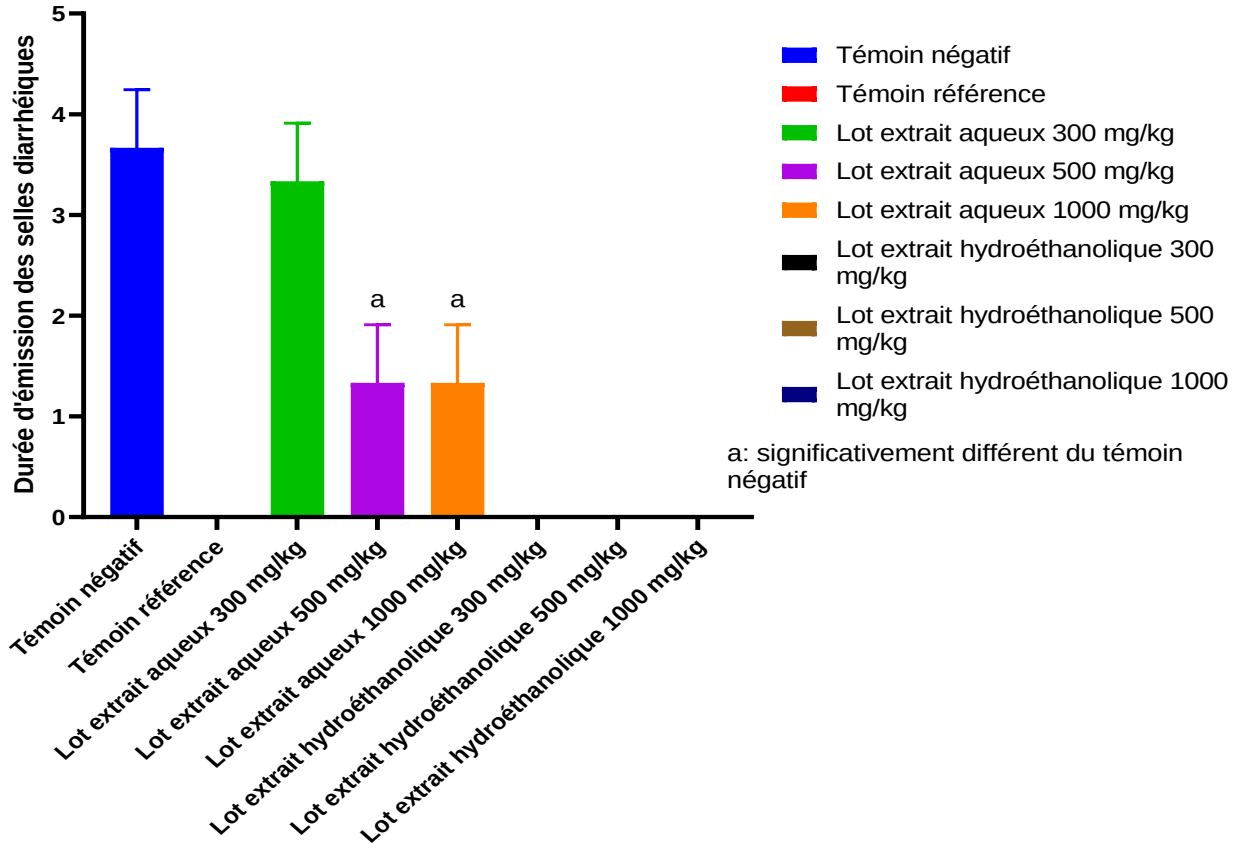

Figure 3:- Average duration of emission of freshdiarrhealstoolsfromdifferentlots.

Effect of differenttreatments on the mass of freshdiarrhealstools

The analysis of Figure 3 revealsthatcompared to the negative control batch, the batchestreatedwith the aqueousextract at doses of 500 mg/kg/bw and 1000 mg/kg/bwshowed a significantreduction ($P < 0.05$) of the mass of freshdiarrhealstools. Furthermore, like the batch treatedwithloperamide, no mass of freshdiarrhealstoolswasobtained for the batch treatedwith the hydroethanolicextract at the doses tested.

