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RESEARCH ARTICLE

PHYTOCHEMICAL, PHYSICOCHEMICAL, ANTIOXIDANT AND TOXICITY STUDIES OF TWO EXTRACTS OF CHAMAECRISTA ROTUNDIFOLIA (PERS.) GRENNE

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Abstract

With a view to promoting Togolese traditional medicine through the revaluation of medicinal endogenous knowledge, phytochemical studies of aqueous and hydroethanolic extracts of the leafy stem of *Chamaecrista rotundifolia*were carried out. Studies of the antioxydant/anti-radical activity and of the extract's safety were undertaken.Thus, 4 main minerals were quantified in the plant's powder.The aqueous (10 mg/mL) and hydroethanolic (5 mg/mL) extracts presentedan anti-radical activity with Inhibitory Concentration 50 percent (IC_{50}) respectively 3.15 1 mg/mL and 2.121 mg/mL versus Vitamin C, IC_{50} =0.1 (1mg/mL). Results of the Ferric Reducing Antioxidant Power test showed an activity of these extracts doses respectively with a ferric reduction rate of 21.73% and 22.27% compared to VitaminC (2 mg/mL) = 80.72%. At the toxicological level, the lethal concentrations of50 percentobtained on shrimp larvae of *Artemia salina* respectively 0.68 ± 0.02 mg/mL and 0.13 ± 0.01 mg /mL of aqueous and hydroethanolic extractswere greater than 0.1 mg/mL indicating that the extracts, at these concentrations, are not cytotoxic. Furthermore, at a single limit dose of 2000 mg/kg/body weight, these extracts are not toxic to the Wistar rat. Phytochemistry and physicochemistry revealed respectively the presence of polyphenols, flavonoids, tannins, and of Calcium, Magnesium, Iron, Zinc at various rates.This study showed that *Chamaecrista rotundifolia*is rich in phytochemical compoundsandvital minerals. **Its**aqueous and hydroethanolic extractshave low free-radical and anti-radical activities,then they are non-toxic at the doses studied. Additional studies are needed for future foundations of phytomedicine's formulation.

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Introduction:-

Traditional medicine (TM) plays a major role in people's choice of primary health care.According to the WHO, 80% of the African population use TM for health care[1].This medicine mainly uses plants and plant recipes in the treatment of a multitude of pathologies.Medicinal plants indeed constitute a large source of chemotherapeutic agents with significant curative potential[2] and are full of substances that could be used for the treatment of diseases or to manufacture phytomedicines and analog structuresof molecules.Thus, there are many conventional drugs which active ingredients are molecules that arepresent in these plants or are analog structures [3]. Although these plants are found in Africanvegetation, the populations of the continent continue to depend on pharmaceutical companies and foreign laboratories [4]and the medicines theyproduced are very expensive for African populations.TM appears in this context as an alternative[5] to fill the gaps in health needs for which populations aspire, especially in recent years, with phytotherapy having consequently a renewed interest[6]. The use in primary health care of natural resources in general and medicinal plants particularly has thus become one of the most important and interesting avenues for the search for new molecules effective against recurrent diseases[7]. Much research has therefore turned to plants as reliable and sustainable alternatives, plants which have proven effectiveness, safety and quality.Although these plants are used by the populations themselves or by traditional medicine practitioners to treat a variety of diseases due to their affordability, availability and acceptability[8, 9]scientific evidence from tests carried out to assess the effectiveness, safety and quality of traditional medicine products are limited[10].It is in this context that *Chamaecrista rotundifolia*, already selected by previous work for its antimalarial[11] and anti-infectious properties[12] was selected to be the subject of this presentstudy.That plant from the Fabaceae family has been identified in Togo in the treatment of infectious diseases such as malaria and liver diseases [13]. In Benin, *Chamaecrista rotundifolia* is used in the treatment of teething disorders, [14] childhood illnesses [15], infections [16], malaria, anemia and diabetes[16, 17, 18, 19]. Despite these studies, little work in Togo has considered the anti-dermatological properties of this specie.This present study, which **sets**itself the objectives **to** evaluating the chemical groups and essential trace elements present in the leafy stem of this species on the one hand and **to** carrying out its toxicological and antioxidant screeningon the other hand, was carried out with the aim tolay foundations for a future formulation of phytomedicines against skin diseases.

Material and Methods:-

Material:-

The plant material used in this studie consists of the leafy stem of *Chamaecrista rotundifolia* (Pers.) Grenne purchased from the herbalists in Akodessewa market in Lome.The identification of these plants was carried out by specialists from the Laboratory of Botany and Plant Ecology (LBEV) of the University of Lome and a voucher number (Voucher N° TOGO15926) is deposited in the herbarium of the said university.

The animal material **consists of** the shrimp larvae (*Artemia salina*), Wistar ratsmaintained at a constant temperature of 22 ± 2°C and subjected to a 12/12-hour light/dark cycle obtained from the animal facility of the Applied Microbiology and Pharmacology of Natural Substances Research Unit of the University of Abomey-Calavi.
Machines used are Rotavapor BUCHI for extraction, MINDRAY type spectrophotometer (BA-88-A) for FRAP test, CECIL CE 2041 Spectrophotometer, and iCE 3000 Series Atomic Absorption Spectrometer. Appropriated various regents [20, 21, 22, 23] are used for phytochemical, physicochemical, toxicological screenings and antioxidant activities valuation.

