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CONTRIBUTION TO THE ETHNO-BOTANICAL STUDY AND THE BIOENERGETIC, COSMETIC, AND PHARMACO-BIOLOGICAL VALORISATION OF J. CURCAS L. (EUPHORBIACEAE) IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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ABSTRACT:
Jatropha curcas is a versatile plant with medicinal, energy, and cosmetic potential. The ethno-botanical survey was carried out using a stratified sampling technique. J. curcas oil was extracted using the standard method, while its characterization was carried out in accordance with Standard DIN 51605 for Rapeseed Oil Fuel. The antiviral activity was evaluated in silico using molecular docking. The study found that the majority of respondents were over 50 years old, with the leaves being the most used parts. Twenty-seven illnesses are treated, of which the 5 most treated are gastritis, cough, haemorrhoids, yellow fever, and tooth decay. The Budja and the Yaka are the only ones who reached a consensus on 6 illnesses. Physico-chemical analyses showed that J. curcas oil complies with the DIN V 51605 standard. The biodiesel obtained gave a better cetane number (109). Molecular modelling indicated that the oil inhibited 2 molecular targets involved in Monkeypox virus DNA replication. J. curcas is a plant with high medicinal, energy and cosmetic potential that should be exploited in the context of sustainable development. J. curcas can be a source of income for rural areas. Bio-clinical studies should therefore be carried out to validate the efficacy and safety of this plant in the treatment of diseases.
KEYWORDS: *Jatropha curcas*, Ethnomedicine, Biodiesel, Monkeypox, Congo Basin

1. INTRODUCTION

Since ancient times, life has been increasingly dependent on energy produced from fossil fuels (Maysa and Hamaizia 2020). Fossil fuels come from the transformation of dead organic matter mixed with various minerals buried deep in the ground for several million years. These include oil, coal, peat, and natural gas. These sources are non-renewable, requiring millions of years to replenish (Rakotoravo, 2016). Thus, the increased use of fossil fuels leads to the depletion of non-renewable natural energy sources because they are used much faster than the time needed to create reserves, but also produce the greenhouse gases (GHGs) at the root of global warming and pose a potential threat to the ozone layer that protects the planet from solar radiation (Bendriss, 2021). This is why the signatory countries of the Kyoto protocol are developing national policies aimed at reducing GHGs either through the ecological restoration of degraded ecosystems (mining sites, etc.), ex-situ or in situ conservation (REDD+ mechanism), or through the use of clean (less polluting) and affordable energy (SDG7). To this end, energy from biomass, in particular biodiesel produced from *J. curcas* oil, is an alternative to fossil fuels. In Africa and in North Ubangi province in particular, the seeds are used by households to light their homes at night. In addition, this plant genetic resource is widely exploited in traditional medicine to treat various diseases, and its anti-COVID-19 activity has been validated *in silico* (Kambu *et al.*, 2009, Kitete *et al.*, 2022). Choosing *J. curcas* as an essential plant species for the creation of a productive ecosystem is therefore a better strategy for any development project in poor rural and peri-urban areas for several major reasons: firstly, the technology for producing biodiesel from *J. curcas* oil has been known for many years and technology transfer is easier. What's more, *J. curcas* has a number of advantages, as it can grow in arid and semi-arid regions. It requires little water or fertilizer and can survive in sterile soil. It is also resistant to pests and has a high seed yield, with a lifespan of 30 to 40 years, and the oil content of *J. curcas* seeds is around 30 to 40 (Fatma *et al.*, 2020).

Indeed, *J. curcas* oil is advantageous in the production of biofuel as a substitute for fossil diesel. This oil can also be used as a lubricant and fuel for diesel engines or trans-esterified into biodiesel with glycerine production as a by-product (Samson, 2013). The trans-esterification of oils is usually carried out with methanol in the presence of basic homogeneous catalysts such as soda or potash. This homogeneous catalysis is effective at low temperatures (Hamad, 2009). This C sink is also a source of raw material for the manufacture of phyto-medicines. The various parts of *J. curcas* (leaves, roots, latex, bark, and seed) are traditionally used in medicinal preparations in Africa, Asia,

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and Latin America (Kambu *et al.*, 2009). With this in mind, an ethno-botanical survey of the various diseases treated by *Jatropha* was launched in Kinshasa.

Hypothesises

- The people of Kinshasa regard *J. curcas* as a plant with multiple medicinal and traditional uses, which could be justified by the fact that its various parts contain secondary metabolites that could justify their use in traditional medicine;
- *J. curcas* seed oil from the *Ubangian ecoregion* has a good yield for bioenergy use;
- This oil contains a chemical compound that forms a thermodynamically stable complex with some key receptors in Monkeypox viral pathogenesis.

Aim: The aim of this study was to assess the ethno-medical knowledge of *J. curcas* in Kinshasa in the Democratic Republic of Congo (DRC).

▪ **Specific objectives**

To achieve our aim, we set the following specific objectives:

- Evaluate the influence of socio-demographic parameters on ethno-botanical data;
- Synthesise and characterise *J. curcas* oil-based biodiesel;
- Study *in silico* the interaction of several compounds contained in *J. curcas* oil with 2 proteins involved in viral DNA replication (MPX).

The present study is of obvious interest because, in accordance with the Kyoto protocol, it constitutes a contribution to the national effort for sustainable development through the use of clean energy that is better adapted to poor environments, with the aim of creating a productive ecosystem and ecological restoration using *J. curcas*. If the antiviral properties of *J. curcas* can be validated *in silico*, a pharmaceutical formulation based on this oil would pave the way for preclinical, toxicological and clinical studies (efficacy and safety) against this virus in endemic regions. An approach based on the development of a value-added oleaginous and medicinal plant could also enable the effective implementation of the access and benefit-sharing process in accordance with the Nagoya protocol in the DRC.

2. MATERIALS AND METHODS

2.1. Study area

In this study, ethno-botanical surveys were carried out in 3 communes of the city of Kinshasa, namely the commune of Limeté (7th industrial street), the commune of Masina (quartier 2), and the commune of Mont-Ngafula in Kindele district (Figure 1).

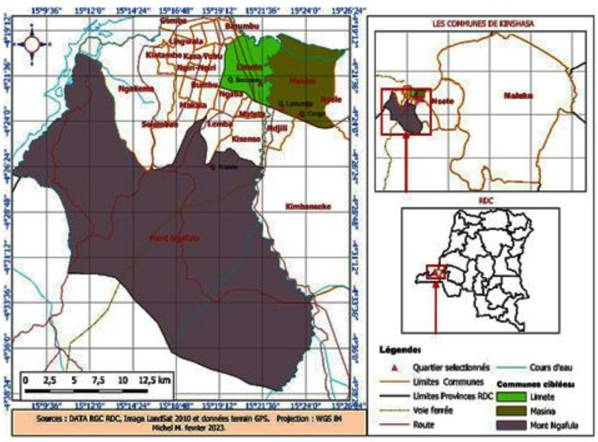

Figure 1. Geographical location of survey sites

2.2. Methods

The ethno-botanical surveys were carried out in 3 communes of the provincial city of Kinshasa using the method of Mongeke *et al.,* (2018). The survey was carried out using a questionnaire containing data on the informant, the name of the plant species; the part used the preparation methods, and the therapeutic and traditional use, on the basis of the free consent of the respondents. The survey was carried out between Nov. and Dec., 2022. Once the survey forms had been tabulated, the data recorded on the survey forms were processed and entered into Excel.