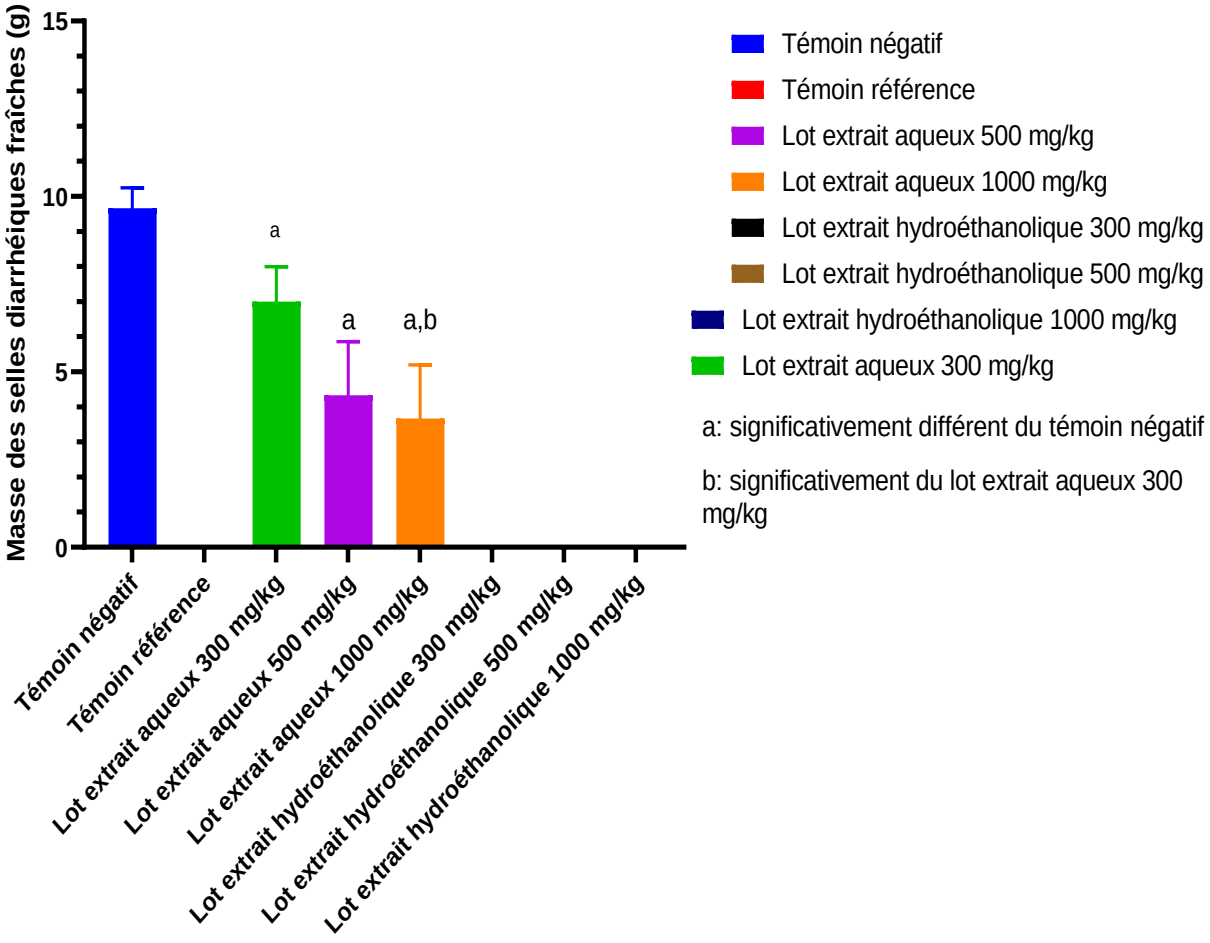

Figure 4:-Average mass of freshdiarrhealstoolsfromdifferentbatches.

Effect of differenttreatments on the frequency of defecation of diarrhealstools

Figure 4 shows the defecationfrequency of diarrhealstools of rats fromdifferentbatches. Fromthis figure, we note thatcompared to the negative control group, the frequency of defecation of diarrhealstoolssignificantlydecreased ($P < 0.05$) for the rats in the group treatedwith the aqueousextract at doses of 500 and 1000 mg/kg. /pc.