Methods:-

Powdering and extraction

The leafy stem of *Chamaecrista rotundifolia* (Pers.) was washed, dried under air conditioning and made into a fine powder using a mill for extraction. The extraction was then carried out to obtain aqueous and hydroethanolic extracts of organs of the two plants at a rate of 100g of powder per 1000 mL of solvent. The extracts were filtered with filter paper (Whatman No. 1) before being evaporated to dryness using a rotary evaporator (Rotavapor BUCHI, Switzerland) under vacuum at 40°C. Consequently, the aqueous extraction was carried out by decoction by heating the powder produced in distilled water and the hydro-ethanolic extraction (80:20 v/v) was carried out by maceration in the 95° ethanol + water mixture. Powder, dry aqueous and hydro-ethanolic extracts are obtained and kept in a freezer for future usages.

Larval toxicity of *Chamaecrista rotundifolia* on shrimp

The cytotoxic effect of the hydro-ethanolic extract and the aqueous extract of the plant studied was evaluated on shrimp larvae*Artemia salina* model[24, 25, 26]. The larvae of *Artemia salina* were obtained by hatching 10 mg of shrimp eggs under continuous agitation in 1L of sea water for 72 h. Dilution series of order 2 of a stock solution of extract with a concentration of 20 mg/ mL were carried out. To 1 mL of each of these diluted solutions was added 1 mL of sea water containing 16 live larvae. A control solution without the extract was prepared. All solutions were incubated with shaking for 24 hours. Counting the number of dead larvae in each solution using an optical microscope made it possible to trace the representative number of surviving larvae of each curve corresponding to the concentration of the extract. The data (concentration-response) were logarithmically transformed and the lethal concentration (LC50) (mean lethal concentrations) was determined. To assess the larval toxicity of the extract, the correlation grid associating the degree of toxicity with the LC50 value [27].

Acute toxicity

Using the acute oral toxicity of the four products was explored following the standard protocol for evaluating the acute oral toxicity of chemical and natural products of the Organization for Economic Co-operation and Development[28], nulliparous and non-pregnant female Wistar albino rats weighing on average 110 g rats were distributed randomly, into three (3) groups of 3 rats each, in standard cages for an acclimation period of 2 weeks before their use. This concerns a control batch treated with distilled water and two test batches treated with extracts at the rate of one extract per test batch. During this period, the animals had free access to food and water and were maintained at a constant temperature of 22 ± 2°C and subjected to a 12/12-hour light/dark cycle. The rats divided into three groups thus acclimated were subjected to the administration of the extracts. The plant extracts to be tested being administered orally, the method described in OECD guideline 423, method by toxicity class, was adopted. A single dose of 2000 mg/kg of body weight was carried out. Twelve hours before carrying out this toxicity test, the rats were deprived of food and water. At the start of the experiment, a blood sample was taken from all rats at the level of the retro-orbital sinus for analyzes of hematological and biochemical constants before administration of the product. Then, the extracts were administered by esophageal gavage in accordance with the selected dose and a period of 14 days was observed to collect data on clinical signs of toxicity (morbidity, mortality, hair loss, eye color, breathing difficulties). At the end of the experiment (D14), a blood sample was again taken for analyzes of hematological and biochemical constants.The essays were triplicated and the SPSS 26.0 software is used to calculate the toxicity degree.

In vitro anti-radicaland antioxidant tests

Anti-radical tests

The DPPH (2,2-Diphenyl-1-picrylhydrazyl)free radical scavenging assay is used for the evaluation of antioxidant activities of medicinal plant extracts[23]. 100 µL of different concentrations of each extract is added to 1900 µL of the ethanolic solution of DPPH (0.4 mg/ mL). The blank is prepared by mixing 100 µL of the extraction solvent with 1900 µL of the DPPH solution. After incubation in the dark for 1 hour at room temperature, the absorbance reading was carried out at 517 nm using a MINDRAY type spectrophotometer (BA-88-A). Vitamin C and BHT were used as reference standards. Samples were prepared in triplicate for each analysis. The percentage of trapping of the DPPH radical was determined. The data generated from the tests performed were subjected to statistical analysis using SPSS 26.0 software. Quantitative variables are presented as mean and standard deviation. Analysis by the probity method was used to determine the 50% inhibitory concentration (IC50) of the DPPH radical.

Antioxidant test

The reducing power of a plant extract is one of the techniques used to evaluate its antioxidant potential. In this study, this reducing power of the extracts was determined by the FRAP method (Ferric Reducing Antioxidant Power)[29]based on the chemical reaction of reduction to Fe 2+ of the Fe 3+ ion present in the K3Fe (CN)6 complex. Thus 0.5 mL of extract at different concentrations is mixed with 1 mL of phosphate buffer (0.2 M; pH = 6.6) and 1 mL of potassium hexacyanoferrate [K3Fe (CN)6] at 1%. After incubating the mixture at 50°C for 30 minutes, 1mL of 10% trichloroacetic acid was added, then the tubes were centrifuged at 3000 rpm for 10 minutes. Then, 1 mL of the supernatant from each tube is mixed with 0.2 mL of a solution of FeCl 3 (0.1%) and left to stand protected from light for 30 minutes. Optical densities were read at 700 nm. Samples were prepared in triplicate for each analysis. The antioxidant activity linked to the reducing power of the extracts is determined by the following formula: PR = 100 (Aa - Ab) /Aa (Aa isthe absorbance of the extract, Ab is the absorbance of the white, PR: Percentage reduction. The IC50 concentration is calculated to express and compare the power of the reducing capacities of the different extracts.