They were analysed using SPSS version 20 software. Frequency curves were obtained using Origin 8.5 Pro software. The sample size was determined using the Dagnelie relationship:

$n = \frac{Z^2 p(1-p)}{\varepsilon^2}$, with Z = 1 96;

p is the proportion of people with knowledge of the plant (at the time of the preliminary survey); Ɛ is the tolerated margin of error (5%). Univariate analyses were carried out on the qualitative variables (socio-demographic and ethno-botanical variables) to obtain descriptive statistics (relative frequencies). The Kolmogorov-Smirnorv test was used to confirm or refute the normality of the distribution of the quantitative variables (age of respondents). Bivariate analyses (Chi-square test) were used to assess the influence of the socio-demographic parameters of the respondents on their ethno-medical knowledge and the availability of the plant in the community. Multivariate analyses (principal component analysis, hierarchical ascending classification and multiple component analysis) were used to assess the degree of correlation between socio-cultural groups, the parts of the plant (*J. curcas*) used and the diseases treated. The informant consensus factor was calculated as previously described in our previous work (Ngbolua *et al*., 2016; Masengo *et al*., 2023).

▪ **Bioenergetics**

The plant material used in this section consists of *J. curcas* seeds. They were harvested at Gbado-Lite in the province of Nord-Ubangi in the Democratic Republic of Congo. The seeds were dried in an oven (Memmert) at a temperature of 105 °C. After drying, they were de-hulled by hand and then ground using a grinder (speed grinder 500A) to obtain pasty flour.

The *J. curcas* oil was extracted using a hot Soxhlet extractor (Figure 2C).

Figure 2. A. J. curcas seeds; B. J. curcas seed powder; C. Oil extraction device (Soxhlet); D. J. curcas oil.

The method used was that of Boulghiti and Hachemi (2020) using 400 g of seed powder for an extraction time of 6 h. The extraction solvent (petroleum ether) was removed using a rotary evaporator at 60 °C and then kept in an oven at 60 °C for 48 h. The yield of oil extracted is calculated by dividing the mass of oil obtained by the mass of seeds multiplied by 100 (Bendiaf, 2018).

Physico-chemical analyses of *J. curcas* oil

- ✓ **Acid index (NFT 60-204)**

The acid number (AI), i.e. the number of mg of (KOH) required to neutralise the free fatty acids present in 1 g of fat, is determined from the principle of dissolving the fat in a solvent (diethyl ether + ethanol) followed by titration of the fatty acids with a solution of alcoholic potash in the presence of phenolphthalein as a colour indicator.

Briefly, 0.5 g of oil is dissolved in 100 mL of 95% ethanol, then the mixture is titrated with a 0.5 N ethanol KOH solution in the presence of 2 to 3 drops of phenolphthalein until a persistent pink colour is obtained. The acid number is calculated from the formula below:

$$AI = \frac{56.1 \times V \times C}{m}$$

Where 56.1: is the molar mass (g/moles) of potassium hydroxide; V: is the volume (mL) of potassium hydroxide used; C: is the exact concentration, in moles per litter, of the KOH standard solution used; m: is the mass in grams of the test sample (Mostefa, 2011).

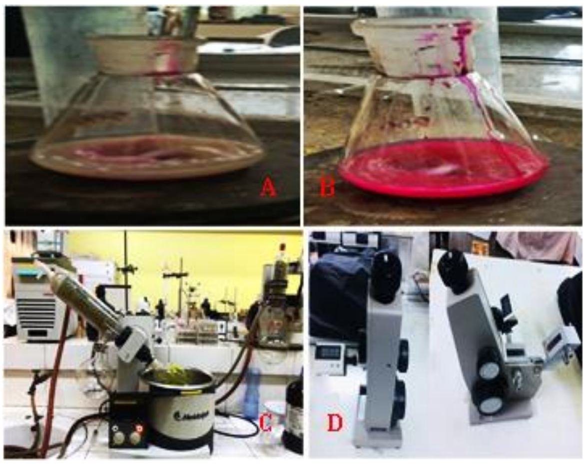

Figure 3. A and B. Oil titration with KOH; C. Heldolph rotavapor; D. Carl Zeiss refractometer.

- ✓ **Saponification index (NFT 60-206)**

The saponification index (SI) is the quantity of potash (KOH) expressed in mg required to saponify 1 g of fat (Alloune and Tazerout 2012). The saponification index (SI) provides information on the behaviour of the oil, revealing whether the oil is more or less easily saponifiable, i.e. whether it will form more esters or more soap (Rhiad, 2017). The oil sample is saponified by refluxing with an excess of alcoholic KOH solution followed by titration with HCl (Ministry of Commerce. 2011). IS is calculated by the formula:

$$SI = \frac{(V0 - V1) \times C \times 56.1}{m}$$

Where V_ (0): is the volume, in ml, of the hydrochloric acid used for the blank test; V_1: is the volume, in millilitres, of the hydrochloric acid used for the determination; C: is the exact concentration, of HCl; m: is the mass, in g, of the test sample (Ministry of Commerce, 2011).

- ✓ **Iodine index**

The iodine value is the number of g of iodine fixed per 100 g of vegetable oil and provides information on the degree of unsaturation of the fat. To prepare solution A, the alcoholic solution of iodine and $HgCl_2$ is mixed to equal volume 24 h in advance. 0.5 g of oil is dissolved in 10 ml of CCl_4 and then 25 ml of solution A is added. The control solution consisted of 10 ml CCl_4 and 25 ml A. The mixture was incubated in the dark for 18 h, and then 20 ml of 10% aqueous KI solution and 400 ml of distilled water were added until dissolution and formation of an orange precipitate.

The mixture was then titrated with a solution of ($Na_2S_2O_3$, N/10) in the presence of starch until complete decolourisation as previously described by Mactar (2009). The iodine value is calculated by the equation: $IV = \frac{(Vb - Ve) \times 0{,}1 \times 12{,}69}{Pe}$;

Where: Vb: Volume of $Na_2S_2O_3$poured into the blank; Ve: Volume of $Na_2S_2O_3$poured into the sample; Pe: Test sample.