However, no defecationwasrecorded for rats treatedwithloperamide and thosetreatedwithhydroethanolicextract at the doses tested.

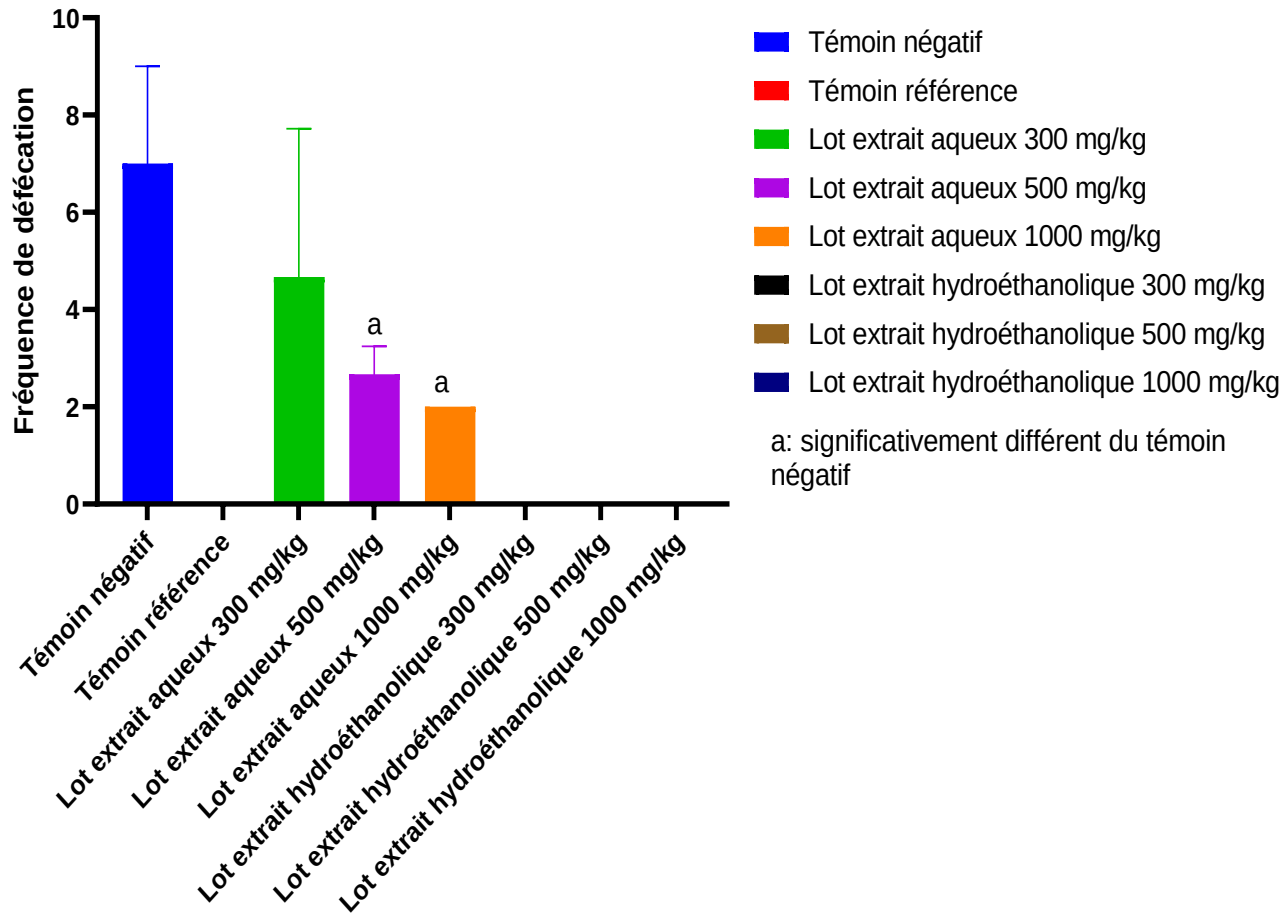

Figure 5:- Frequency of defecation of freshdiarrhealstools of rats fromdifferentlots.