Qualitativeand quantitative phytochemical screenings

Qualitative screening

Qualitative phytochemical screening is based on the differential reactions (coloring and precipitation) of the main groups of chemical compounds contained in the products according to the classic method [30]and which is widely used in the literature with success[22, 23]. This analysis involves searching for 18 chemical groups.

Quantitative screening

Quantifications of total polyphenols, total flavonoids and total tannins are assays realized.

Total polyphenols in the different extracts of the plants werequantified by the commercial FolinCiocalteu reagent. The FolinCiocalteu reagent, consisting of a mixture of phosphotungstic acid (H3PW12O40) and phosphomolybdic acid (H3PMO12O40) reducing method [31] using the reducing during the oxidation of phenols to a mixture of blue oxides of tungsten and molybdenum.

The total polyphenol contents were determined using the equation from the gallic acid calibration curve (0-200 µg/mL) taken as a reference standard. Samples were prepared in triplicate for each analysis. The content of total polyphenols was determined in mg of gallic acid equivalent/g of extract (mg EAG/g)[32].

The quantification of flavonoids from the plant extracts studied was carried out by conventional methods adapted from[33, 34]. The total flavonoid content was determined in mg of rutin equivalent/g of extract (µg RuE/g) [32]

For total tannins determination, the condensed tannins content was determined by the vanillin method [35]. Different concentrations between 0 and 1000 µg/mL prepared from a stock solution of catechin will allow the calibration curve to be drawn. The calibration curve Y = 0.1165X + 0.00003 with R2 = 0.9996 plotted using the tanic acid stock solution as a standard.

Dosage of minerals

The samples were heated to 150˚C in a mixture of sulfonitric acid. After solubilization, the samples were filtered with filter paper. The determination of Calcium, Magnesium, Iron and Zinc was carried out in the filtrate recovered using an iCE 3000 series atomic absorption spectrometer[20].

Results:-

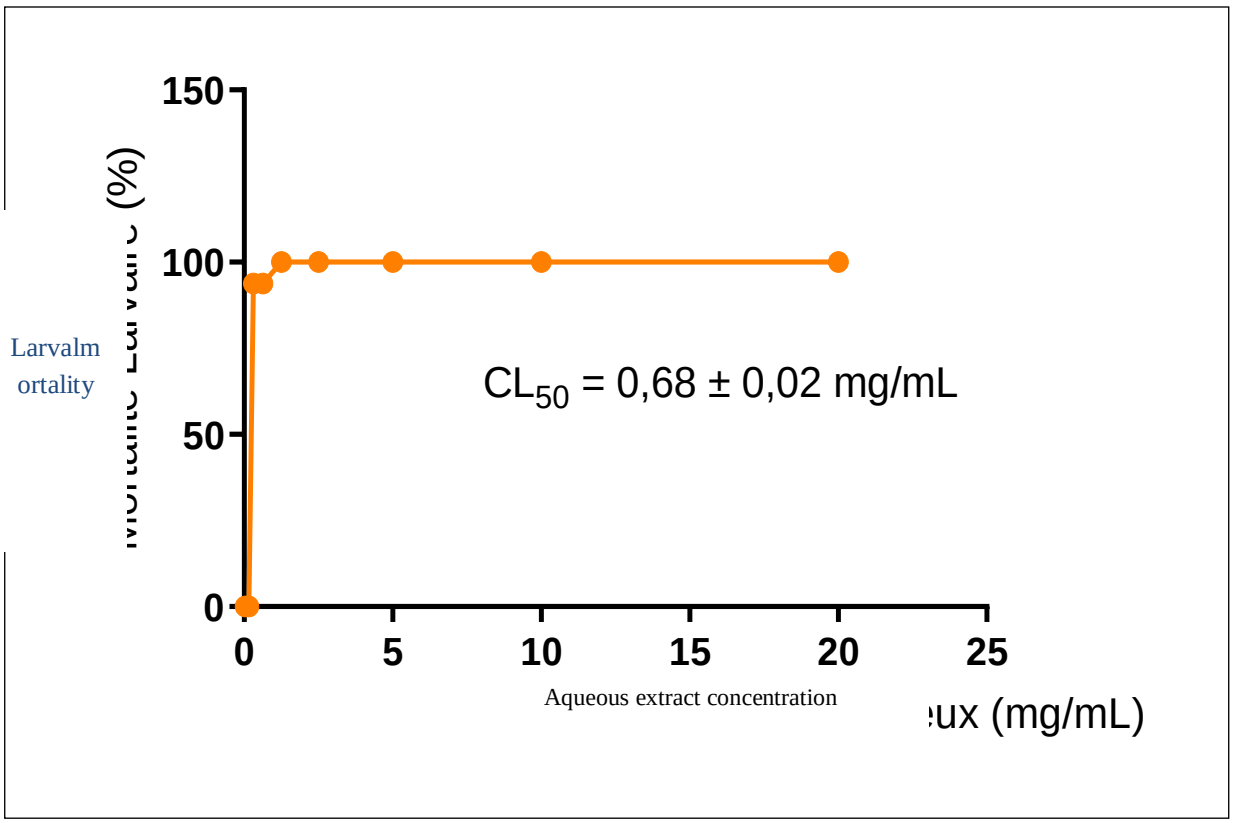
Extraction yield

Aqueous extraction made it possible to obtain 15.9 g of concentrated material, a yield of 15.9%. As for the hydroethanolic extraction, it made it possible to obtain 17.4% of concentrated material, i.e. a yield of 17.4%.