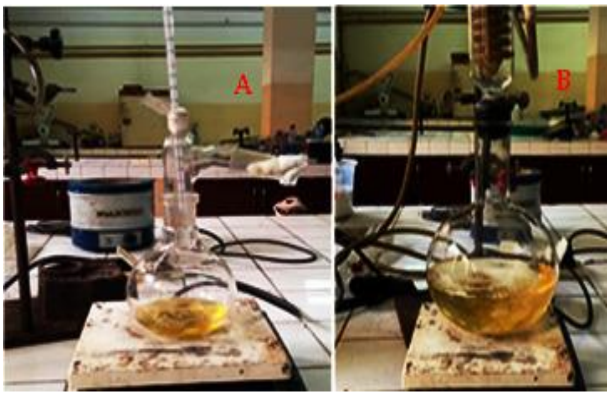

Figure 4: Trans-esterification reaction of J. curcas oil.

- ✓ **Ester index**

The ester number value is calculated as the difference between the saponification number and the acid number (Kerras *et al*., 2018).

Ester index = *IS* – *IA*

- ✓ **Density (NFT 60-214)**

Density is the ratio of the density of oil to that of water under fixed pressure and temperature conditions (Rhiad, 2017). In this study, measurements were carried out at 20 °C (Kerras *et al*., 2018). For this purpose, a 10 ml pycnometer is weighed empty (m_0) and then filled with water and weighed (m_1), then it is filled with oil and its weight is determined (m_2). The density is given by : $D^{20} = \frac{m_2 - m_0}{m_1 - m_0}$, where $\boldsymbol{m_0}$: mass of empty pycnometer ; $\boldsymbol{m_1}$: mass of the pycnometer filled with distilled water and $\boldsymbol{m_2}$: mass of pycnometer filled with *J. curcas* .

- ✓ **Viscosity**

Viscosity is the resistance to flow and is measured using a viscometer. A stopwatch is used to measure the time taken for the oil to travel the distance between the 2 lines. The viscosity is then given by the formula: $\mathbf{v} = \frac{V}{d \times t}$; where **v** : is the kinematic viscosity ($mm^2 . S^{-1}$); **V** : oil volume (mm^3); **d** : distance between the 2 lines (mm) ; **t** : the time required for the oil to flow between the lines.

- Refractive index (NFT 60-212)

The refractive index of *J. curcas* oil is evaluated according to the ISO 6320 method at 20 °C using a Carl Zeiss refractometer, thermostatically controlled as previously described by Mostefa (2011). The higher this index, the more the liquid deflects the light passing through it. This index is calculated from the relationship (at 20 °C): $\eta^t_D = \eta^{t'}_D + 0.00035\ (t' — t)$ si t'>t (1) ou $\eta^t_D = \eta^{t'}_D + 0.00035\ (t — t')$ si t'< t (2).

✓ **Cetane index**

The cetane number is calculated from the iodine number and the saponification number of the esters obtained experimentally as follows: $CI = 46{,}3 + \frac{5458}{x} - 0{,}225 * y$; with x = iodine index and y = saponification index (Ramanamihaja, 2006).

✓ **Preparing and characterising biodiesel**

Two hundred and sixty-five g of *J. curcas* oil was placed in a flask. KOH of 2.65 g (1% by weight) previously dissolved in 61.97 g (6 moles) of methanol was then added to the *J. curcas* oil. The mixture was heated to 65 °C with stirring at 120 rpm. After 120 min of reaction, the mixture was cooled to room temperature before being transferred to a separating funnel and left to stand overnight.

✓ ***In silico* molecular modelling**

Molecular modelling is carried out according to the protocols previously described by several authors (Chih-Hao Lu *et al*., 2012; Yu-Feng Lin *et al*., 2016; Murail *et al*., 2021; Chih-Hao Lu *et al*., 2022).

✓ **Ethical issues**

The research protocol for this study was approved by the Ethics Committee of the Life Sciences Department of the University of Kinshasa (CDB/DB-Sci-UNIKIN/PMM 007/2023). The study complied with the principles of the Declaration of Helsinki (free consent of respondents, etc.). All the rules of confidentiality and ethics as well as the rules of access and benefit sharing (ABS) linked to the use of plant genetic resources in force in the Democratic Republic of Congo and the code of ethics of the International Society of Ethnobiology (ISE 2006) were respected in this study. Respondents were informed that participation in the survey is voluntary and not subject to any coercion. They were informed that the results of the study would be returned to them in the form of open-access articles for dissemination by local leaders.

3. RESULTS AND DISCUSSION

3.1. Socio-demographic characteristics

Table 1 gives the socio-demographic parameters of the surveys.

Table 1. Socio-demographic parameters of respondents

Socio-demographic parameters		Frequency	%
1. Age group			
	18-35 years	41	27.3
	36-50 years	50	33.3
	>50 years	59	39.3
	Total	150	100.0
2. Sex			
	Females	74	49.3
	Males	76	50.7
	Total	150	100.0
3. Tribes			
	Budja	7	4.7
	Luba	26	17.3
	Mboma	1	0.7
	Mbunda	2	1.3
	Mongo	11	7.3
	Murega	2	1.3
	Mushi	2	1.3
	Nande	3	2.0
	Ndibu	3	2.0
	Ngbaka	5	3.3
	Ngbandi	8	5.3
	Ngombe	2	1.3
	Nianga	9	6.0
	Pende	6	4.0
	Songe	11	7.3
	Suku	8	5.3
	Tandu	4	2.7
	Yaka	22	14.7
	Yansi	6	4.0
	Yombe	9	6.0
	Zombo	3	2.0
	Total	150	100.0
4. Education level			
	Primary	4	2.7
	Secondary	80	53.3
	University	66	44.0
	Total	150	100.0
5. Profession			
	Fitter	1	0.7
	Driver	5	3.3
	Trader	29	19.3
	Designer	8	5.3
	Resourcefulness	13	8.7
	Teacher	15	10.0
	Students	19	12.7
	Civil servant	24	16.0
	Housewife	25	16.7
	Carpenter	4	2.7
	Tradipracticist	7	4.7
	Total	150	100.0
6. Marital status			
	Single	34	22.7
	Divorced	1	0.7
	Married	114	76.0
	Widower	1	0.7
	Total	150	100.0

Table 1 shows that the use of *J. curcas* in traditional medicine is common practice among people aged 36 to over 50, with a predominance of people over 50 (39.3%), followed respectively by people aged 36-50 (33.3%) and 18-35 (27.3%). Thus, according to the gender breakdown of the studied population, 50.7% of informants were males and 49.3% females. The Luba tribe is the most frequently cited (17.3%), followed by the Yaka tribe (14.7%). In terms of level of education, 53.3% of informants had a secondary education, 44.4% were university graduates and barely 2.4% had a primary education. In most cases, they were shopkeepers (19.3%), housewives (16.7%) and civil servants (16%). Finally, the sample studied according to marital status showed that married people were in the majority (76%), followed by single people (22.7%), divorcees (7%) and widowers (7%). Figure 5 shows that for a sample size of 150, the minimum age was 24, the maximum age was 71 and the mean age was 47.92 ± 16.845. Statistical analysis according to Kolmogorov-Smirnov [ddl (150) = 0.237, P = 0.000] or Shapiro-Wilk [ddl (150) = 0.843; p=0.000] shows that the age distribution of respondents does not follow a normal distribution (P<0.05) in the population of the survey zone.