Water content of diarrhealstoolsfromdifferentlots

Figure 5 summarizes the data on the water content of diarrhealstools. Fromthis figure, itappearsthatcompared to the negative control batch, a dose-dependentreduction in the water content of diarrhealstoolswasobtained for the batchestreatedwith the aqueousextract. Furthermore, just like the loperamide batch, no water content of the diarrhealstoolscouldbedetermined for the batchestreatedwith the hydroethanolicextractgiventhattherewas no emission of diarrhealstools for thesebatches.

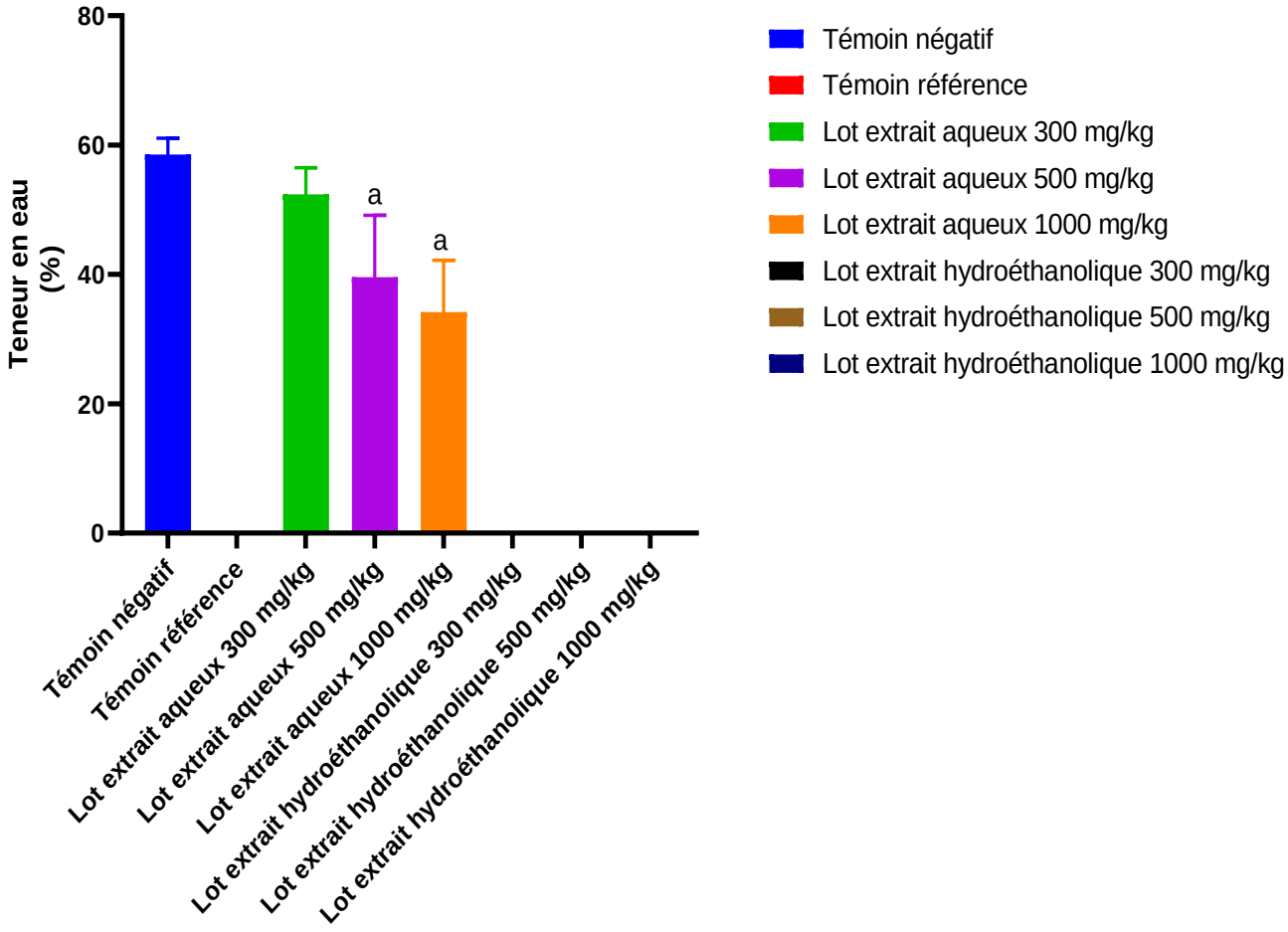

Figure 6:- Water content of diarrhealstoolsfrom rats fromdifferentlots.