Larval toxicity of *Chamaecrista rotundifolia* on shrimp (*Artemia salina*) larvae

Figure 1 shows the results of the toxicity test of the aqueous extract of *C. rotundifolia* on *Artemia salina* larvae. These resultsshow that the lethal concentration which causes 50% mortality (LC 50) of the larvae is 0.68 ± 0.02 mg/ mL.

Figure 2: - Sensitivity *of Artemia salina* larvae to the aqueous extract of *Chamaecrista rotoundifolia.*



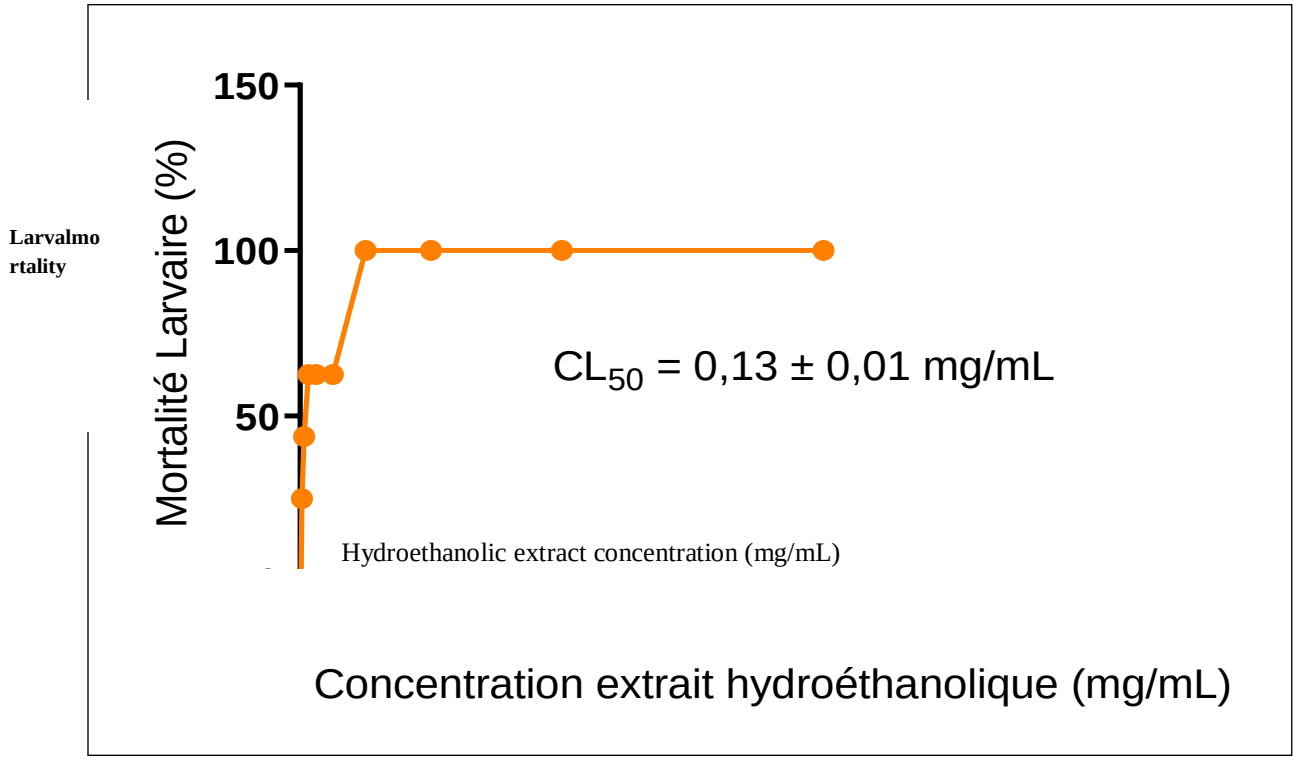


Figure 3:- Sensitivity *of Artemia salina* larvae to the hydroethanolic extract of *Chamaecrista rotoundifolia.*

Acute oral toxicity of *Chamaecrista rotundifolia*

Weight gain

Figure 3 shows the weight change of the Wistar rats over the 14 days of the animal experiment and Figure 4 shows the weight gain of the Wistar rats.