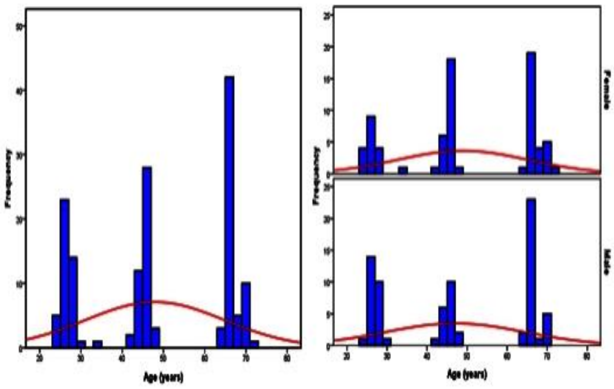

Figure 5. Age distribution of respondents in the study area

3.2. Ethno-botanical data

Figure 6a shows the different parts of *J. curcas* used in traditional medicine and shows that the organ of the plant most commonly used in traditional medicine is the leaf (62%), followed respectively by the bark (12.7%), the seed (10.7%), the root (10.7%) and the stem (6%). It turns out that the leaves are used at least 4 times more than the other parts of the plant. This can be explained by the fact that they are rich in active ingredients and are considered to be the most accessible parts of the plant. The use of leaves in traditional medicine is a sustainable practice and does not lead to the disappearance of plant biodiversity. Figure 6b lists the diseases treated with *J. curcas* and shows that 27 illnesses are treated with *J. curcas*. The 5 illnesses most frequently treated were: gastritis (17 mentions), cough (16 mentions), haemorrhoids (15 mentions), yellow fever (12 mentions) and tooth decay (10 mentions). However, the Budja (ICF= 0.8) and the Yaka (ICF= 0.52) only reached consensus on 6 diseases: hernia (ICF= 1), splenomegaly (ICF= 0.67), haemorrhoid (ICF= 0.57), infection (ICF= 0.5), siege matrix (ICF= 0.5) and prostatitis (ICF= 0.5).

Figure 6c shows the different ways in which therapeutic recipes based on *J. curcas* can be prepared. The recipe preparation methods revealed that decoction is the most frequently cited method of preparation (72.7%), followed by expression and maceration (24.7% and 2.7%, respectively). The forms of administration are derived from the methods used to prepare the recipes. Figure 6d shows how therapeutic recipes are administered. This figure shows that oral administration is the most commonly used route (81.1%). Massage was the second most common route (12.6%), followed by ophthalmic (3.5%) and anal (2.8%).

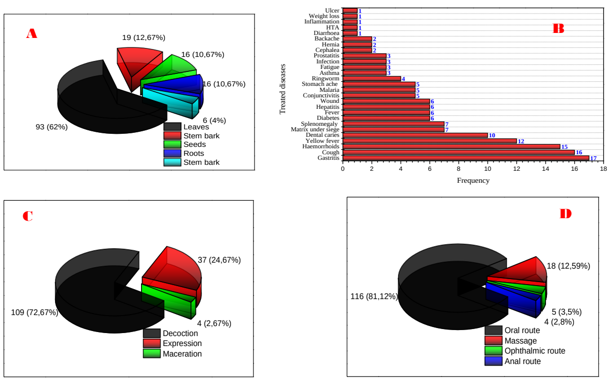

Figure 6. Ethno-botanical data: A. J. curcas used parts; B. Frequency of diseases treated by J. curcas; C. Recipes preparation modes; D. Route of recipes administration**.**

According to the composition of the therapeutic recipes (Figure 6), the survey revealed that the majority of respondents (96.7%) use *J. curcas* alone to treat common illnesses. Only 3.3% of respondents combined *J. curcas* with other ingredients in preparing their recipes. The ingredients associated with *J. curcas* recipes included charcoal, palm oil and cassava flour. Thus, according to the informants, the recipes are prepared in a poly-specific composition for reasons of bio-optimisation of the activity targeted in the treatment of the disease.

Figure 7 shows the population's opinion on the availability of the species.

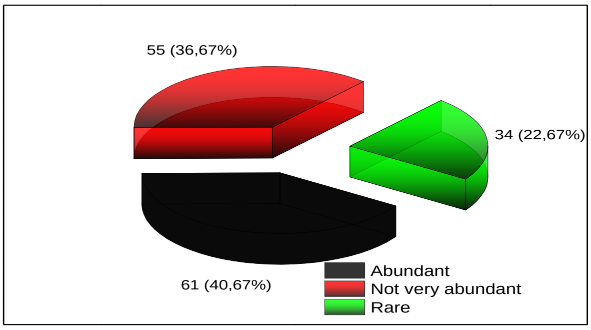

Figure 7. J. curcas availability frequency

With regard to the availability of the species, the survey revealed that 40.7% of respondents thought the species was abundant, followed by 36.7% who thought it was scarce and 22.7% who thought it was rare (Figure 7). Given the current challenges posed by climate change, *J. curcas* could provide an opportunity for development projects through the creation of productive ecosystems (ecological restoration of degraded sites, raw material for the manufacture of biodiesel, phytomedicines and soap). By integrating it into an agroecological system, this objective can be achieved in the context of sustainable development.

Statistical analysis

Chi-square test, multiple component analysis and principal component analysis were applied. Table 2 gives the results of the bi-variate statistical analyses (Chi-square).

Table 2. Influence of socio-demographic parameters on ethno-botanical data.

Legend: UP (used parts); ST (Statistics); TD (treated diseases); MP (method of preparation); RA (route of administration); RC (recipe composition) DoF (degree of freedom); AV (Availability); SDP (Socio-demographic parameters); 1 (Age group); 2 (Sex); 3 (Education level); 4 (Profession); 5(Marital status); 6 (Tribes).