Percentage of diarrhea inhibition of differentlot

The data relating to the percentages of inhibition of diarrheafrom the treatments are summarized in Figure 6. From the analysis of this figure, itappearsthatcompared to the negative batch, all the extracts and loperamide (referencemolecule) significantlyinhibitedinduceddiarrhea ($P < 0.05$). Furthermore, the diarrheainhibitoryeffectexerted by loperamide (referencemolecule) issignificantlybetter ($P < 0.05$) thanthat of the aqueousextract. However, thiseffect of loperamideisstatisticallyidentical to that of the hydroethanolicextract.

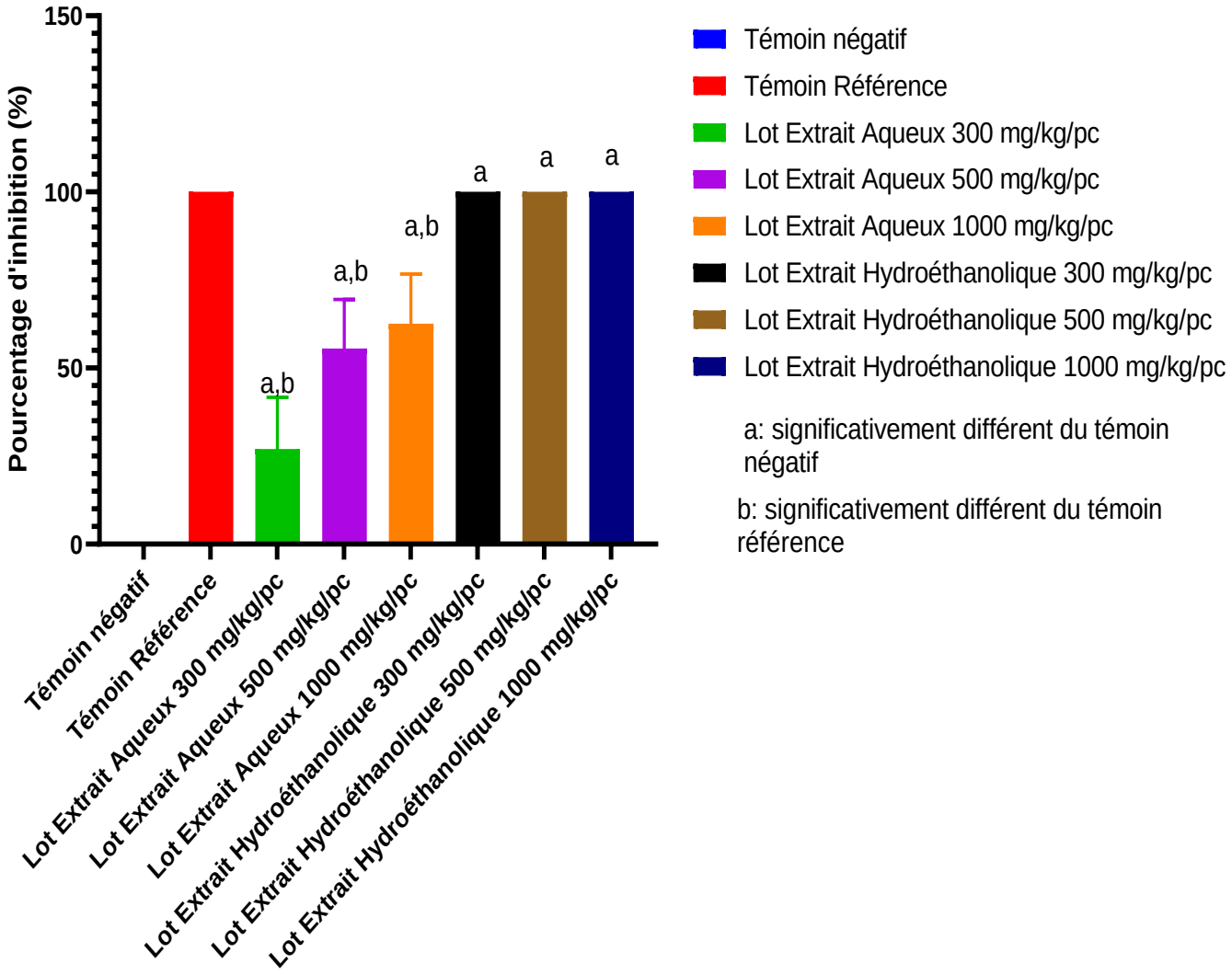

Figure 7:- Percentage of inhibition of diarrhea in rats fromdifferentlot.