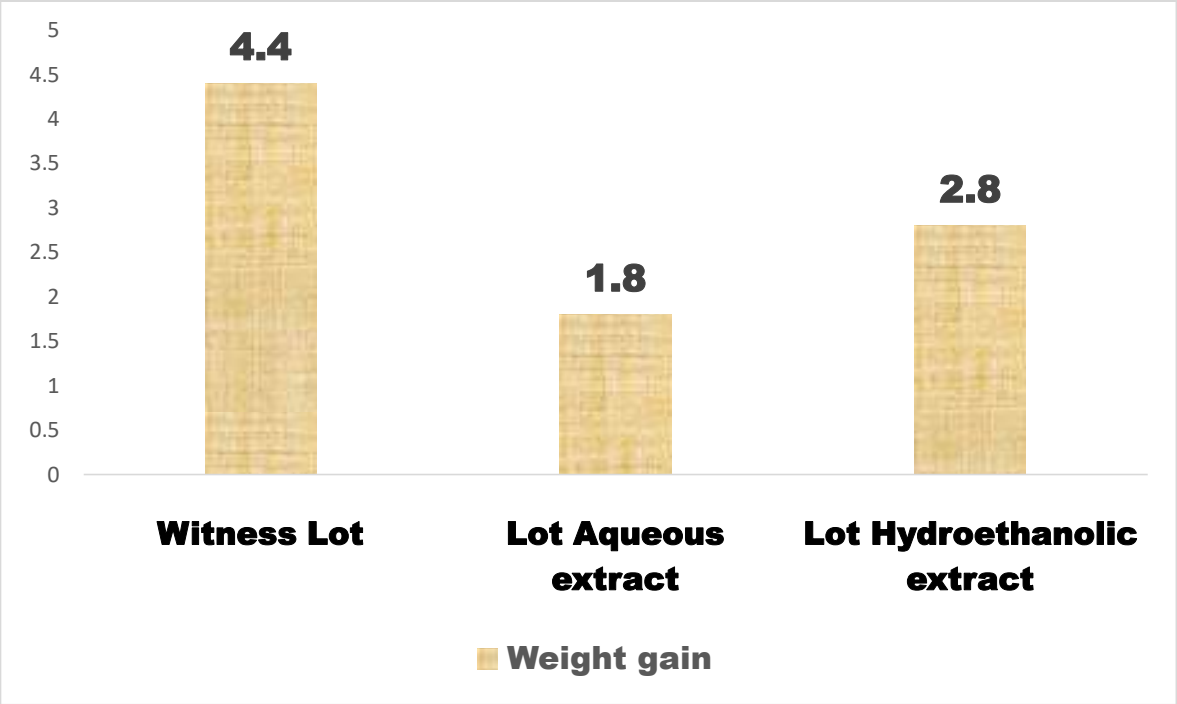


Figure 4:- Effect of products on the weight load of rats.

Influence of the extracts tested on the hematological parameters of Wistar rats

Table 2 summarizes the statistical analysis of the data relating to the hematological parameters of rats from the different batches of rats. The parameters considered are: White blood cell, Red blood cell, Hemoglobin, Average corpuscular volume, Average corpuscular hemoglobin concentration. The various results show no significant difference in the various parameters between day zero (Start of the experiment) and day fourteen (End of the experiment) for the control batch as well as for the experimental batches having received a dose of aqueous extract or hydroethanolic.

The data relating to the biochemical parameters of Wistar rats are presented in Table 3 The parameters considered are: Aspartate Aminotransferase, Alanine- Amino -Transferase, GlutamoPyruvate Transferase, and GlutamoOxaloacetate Transferase. The various results show no significant difference in the various parameters between day zero (Start of the experiment) and day fourteen (End of the experiment) for the control batch as well as for the experimental batches having received a dose of aqueous extract or hydroethanolic.

Table 2:- Effect of extracts on hematological parameters of Wistar rats.

Setting	**No**	**witness**			**Aqueous extract**			**Hydroethanolic extract**			
		Avg	**E**	**sample Student 's t test at the 5% threshold (P)**	Avg	**E**	**sample Student 's t test at the 5% threshold (P)**	Avg	**E**	**sample Student 's t test at the 5% threshold**	**ANOVA test between batches on D14 at the 5% threshold**
GB J0	3	7.47	0.67	0.417	6.93	0.29	0.796	9.60	1.13	0.700	
GB J14	3	6.97	0.57		7.27	1.68		6.45	1.63		

GR J0	3	7.78	0.67	0.100	7.23	0.67	0.207	7.53	0.67	0.299	P > 0.05 No significant difference for each of the parameters following the comparison between the control batch and the test batch on D14
GR J14	3	6.69	0.12		6.63	0.20		6.43	0.11		
HB J0	3	14.33	0.81	0.916	14.20	1.54	0.081	13.75	1.20	0.686	
HB J14	3	14.40	0.17		13.43	1.87		14.45	0.64		
HCT J0	3	39.43	1.80	0.139	36.63	3.40	1.00	40.25	1.48	0.248	
HCT J14	3	35.23	1.32		36.63	2.46		32.10	6.22		
VGM J0	3	51.83	2.14	0.076	52.63	1.97	0.4	53.65	2.90	0.751	
VGM J14	3	52.77	1.89		53.50	1.87		54.35	5.30		
MCH J0	3	20.47	0.31	0.121	20.87	0.25	0.398	21.05	0.35	0.217	
MCH J14	3	21.53	0.51		21.10	0.40		22.60	0.42		
CCMH JO	3	37.70	1.73	0.101	37.50	1.35	0.255	39.85	0.64	0.348	
CCMH J14	3	40.80	0.85		39.47	0.97		42.15	2.62		
PLT J0	3	765.67	15.18	0.957	759.67	15.89	0.069	750.00	33.94	0.512	
PLT J14	3	767.00	28.48		723.00	13.45		776.00	4.24		

Table 3:- Effect of extracts on renal and hepatic parameters of Wistar rats.