SDP	ST	Ethno-botanical data					
		UP	TD	MP	RA	RC	Av
1	χ^2	16.274	81.580	8.928	10.247	1.697	4.604
	DoF	8	54	4	8	2	4
	p-value	0.039	0.009	0.063	0.248	0.428	0.330
2	χ^2	9.824	32.519	0.226	1.597	1.781	0.576
	DoF	4	27	2	4	1	2
	p-value	0.043	0.213	0.893	0.809	0.182	0.750
3	χ^2	7.354	45.957	1.601	7.352	0.200	2.335
	DoF	8	54	4	8	2	4
	p-value	0.499	0.774	0.809	0.499	0.905	0.674
4	χ^2	48.814	264.813	22.067	28.757	11,263	24.275
	DoF	80	270	20	40	10	20
	p-value	0.160	0.578	0.337	0.907	0.337	0.231
5	χ^2	11.094	71.507	5.981	15.542	0.096	6.286
	DoF	12	81	6	12	3	6
	p-value	0.521	0.766	0.425	0.213	0.992	0.392
6	χ^2	90,192	581.915	35.904	72.279	32.915	77.067
	DoF	80	540	40	80	20	40
	p-value	0.204	0.103	0.655	0.718	0.034	0.00

However, multiple component analysis showed that the disease treated depended on the method of preparation, the route of administration and the plant organ. This practice is linked to the ethnicity, profession, family situation and age group of the respondents. The results of the principal component analysis test on the organs used of *J. curcas* in phytotherapy showed that the first principal component is highly correlated with 2 original variables. This component increased with the use of *J. curcas* bark and roots. This indicates that these 2 variables vary together. If one is prescribed (correlation coefficient: 0.826), the other tends to be prescribed (correlation coefficient: 0.783). However, we find that the second component is most strongly correlated with the leaves (correlation coefficient: 0.817). Leaves are prescribed on their own when treating illnesses. This means that the bark and roots are not prescribed at the same time as the leaves of this plant. Furthermore, the stems and seeds are not correlated with anyother organ of *J. curcas*. On the other hand, the results of principal component analysis on the diseases used show that the first principal component is strongly correlated with 16 original variables represented by the 16 diseases (infection, cough, ringworm, hepatitis, diabetes, hernia, siege matrix, weight loss, wound, headache, yellow fever, tooth decay, malaria, fever, conjunctivitis, haemorrhoid). This component increased with the treatment of these 16 diseases (correlation coefficient ≥ 0.682). This indicates that these 16 variables vary together. If one is treated, the other 15 tend to be treated as well. However, we find that the second component is most strongly correlated with 5 diseases (inflammation, prostatitis, ulcers, splenomegaly and asthma) with a correlation coefficient equal to 0.952. These diseases have the same tendency to be treated by traditional medicine. In addition, 6 illnesses (fatigue, gastritis, backache, diarrhoea, stomachache and high blood pressure) were not correlated with any other illnesses treated.

Hierarchical ascendant classification

Figure 8 shows the relationship between diseases (observations) and ethnic groups (variables).

The 5 classes of illness are treated for the different ethnic groups, namely Class 1 (asthma, tooth decay, headache, conjunctivitis, diabetes, diarrhoea, fatigue, fever, hepatitis, hernia, HTA, infection, inflammation, malaria, siege state, backache, stomachache, weight loss, wound, prostatitis, splenomegaly, ringworm; ulcer); Class 2 (yellow fever); Class 3 (gastritis); Class 4 (haemorrhoids); Class 5 (cough).

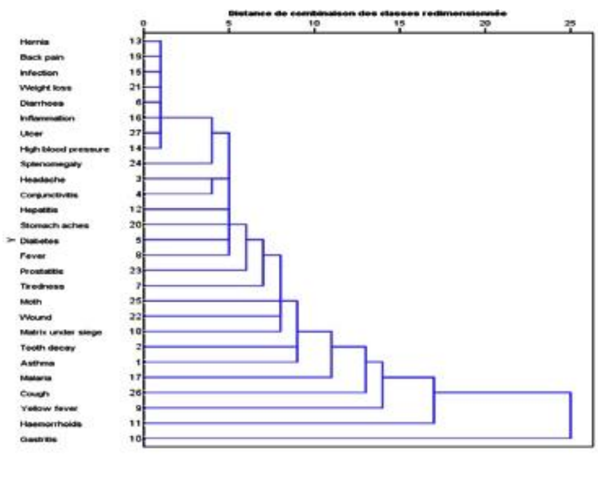

Fig. 8. Hierarchical classification of diseases in relation to ethnic groups by combination distance of resized classes.

Figure 9. shows the hierarchical tree of the different parts of *J. curcas* used in traditional medicine.

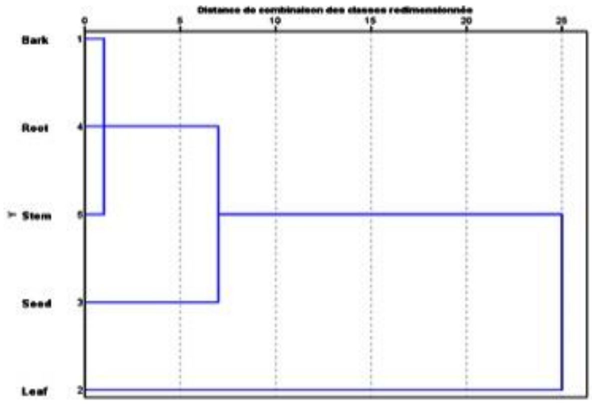

Figure 9. Hierarchical tree of J. curcas used parts by combination distance of resized classes

Figure 9 shows that the five organs of J. curcas are grouped into four classes. These are Class 1 (Bark and root); Class 2 (leaf); Class 3 (seed) and Class 4 (stem).

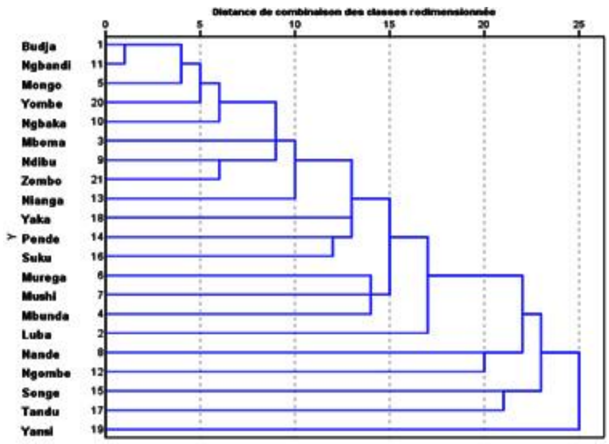

Figure 10. Hierarchical tree of socio-cultural groups using *J. curcas* according to combination distance of resized classes

Figure 10 shows the hierarchical ascending classification of socio-cultural groups using *J. curcas* to treat common illnesses. The ascending hierarchical classification (Figure 10) divides the different socio-cultural groups into 5 classes: Class 1 (Budja, Luba, Mboma, Mbunda, Murega, Mongo, Mushi, Ndibu, Ngbaka, Ngbandi, Nianga, Pende, Suku, Yaka, Yombe, Zombo); Class 2 (Nande, Ngombe); Class 3 (Songe), Class 4 (Tandu) and Class 5 (Yansi). Class 1 is the most diverse, with 17 tribes including Sudanese (Ngbaka and Ngbandi) and Bantu.
The ethno-botanical surveys carried out in the field enabled 150 people to be interviewed in order to assess their level of knowledge about *J. curcas*. This study showed that the frequency of use of this plant is linked to the profile of the people surveyed. Older people were more likely than younger people to know how to use *J. curcas*. These results corroborate those of Ouro-djeri *et al.,* (2022), and Nesrine *et al.,* (2022), who showed that older people were the most likely to have access to knowledge about traditional medicine. This is probably the main reason why this profession is practised by older people. However, women and men showed a shared knowledge of *J. curcas*, with a slight difference in the % of use between the 2 genders, with a slight advantage for men. Our results are close to those obtained by Nesrine *et al.,* (2022), who also found a slight difference in % use between the 2 genders,

but with a slight advantage going to women. With regard to marital status, our results are close to those obtained by Nesrine *et al*., (2022), with a rate of 56% of married people compared with 30% who are still single. Our results also indicate that the level of education has an influence on society's attachment to traditional care. This is confirmed by the work of Ngbolua *et al*., (2016), who found that high school informants represented those who used medicinal plants the most. However, our results are at odds with those of Ouadeh *et al*., (2021), who found that the vast majority of medicinal plant users were university graduates (59%), followed by secondary school graduates (17%) and primary school graduates (13%).