Purge Indices

Figure 7 shows the purge indices in the differentbatches. From the analysis of this figure, itappearsthatcompared to the negative control batch, we note a low purge index for the batchestreatedwith the aqueousextract. This weakeffectisproportional to the doses tested (300 mg/kg/bw and 500 mg/kg/bw and 1000 mg/kg/bw). As no emission of diarrhealstoolswasobtained for the loperamide batch and the batchestreatedwith the hydroethanolicextract at the doses tested (300 mg/kg/bw and 500 mg/kg/bw and 1000 mg/kg/bw), the diarrhea purge index of thesebatcheswas 0.

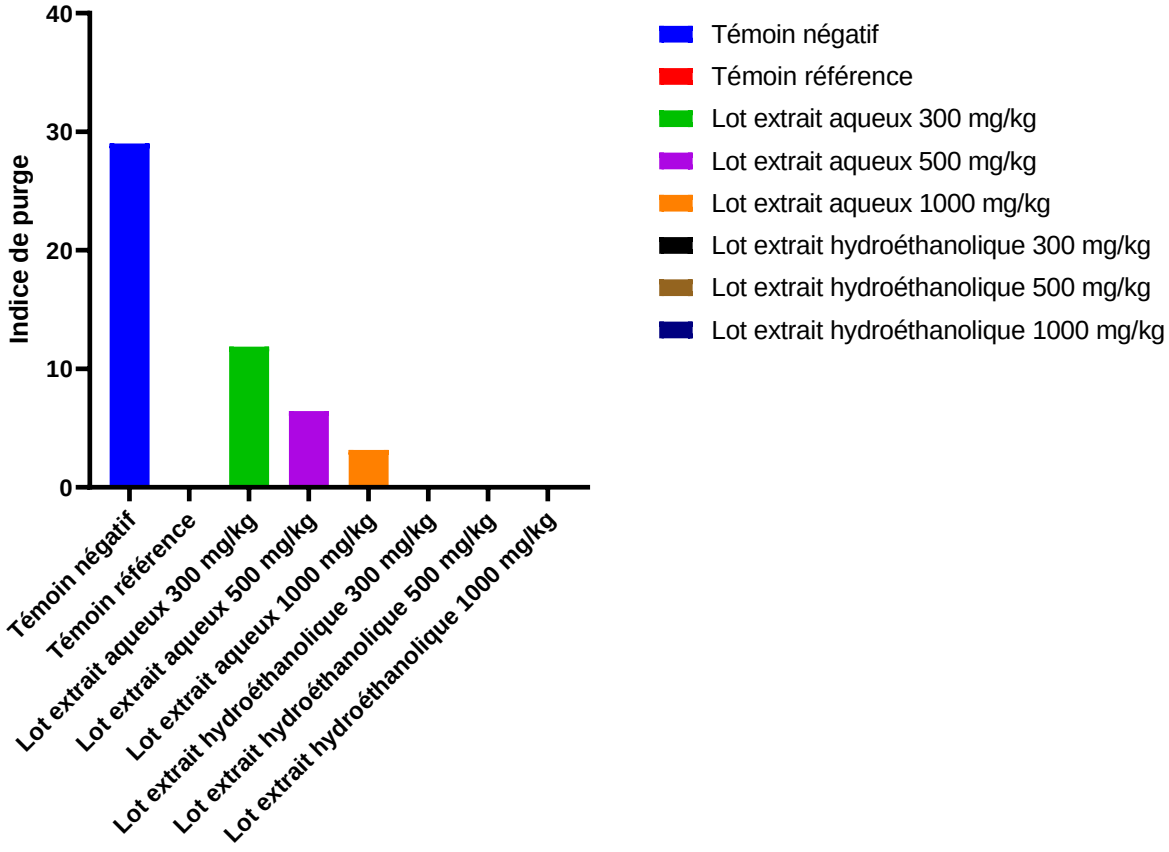

Figure 8:- Purge Indices.