Settings	No	witness			Aqueous extract			Hydroethanolic extract			
	3	Avg	E	Sample Student 's t - test matched at the 5% threshold **(P)**	Avg	E	Sample Student 's t - test matched at the 5% threshold**(P)**	Avg	E	Sample Student 's t - test matched at the 5% threshold**(P)**	ANOVA test between batches on D14 at the 5% threshold
Uremia (mg/dl) D0	3	0.69	0.08	0.377	0.72	0.12	0.379	0.65	0.04	0.156	P > 0.05 No significant difference for each of the parameters following the comparison between the batches on D14
Uremia (mg/dl) D14	3	0.64	0.02		0.64	0.03		0.69	0.05		
Creatinemia (mg/dl) D0	3	5.39	1.31	0.051	5.58	0.33	0.702	6.55	0.49	0.479	
Creatinemia (mg/dl) D14	3	6.40	1.29		5.62	0.47		5.59	0.78		
AST/GOT (U/L) J0	3	82.67	11.93	0.821	82.00	5.20	P = 0.319	86.50	0.71	0.093	
AST/GOT (U/L) J14	3	81.31	3.92		78.77	4.77		72.46	2.22		
ALT/GTP (U/L) J0	3	50.66	5.81	0.997	47.87	6.42	0.680	49.47	3.74	0.511	
ALT/GTP (U/L) D14	3	50.67	4.73		48.33	5.03		52.50	0.71		

Antioxidant activity

DPPH test

Table 4 presents the data relating to antioxidant activity by the DPPH test. From this table, it appears that all the extracts produced an inhibitory effect on the DPPH radical. However, the effect of the hydroethanolic extract is significantly better compared to that of the aqueous extract. The reference standard (Vitamin C) produced a significantly better effect than the two extracts.

Table 4:- Anti-radical effect of extracts and reference standard.

Settings	Aqueous extract (10 mg/ mL)	Hydroethanolic extract (5 mg/ mL)	VITAMIN C (1mg/ mL)
% inhibition of the DPPH radical	76.05	71.44	98.95
IC_{50} in mg/mL	3.15 ± 0.04	2.12^{a} ± 0.11	0.1a ,b±0.001

FRAP test

Table 5 presents the ferric reducing activity of the products tested. From this table, it appears that the extracts tested presented ferric reducing activity in different proportions.

Table 5:- Ferric reducing effect of extracts and reference standard.

Settings	Aqueous extract (10 mg/ mL)	Hydroethanolic extract (5 mg/ mL)	VITAMIN C (2 mg/mL)
% ferric reduction	21.73	22.27	80.72

Phytochemical screening

Qualitative tests

Table 6 presents the screening results of the major phytochemical groups. A total of eighteen (18) groups were subject to the qualitative assay. Anthocyanins, flavonoids, steroids, catechin tannins are groups present both in aqueous extracts and in hydroethanolic extracts.

Table 6:- Large group of phytochemicals present in Chamaecrista rotundifolia extracts.

Chemical groups	**Hydroethanolic extract**	**Aqueous extract**
Alkaloids	-	+
Anthocyanins	+	+
Free anthracenics	-	-
C- Heterosides	+	-
Reducing compounds	-	+
Coumarins	-	-
Cardiotonic derivatives	-	-
Cyanogenic derivatives	-	-
Quinone derivatives	-	-
Flavonoids	++	++
Leuco- anthocyanins	-	+
Mucilages	-	++
O- Heterosides	+	-
Saponosides	-	++
Steroids	+	+
Catechical tannins	++	++
Gallic tannins	-	++
Triterpenes	-	+

Content of the tested extracts in total polyphenols, total flavonoids and condensed tannins

The aqueous extract contains polyphenols, flavonoids, and condensed tannins with respective concentrations of 36.79 ± 1.15mg EAG/g, $5.66.10^{-2}$ ± 0.001mg RuP /g, $26.72.10^{-2}$ ± 0.018mg CaP /g. The hydroethanolic extract also contains polyphenols, flavonoids, and condensed tannins with respective concentrations of 71.09 ± 2.01mg EAG/g, $12.57.10^{-2}$ ± 0.002mg RuP /g, $37.82.10^{-2}$ ± 0.02 mg CaP /g

Table 7:- Content of total polyphenols, total flavonoids and total tannins.