The predominant use of leaves in our survey can be explained by the fact that they are rich in active ingredients. They are considered to be the most accessible parts of the plant. The interest in leaves can be explained by the fact that they are the storage site for secondary metabolites, which are responsible for the plant's biological properties (Nesrine *et al*., 2022). However, studies carried out by Ouro-djeri *et al*., (2022) and Ouadeh *et al*., (2021) showed that leaves were the most frequently used drugs in the preparation of traditional medicinal recipes, with %s of 53.59% and 22%, respectively, which is confirmed by the results of our survey. Finally, Mawunu *et al*., (2022) report that *J. curcas* leaves are used to treat teigns, dermal infection and stomach ache in the small town of Songo in northern Angola.

With regard to the method of preparation of the recipes in our survey, decoction was the most frequently cited method with 72.7%, followed by expression and maceration with (24.7%), and (2.7%), respectively. Our results are similar to those of Ngbolua *et al*., (2019) and Houmenou *et al*., (2017), who showed that decoction is the most commonly used preparation method, followed by maceration. This method of preparation yields the most active ingredients and reduces or eliminates the toxicity of certain recipes (Salhi *et al*., 2010). Among the routes of administration, oral use was the most frequently cited in our survey. With regard to the diseases cited by field informants, our survey revealed that *J. curcas* is a plant with multiple traditional medicinal uses. Our findings corroborate those of Kambu *et al*., (2009), who found that decoction of the leaves cures headaches, stomach aches, tooth decay, diabetes, malaria, coughs, etc. Latex from the leafy stem, when applied to the eyes, treats conjunctivitis. The decoction of root bark treats gastritis, etc. For a development project, *J. curcas*is a plant species of choice for agro-ecology as a strategy for sustainable community development.

3.1 Bioenergy

✓ **Drying kinetics of *J. curcas* seeds**

The seeds were oven-dried at 105 °C and the weight of the seeds was measured over time (Figure 11).

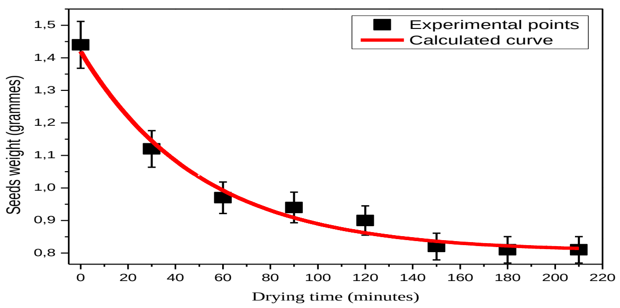

Figure 11. Evolution of *J. curcas* seed weight as a function of drying time (obtained by non-linear regression using the decreasing exponential equation of the form. y=yo+A1e-x/t1 avec yo=0.804±0.020; A1=0.616±0.042; t1=50.652±7.554; Khi-deux = 0.382x10-4; R2=0.98)

Figure 11 shows that constant weight is obtained after 3 h of steaming *J. curcas* seeds at 105 °C.

✓ Major mineral elements in *J. curcas* seeds

Figure 12 shows some minerals contained in *J. curcas* eeds.

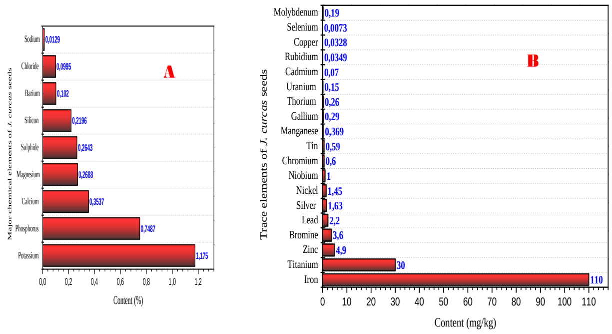

Figure 12A: Major mineral elements and Figure 12B: Trace elements in J. curcas seeds

Figure 12 shows that *J. curcas* eeds contain the following minerals: K, P, Ca, Mg, S, Si, Ba and Na (A); Fe, Ti, Zn, Br, Pb, Ag, Ni, etc. (B). Some of these chemical elements may play an important role in the interaction with viral proteins. Orthopoxviruses code for a protein close to Cu-Zn superoxide dismutase or a functional GPx enzyme dependent on Se, the presence of which leads to a decline in the level of antiradical activity in infected cells (Chaturvedi and Shrivastava, 2005). These mineral elements also play an important role in the physiological function of the skin, in particular the skin's bioenergetics process, redox balance, epidermal barrier function and dermal remodelling. These ions are involved in skin regeneration and ageing processes (Marek *et al*. 2022). This oil may therefore be useful in the treatment of skin infected by the monkeypox virus.

✓ **Oil yield from J. curcas**

J. curcas seeds gave an oil yield of 26.5% (powder mass: 1 kg; oil mass: 265 g), which is higher than the value found in the work of Aboubacar (2007) (23%), but lower than the value found in the work of Rakotoravo in 2016 (38.2%). According to the literature, the harvesting period, extraction conditions and edaphic or climatic parameters related to the species can influence vegetable oil extraction yields (Razafindralambo, 2013).

✓ **Physico-chemical characteristics of J. curcas oils**

Table 3 gives the physico-chemical characteristics of *J. curcas* oil.

Table 3. Physico-chemical characteristics of *J. curcas* oil

Parameters	Experimental values
Acid index	1.8 mg/KOH/g
Density (20 °C)	910 Kg/m^3
Viscosity (40 °C)	36mm^2/s
Saponification index	199
Esters index	197.2 mg
Moisture content	14.43%
Refraction index	1.4769

These are acid number, density, viscosity, saponification number, ester number, water content and refractive index.