Flour formulation

Effective dose for humans

Based on the CCC calculationmethod, the human dose wasobtained by extrapolation. This dose corresponds to 48.65 mg/kg. This isequivalent to 486.5 mg of granules to bereconstituted in water to treatdiarrhea in a childweighing 10 kg. Table III presents the qualitative and quantitative characteristics of the constituents for 50 g of granules.

Table III:- Doses and quantities of constituents for 50 g of granules.

Names of components	Experimental dose(mg/kg)	Dose on a humanscale(mg/kg)
Dry extract	300	2,43
Sorbitol	-	50
Pregelatinized corn starch	-	2,50 g
Purified water	-	Qsp

Legend: -: not considered; Qsp: sufficient quantity for

Pharmacotechnical data

The results of the pharmacotechnicalcharacterization tests of the granules (flour) indicatethat the formulated granules are beige in color (Figure 8a and 8b), 250 and 500 µm in size with a redissolution time of 14 seconds. The granules are sweet and slightlytart in flavor. The pH of the reconstituted solution is 3.74. The water solubility of the granules isvery good and isbetween 1000 and 200 g/l. With a lowadhesiveness of 1%, the granules are packaged in the form of a 486.5 mg aluminum film sachet (figure 9).

Figure 9:- Granules based on dry extract of Combretumgrandiflorum in bulk.

Figure 10:- Granules based on dry extract of Combretumgrandiflorumpackaged in the form of an aluminum film sachet.

Discussion:-

The aim of thisstudywas to determine the in vivo antidiarrhealactivity of the crudeextract of Combretumgrandiflorum plant extracts, a formulation of antidiarrhealflour. Castor oilis one of the mostcommonlyused agents to inducediarrhea in animals for the in vivo study of the antidiarrhealactivity of medicinal plants. Castor oilstimulates intestinal peristalsis, leading to diarrhea by preventing the absorption of fluids and electrolytes [23]. Therefore, prevention of castor oil-induceddiarrheais of paramount importance in the management of diarrhea. After administration of the aqueousextract of Combretumgrandiflorum, therewas a significantdelay in the emission of diarrhealstoolscompared to the negative control batch. For the test batchestreatedwith the hydroethanolicextract and the referencemolecule (loperamide), no emission of diarrhealstoolswasobserved. Diarrhealstoolsappeared 1 hourafter the induction of diarrhea for the batchestreated at doses of 300 mg/kg/bw and 500 mg/kg/bw and 2 hours for the batch treated at a dose of 1000 mg/kg /pc ($P<0.05$). The appearance of diarrhea and a decrease in the frequency of feces, indicatingthattheyhad an antidiarrhealeffect at the test doses thatwereused. One potentialexplanation for the antidiarrhealeffectiveness of Combretumgrandiflorumcrudeextractistheirability to improve the absorption of fluids and electrolytesfrom the gastrointestinal tract. According to reports fromnumerous investigations, phytochemicalssuch as alkaloids, tannins, saponins, phenols, terpenoids and flavonoidsmayberesponsible for the antidiarrhealeffects of Combretumgrandiflorumcrudeextract [24, 25.26].

Concerning the mass of freshdiarrhealstools, the batchestreatedwith the aqueousextract at doses of 500 mg/kg/bw and 1000 mg/kg/bwshowed a significantreduction ($P < 0.05$) in the mass of diarrhealstools. fresh. Furthermore, like the batch treatedwithloperamide, no mass of freshdiarrhealstoolswasobtained for the batch treatedwith the hydroethanolicextract at the doses tested. The frequency of defecation of diarrhealstoolssignificantlydecreased ($P < 0.05$) for rats in the group treatedwith the aqueousextract at doses of 500 and 1000 mg/kg/bw. However, no defecationwasrecorded for rats treatedwithloperamide and thosetreatedwithhydroethanolicextract at the doses tested. The dose-dependentreduction in the water content of diarrhealstoolswasobtained for the batchestreatedwith the aqueousextract. Furthermore, just like the loperamide batch, no water content of the diarrhealstoolscouldbedetermined for the batchestreatedwith the hydroethanolicextractgiventhattherewas no emission of diarrhealstools for theselots.