Extracts/Contents	Total polyphenol content (mg EAG/g)	Total flavonoid content (mg RuP /g)	Condensed tannin content (mg CaP /g)
Aqueous extract	36.79 ± 1.15	$5.66.10^{-2} \pm 0.001$	$26.72.10^{-2} \pm 0.018$
Hydroethanolic extract	$71.09^{to} \pm 2.01$	$12.57^{to}.10^{-2} \pm 0.002$	$37.82^{to}.10^{-2} \pm 0.025$

a: significantly different from the aqueous extract at the 5% threshold

Mineral content

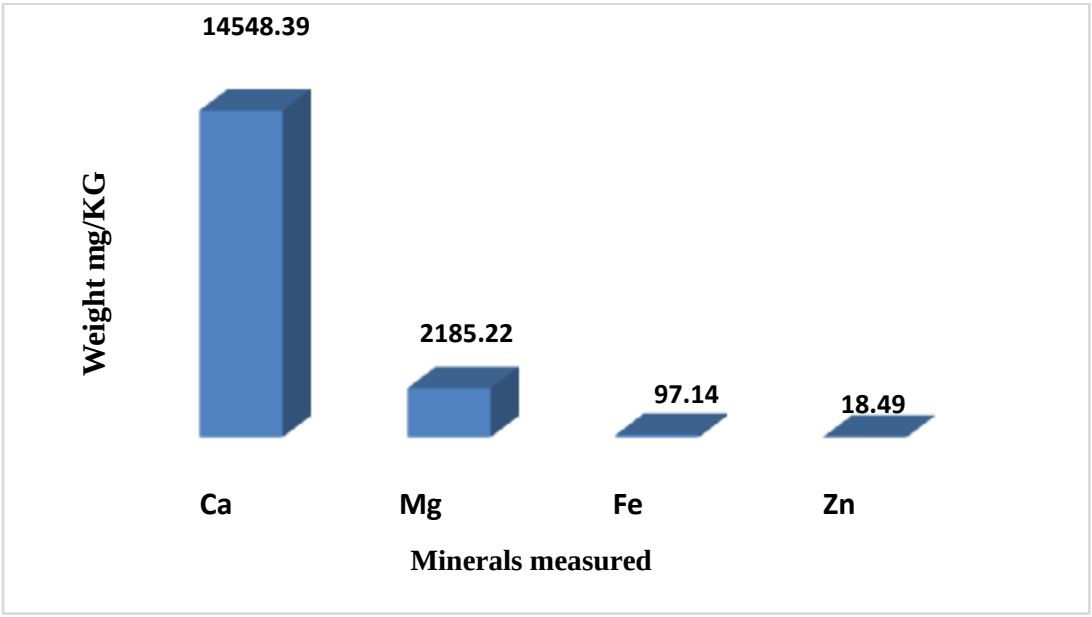


Figure 5:- Mineral content.

Discussion:-

The study of safety allows us to assess the degree of toxicity of an extract. This study is important from the perspective of formulating a phytomedicine because, even if a drug is effective, it must be non-toxic. In the present study, preliminary toxicity on shrimp larvae was evaluated. Acute toxicity on Wistar rats was evaluated. The LC_{50} obtained is respectively 0.68 mg/ mL for the aqueous extract and 0.13 mg/ mL for the hydroethanolic extract in the larval toxicity test. These $CL_{50\ values}$ reported on the Mousseux scale allow us to conclude that the products tested are not cytotoxic (because $CL_{50} \geq 0.1$ mg/ mL) at the concentrations tested. The LC_{50} obtained show that the hydroethanolic extract would potentially be more toxic at high concentrations (0.68 mg/ mL > 0.13 mg/ mL). The absence of toxicity of this plant could be confirmed by its use as fodder[36]. In the literature, no cases of larval toxicity have been reported for this plant. Oral administration of the tested extracts did not induce rat mortality. Their average lethal dose (DL_{50}) is therefore greater than 2000 mg/kg/BW. The results show a weight growth of rats in all batches during the 14 days of the monitoring period. However, no significant difference in weight gain was noted between the control batch and each of the test batches. This suggests that extract treatment does not exert any negative influence on the development of body weight in Wistar rats.

The analysis of the data relating to the hematological parameters of the rats from the different batches shows that the products tested had no significant effect on the different constants of the parameters of the leukocyte and erythrocyte lines ($p>0.05$). The extracts tested therefore did not induce anemia in the experimental rats.In addition, from the analysis of data relating to the biochemical parameters of Wistar rats, it appears that at the threshold of 5%, no significant influence was noted for both the biochemical parameters of the kidneys (urea and creatinine) and those liver (ALT, AST) ($p>0.05$). The extracts tested therefore did not affect the functions of the kidneys and liver of rats. The absence of toxicity of the extracts helps to lay the foundations for the formulation of a phytomedicine. However, studies have shown that this species has high lead and cadmium content[37, 38, 39]. This shows that prolonged consumption could lead to health consequences through chronic intoxication. If the present study showed that extracts of *Chamaecrista rotundifolia*are not toxic, another species of the same genus is. Indeed, *Chamaecrista mimosoides*(L.) Greene contains toxic principles that can cause muscular and neurological damage when consumed

by livestock in large doses[40]. *Chamaecrista fasciculata*, another species of the genus is said to be toxic to humans[41].