By comparing the values of the physico-chemical parameters of *J. curcas* oil with those of the DIN V 51605 standard for pure vegetable oils that can be used as fuel (Table 3), we can see that *J. curcas* oil from the Ubangi ecoregion has energy properties that meet the standard and can therefore be used for bioenergy recovery. Density is one of the criteria for oil purity, and depends on the chemical composition and temperature of the oil. The density found in the present work is 910. This value is higher than that of Fatma *et al*., (2020), who found 880, while it is lower than that of Aboubacar (2007), who found 920. According to Blin *et al*., (2014), the ASTM international standard recommends that the density of a vegetable oil intended for biodiesel production should vary between 900 and 960. At this point, *J. curcas* oil from the Ubangian ecoregion has a density close to this range and is therefore exploitable for bioenergy recovery.

The viscosity of an oil reflects its resistance to flow, and the value obtained in this study is 36 (mm^2/s). This value is lower than that of Aboubacar (2007) but higher than that of Fatma *et al*., (2020), who found 58 and 40 (mm^2/s), respectively. This difference can be justified by the fact that the viscosity of oil depends on the structure of the oil chains, the nature of the fatty acids and also the temperature. Temperature is a factor that has a direct effect on viscosity variation (Courtois *et al*., 2012).

The acid number of oil is a measure of the fraction of free fatty acids. The value obtained in the present work is 1.8 of that found by Aboubacar (2007) who found 4.2. According to DIN V 51605, the limit value for the acid index is 2 mg KOH/g. We can see that *J. curcas* oil has a low content of free fatty acids, which are formed by the hydrolysis of triglycerides when the seeds contain high moisture content (Blin *et al*. 2014).

The refractive index of oils varies according to their unsaturation. The value found in this study is 1.476. This value is the same as that found by Aboubacar (2007). This means that the *J. curcas* oil used in this study is classified as non-drying oil, i.e. one that does not dry out when exposed to open air. However, the results of the saponification index are slightly higher than those found in the work of Fatma *et al*., (2020) and Aboubacar (2007), who obtained 195 and 190 respectively.

The standard also specifies that the saponification value of *J. curcas* oil should be between 190 and 200, which is sufficient proof that the oil has not undergone any artificial additives, such as preservatives, colorings or oils of different kinds.

Biodiesel

Figure 13 gives the biodiesel synthesised from *J. curcas* oil. The biodiesel is the less dense main product (top or supernatant) and the glycerol, the denser by-product (bottom or pellet) still to be recovered at a later stage. After 8 hours of trans-esterification of *J. curcas* oil (50 ml) with methanol in the presence of KOH as a catalyst, the reaction gave a biodiesel yield of 80% (i.e. 40 ml). Ramanamihaja (2006) found that the ratio (3:1) in the presence of 1% KOH gave a biodiesel yield of 66.33% from castor oil. The higher yield obtained in our work is due to the longer reaction time. In the trans-esterification reaction, the molar ratio between the oil and the alcohol, the reaction time and the catalyst are important parameters for optimizing the yield.

Figure 13. Biodiesel

✓ **Physico-chemical characteristics of biodiesel**

Table 4 shows the physico-chemical characteristics of biodiesel produced from *J. curcas*.

Table 4. Physico-chemical characteristics of synthesised biodiesel

Parameters	Experimental values	EMHV de ricin	EMHV Norme
Acid index (mg/KOH/g)	0.21	0.3 – 0.4	<0.5
Density (20 °C) $(\mathbf{Kg/m^3})$	857	880	860-900
Viscosity (40 °C) $(\mathrm{m}\mathbf{m^2/s})$	2.64	16.06	3.5 -5.0
Iodine index	59.6	-	<120
Cetane index	109	53	>51

Overall, the results of the ***J. curcas*** biodiesel analyses (Table 4) are in line with the usual standards for vegetable oils. ***J. curcas***biodiesel can therefore be used as an energy source for diesel engines. However, it should be noted that these parameters may vary depending on the quality of the oil and the production and storage conditions. The results of analyses of ***J. curcas*** biodiesel give a low acid number of 0.21 mg/KOH/g, which is lower than that of castor biodiesel (0.3 mg/KOH/g) found in the work of Ramanamihaja (2006). This suggests that biodiesel from ***J. curcas*** oil is less acidic and therefore less corrosive than other vegetable oil methyl esters (Bagan *et al*., 2012). The density of ***J. curcas*** biodiesel at 20°C is 857 kg/m^3, which is lower than that of Fatma *et al*., (2020), who found 880 kg/m^3 for soybean biodiesel. The viscosity at 40°C of ***J. curcas*** biodiesel is 2.64 mm^2/s, indicating a viscosity higher than that of sunflower biodiesel, but lower than that found by Ramanamihaja (2006) on castor biodiesel.

The iodine value provides information about the level of unsaturation in a fat. The iodine value of ***J. curcas*** biodiesel is 104, indicating average resistance to oxidation. The cetane number is a measure of a diesel fuel's ability to ignite rapidly when injected into a diesel engine. Thus, the higher the cetane number, the more efficient the combustion and the lower the pollutant emissions (Mactar 2009).

The cetane number of ***J. curcas*** biodiesel is 59.6, which is higher than the cetane number of castor biodiesel in the work of Ramanamihaja (2006), who found 53. This means that ***J. curcas*** biodiesel from the Ubangi ecoregion has all the characteristics needed to be used as a fuel in diesel engines.

3.2 Vegetable oil and monkeypox skin infections

Monkeypox virus infection is a zoonosis transmitted to humans by animals, and its biological agent belongs to the same family as human smallpox (Ngbolua *et al.,* 2020). It occurs mainly in the forests of central tropical Africa (including the North Ubangi forest block: Figure 14A) and tends to become a pandemic through the process of biological invasions. The Monkeypox virus can be transmitted to humans, causing a syndrome with clinical manifestations similar to those of smallpox, including a pustular rash (Figure 14C), fever, respiratory symptoms, etc. (Ngbolua *et al.,* 2022). Cosmetological products can be used to treat infected individuals. Jatropha oil, because of the presence of phorbol esters (Haas *et al.,* 2002), can be used externally to treat the virus that causes this skin disease.