All extracts and loperamide (referencemolecule) significantlyinhibitedinduceddiarrhea ($P < 0.05$). Furthermore, the diarrheainhibitoryeffectexerted by loperamide (referencemolecule) issignificantlybetter ($P < 0.05$) thanthat of the aqueousextract. However, thiseffect of loperamideisstatisticallyidentical to that of the hydroethanolicextract. In the castor oil-induceddiarrhea model, the extractdelayed the onset of diarrhea and reduced the total number of stools, number of wetstools, and wetstoolweight. This is consistent with a study on a hydroalcoholicextract of an Ethiopian plant [27] whichsuggested the presence of bioactive secondarymetabolitesresponsible for the reportedeffects. It has also been reportedthatnonsteroidal anti-inflammatorydrugs can inhibit the castor oil-induced type of diarrhea [29]. It istherefore plausible to assertthat the antidiarrheal action of the plant studiedcouldbe due to antiprostaglandin-type effectssimilar to those of analgesic and anti-inflammatoryeffects.

Concerning the formulation of the antidiarrhealflour, the hydroethanolicextract of Combretumgrandiflorumwhichshowed a betterresultwasused. Initial human dose selectionis one of the most important steps in the clinicaldevelopment of new investigationaldrugs. Differentapproaches are applied and tend to generate the most conservative initial dose [30, 31, 32]. However, a betterestimate of the potentialhumanequivalent effective dose wouldbeuseful for proposing dose escalation in clinical trials and wouldprovide a targetreference in phase II/III dose selection. In ourstudy, this dose corresponds to 48.65 mg/kg. This isequivalent to 486.5 mg of granules to bereconstituted in water to treatdiarrhea in a childweighing 10 kg.

Assumingthat the pharmacodynamic (PD) model used in animalscorrelateswellwith the PK/PD relationship in humans, the basis of dose conversion betweenspeciesisthought to be a function of differences in metabolic rate of a animal which are reflected in its size. In ourstudy, the results of the pharmacotechnicalcharacterization tests of the granules (flour) indicatethat the formulated granules are beige in color, 250 and 500 µm in size with a redissolution time of 14 seconds. The sameresultswerefound by Mahouli et al [33] on infant flourbased on parboiledrice (Oryza sativa), spirulina (Spirulinaplatensis) and cashew nuts (Anacardiumouest). Theseauthorsfound a flour size of 300 µm.

The granules are sweet and slightlytart in flavor. The pH of the reconstituted solution is 3.74. The water solubility of the granules isvery good and isbetween 1000 and 200 g/l. With a lowadhesiveness of 1%, the granules are packaged in the form of a 486.5 mg aluminum film sachet. The pH value of a food or preparation influences its conservation becausepathogenicbacteria do not develop in an environmentwhose pH islowerthan 4.5. Foodswith a low pH (mainly plant-based) are therefore more stable thanfoods of animal originwith a higher pH.

Conclusion:-

The results of the presentstudyrevealedthatCombretumgrandiflorumextracts have antidiarrhealactivity. The antidiarrhealactivitiescouldbeattributed to the presence of bioactive secondarymetabolites, includingflavonoids, tannins, terpenoids, saponins, phenols and alkaloids, whichactindividually or collectively to produce the overallantidiarrhealeffect. Theseresultsprovidescientific support for the folkloric use of Combretumgrandiflorum plants as a treatment for diarrhealdiseases. The formulation of antidiarrhealflourwith the hydroethanolicextract of Combretumgrandiflorumwillprovide an antidiarrhealfood of good nutritional value and acceptable rheologicalproperties. Furtherresearchisthereforeneeded to findantidiarrhealflours to solve public problems.

Aknowlegment:-

The authorsthankthelaboratorywhichcarried out the differents manipulations of thismanuscript

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