Antioxidants are naturally present in plants and prevent free radicals from oxidizing our body's cells. Thanks to them, it is possible to break the chain of creation of free radicals and therefore limit or even stop the degradation and premature aging of cells. They increase immunity. The aqueous and hydroethanolic extracts of *C. rotundifolia*presentedanti-radical activity. This activity would be due to the phytochemical compounds revealed by the phytochemical screening. The aqueous (10 mg/ mL) and hydroethanolic (5 mg/ mL) extracts of C. rotundifolia presentedan anti-radical activity with respectively IC 50 =3.15, and CI 50 =2.12 while vitamin C (1 mg/ mL) has IC 50 = 0.1. These results show that the hydroethanolic extract (5 mg/ mL) has better anti-radical activity. However, both extracts exhibit low free-radical scavenging activity. These results are similar to those of Hamid et al 2023 where the activityantioxidant(usingtrappingof theradical1.1 - diphenyl2 - picrylhydrazyl (DPPH))hasrevealedthatthe extractacetateethylof *C. rotundifolia* has weak antioxidant properties. However, the extractof methanol presentactivityantioxidantwithavalueIC 50of5µg/ mL,comparablehasthatofascorbic acid,2.55µg/ mL. These results show that the antioxidant activity of *C. rotundifolia* extracts depends on the type of solvent and therefore on the compounds available in the extract.

Data relating to antioxidant activity by the testof FRAP show that the extracts tested present a ferric reducing activity in different proportions and lower than that of the reference molecule, vitamin C. These results show the same trend as those obtained with the test using the DPPH radical

The biological and pharmacological activities of plant extracts are linked to their phytochemical compounds. It appears from the work that the hydroethanolic extract contains six (06) compounds compared to eleven (11) for the aqueous extract. Four (04) compounds are common to them: anthocyanins, flavonoids, steroids and catechictannins. The difference observed in the extracts would be due to the different solvents used. The anti-radical activity recorded would be due to the presence of different chemical groups, especially polyphenols. The different compounds are known for their pharmacological activities[42,43,44]. The works ofMogode, (2005)showthat*Cassia nigricans* and *Cassia alata* contain compounds found in *C. rotundifolia*. This is the case of flavonoids which are recognized for their antimicrobial activity. Aqueous extracts of *Cassia siamea*Linn&Bar, a species of the same genus, are rich in flavonoids, tannins, sterols and triterpenes which are known for their analgesic, anti-inflammatory and antioxidant properties. Species of the *Cassia genus* appear not to be rich in alkaloids[45,46]. *C. rotundifolia* extracts did not have antimicrobial activity despite the similarity in chemical composition with other species of the same genus. This could be explained by a difference in concentration of the different compounds which could also be linked to an ecotypic difference.

Quantitative dosages of polyphenols, flavonoids and total tannins confirm the presence of these compounds in the aqueous and hydroethanolic extracts. These results show that the extracts are richer in polyphenols followed by condensed tannins and flavonoids. However, the hydroethanolic extract is richer in these different compounds. The ratio is approximately twice as many total polyphenols and total flavonoids in the hydroethanolic extract. These compounds have also been found in other species of the same genus[45,46].

The analysis of *Chamaecrista rotundifolia* powder revealed that this plant contains minerals such as calcium, magnesium, iron and zinc in different proportions with concentrations respectively of 14548.39 mg/Kg, 2185.22 mg /Kg, 97.14 mg/Kg, 18.49 mg/Kg. It actually contains more calcium. Trace elements (trace elements) are essential for many biological and physiological functions of the body. They are provided by food or artificial nutrition[47]Calcium plays a key role in skeletal mineralization and structure. It is necessary for many biological functions such as muscle contraction, blood clotting, hormone release and even enzyme activation. Calcium is essential for the formation of bones and teeth, the contraction of muscles, the normal functioning of many enzymes, blood clotting and normal heart rhythm. It is also a potential central regulator in skin wound healing [48]. The importance of calcium on the skin is undeniable[49]. The body precisely controls the amount of calcium in cells and blood. To maintain normal blood calcium levels without weakening bones, you need to consume at least 1,000 to 1,500 mg of calcium per day. The normal functioning of many enzymes in the body also depends on magnesium. It is also linked to calcium metabolism and potassium metabolism[50]

Conclusion:-

The study of the larval toxicity of aqueous and hydroethanolic extracts of *Chamaecrista rotundifolia* showed that these extracts show any sign of toxicity at the doses tested. The same applies to the study of subacute oral toxicity, the results of which show no significant variation in blood, liver and kidney parameters. This plant could therefore be used without risk of toxicity in traditional medicine if conclusive work is done on the pharmacological aspect. The high levels of lead and cadmium in this species according to certain studies, however, show that we should be careful about the quantity and duration of consumption. The content of these heavy metals depends on the environment, it is therefore necessary to check the origin of the plant before consumption because it fixes minerals.

The aqueous and hydroethanolic extracts of *Chamaecrista rotundifolia*presentedanti-radical activity. The hydroethanolic extract (5 mg/ mL) has better anti-radical activity. However, the two extracts have low activity compared to the activity of the reference molecule. The existence of antioxidant activity with another extract assumes that the plant contains antioxidant molecules which must be isolated in order to carry out various pharmacological tests.

The qualitative analysis of the aqueous and hydroethanolic extracts showed the presence of several phytochemical compounds of which four (04) are common to them (anthocyanins, flavonoids, steroids and catechictannins). This observation shows that the component of the extract dependent on the solvent use for extraction. Very few studies have focused on the quantitative and qualitative phytochemical dosage of major groups of *C. rotundifolia* extracts. The levels of trace elements present are at values which would justify the biological properties recorded at the extract level. This study constitutes one of the first phytochemical studies on *C. rotundifolia* in Togo.

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