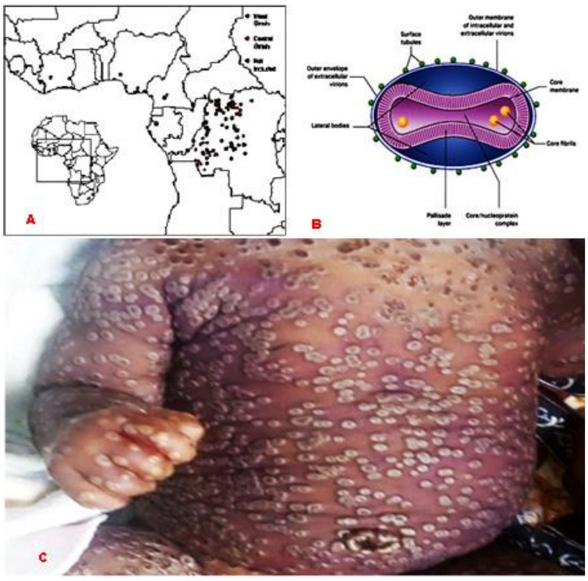

Figure 14. A.Monkeypox occurrence endemic zones in Africa (Levine et al., 2007); B. Genome of Human Monkeypox Virus [The HMPXV genome is a linear, double-stranded DNA, approximately 197 kb, with inverted terminal repeats (ITRs) at its ends, and encodes more than 190 ORFs] (Sklenovská, 2020).; C. A child suffering from monkeypox in the province of North Ubangi in the Democratic Republic of the Congo (Ngbolua et al., 2022)**.**

This scientific evidence is demonstrated by the results of molecular simulation/modelling. Indeed, 12-Deoxy-16-hydroxyphorbol forms a thermodynamically stable complex with viral proteins such as Poxvirus Thymidylate Kinase (PDB ID: 2V54) and protein A42R (PDB ID: 4QWO) and are stabilized by weak molecular interactions including hydrogen bonds. The 2V54/12-Deoxy-16-hydroxyphorbol complex (-6.49±0.39 kcal; Chain B: T18; N37; F38; T18; Y35; K14; K17; K14; R41; N37) is more stable than the 2V54/Cidofovir complex (-6.18±0.27 kcal; Chain A: D13 ; D13; F38; G98; Y101; E142 ; R93). This trend is observed with the second molecular target, as the complex formed between 4QWO et 12-Deoxy-16-hydroxyphorbol [-6.85±0.39 kcal: E77(B); T126(B); R127(A); N78(B); R127(A); R129(B); H100(B); R129(B)] is more stable than the positive control [Complexe 4QWO/Cidofovir: -6.42±0.18 kcal: N78(A); Y80(A); D10(B); D10(B); T120(B); T120(B); R127(B); R127(B); R129(A); R129(A); R127(B)].

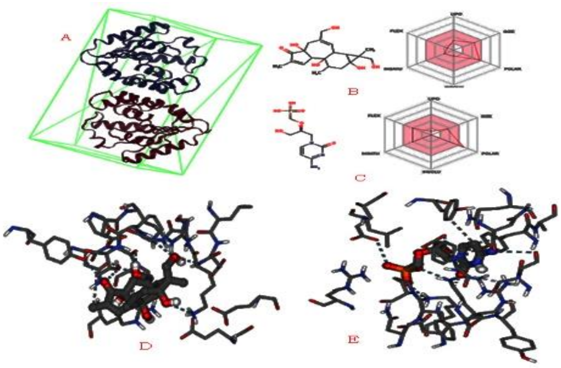

Figure 15A. Structure of ligands, receptors and complexes formed (A: Poxvirus Thymidylate Kinase (PDB ID: 2V54), B: 12-Deoxy-16-hydroxyphorbol, C: Cidofovir, D: 12-Deoxy-16-hydroxyphorbol-enzyme complex, E: Cidofovir enzyme complex).

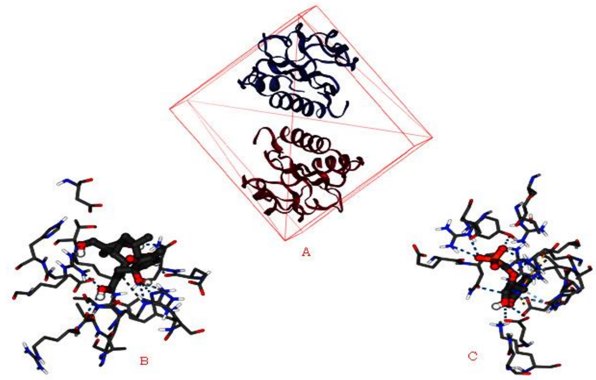

Figure 15B (continued). Structure of the receptor and complexes formed [A: A42R protein (PDB ID: 4QWO), B: 12-Deoxy-16-hydroxyphorbol receptor complex, C: Cidofovir receptor complex].

P*oxviruses* are DNA viruses that replicate in the cytoplasm of host cells thanks to their ability to encode the enzymes required for genome replication and transcription. This is particularly true of thymidylate kinases, whose sequences are similar (42% identity) to those of humans but differ in their homodimeric association and the geometry of the active site. Indeed, the arrangement of the vaccinia thymidylate kinase dimers is orthogonal and not antiparallel as in the human enzyme (Caillat *et al*., 2008). These viruses also encode the A42R protein which, although structurally similar to profilins (cellular proteins regulating actin cytoskeleton assembly), binds only weakly to actin and does not bind to poly (L-proline) (Minasov *et al*. 2022). Inhibition of these two enzymes by *Jatropha curcas* oil may therefore have an impact on orthopoxvirus replication in the host cell.

Large-scale cultivation of *J. curcas* should therefore be considered as part of a project to create a productive ecosystem for a sustainable supply of raw materials. In the current context of biodiversity loss, linked to human activities and environmental factors, the safeguarding of natural resources must necessarily involve the implementation of effective management and conservation strategies. Setting up a plant to process this non-conventional oil plant will also facilitate socio-economic development through a community-wide pharmaceutical and bioenergy industrial initiative. Such an initiative would make it possible to combat climate change by creating a carbon sink aimed at reducing GHGs, as recommended by the Kyoto protocol, while developing high value-added products of public utility derived from biomass (Ngbolua, 2020). The results of this study show that by combining the traditional medicinal heritage handed down by various peoples over the years with modern scientific research, Africa in general, and the Democratic Republic of Congo in particular, has all the keys in hand to solving its health problems in terms of accessibility and low cost, in a context of support for the universal health cover advocated by the WHO (2023).

4. CONCLUSION AND SUGGESTIONS

The aim of this study was to assess the ethnomedical and socio-cultural knowledge of *J. curcas* in Kinshasa, to predict the antiviral activity of its oil, and to characterize the biodiesel produced from this vegetable oil. The results of the surveys showed that 27 illnesses are treated by *J. curcas*, the most commonly cited being gastritis, coughs, hemorrhoids, yellow fever, and tooth decay. The leaves are the most commonly used organ. However, the most frequently cited method of preparation is decoction, used mainly in single-species compositions. In addition, the recipes are mostly administered orally, and the majority of the population surveyed believe that the plant is abundant in their environment. In addition, the extraction of oil from the seeds produced a significant yield. Physico-chemical analyses showed that *J. curcas* oil from the ecoregion meets the DIN V 51605 standard and can therefore be used for bioenergy purposes. The biodiesel obtained by trans-esterification of the oil gave a better cetane number. Molecular modeling has predicted that the oil from this plant may have antiviral properties against 2 molecular targets involved in the replication of the DNA of the Monkeypox virus (Poxvirus Thymidylate Kinase and A42R protein). Bioclinical studies are therefore needed to validate the efficacy and safety of this plant in the treatment of diseases. There is also the possibility of testing the use of its biodiesel as a household energy source.

Acknowledgments

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