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Research Article
In silico Investigation and BSA Denaturation Inhibitory Activity of Ethyl Acetate and N-Butanol Extracts of *Centaurea tougourensis* Boiss. and Reut.

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Abstract

Background and Objective: Phytotherapy is considered nowadays an important discipline that allowed both the scientific community and pharmaceutical companies to innovate in the drug discovery and development process. In this study, we aimed to evaluate the anti-inflammatory activity of the n-butanol (n-BuOH) and ethyl acetate (EA) extracts of a plant species named *C. tougourensis* as well as to investigate for the first time the physico-chemical and biological characteristics of some compounds identified in this plant using a bioinformatics approach. **Materials and Methods:** In this study, the *in silico* approach was applied to predict the physicochemical characteristics, pharmacokinetics, bioactivity, cytotoxicity but also the possible changes in the expression of some gene using four newly identified compounds. Thus, two compound were tested from the n-BuOH extract named, respectively, Silanediamine, 1,1-dimethyl-N,N'-diphenyl- (compound 1), Methanone, 1,3-dithian-2-ylphenyl-(compound 2) and two compounds from EA extract, named, respectively, 4-Fluoro-1-methyl-5-carboxylic acid, ethyl (ester) (compound 3), propionic acid, 3-iodo-, tetradecyl ester (compound 4). The anti-inflammatory activity of these extracts was also tested using bovine serum albumin (BSA) denaturation approach. **Results:** The *in silico* study indicated that the tested compounds may be involved in the regulation of gene expression but may also exert a noticeable cytotoxicity against several tumour cell lines. Furthermore, these compounds could also be considered a potent candidate to treat metabolic disorders linked to diabetes and obesity, but also oxidative stress, immune system dysfunction and skin pathologies. The BSA assay revealed that both extracts have almost the same moderate inhibition effect on protein denaturation. The EC_{50} values were (335 ± 0.03 µg mL^{-1}) for n-BuOH and (338 ± 0.07 µg mL^{-1}) for EA extracts. The difference was considered highly significant ($p<0.001$) when compared to diclofenac sodium (115.76 ± 0.19). **Conclusion:** These preliminary results indicate that the actual biocompounds contained in *Centaurea tougourensis* could be a good candidate for the elaboration of more effective and natural drugs.

Key words: Anti-inflammatory, BSA, *Centaurea tougourensis*, *in silico*, phytotherapy

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Several bioinformatics means are now-a-days used to predict with high accuracy the physicochemical and pharmacological properties of specific compounds due to the emergence of a discipline called *in silico*, which is principally based on computer-aided drug design softwares and collaborative platforms[1]. This bioinformatics approach can be very helpful, especially before conducting *in vitro* and *in vivo* experiments[2]. Indeed, several fields of research including genetic and evolutionary biology have considerably evolved due to *in silico* and molecular docking process[3], as well as the possible interactions of a molecule with a specific receptor at the atomic level, based on energy transfer process[4], helping the scientific community to develop more effective therapies, especially against cancer.

Human health is constantly threatened by pathogens including; microorganisms, chemicals, radiations, physical agents, which may also have a negative repercussion on the genetic level[5,6]. However, our body has many natural means to maintain its physiological state. Among them, the inflammatory reactions are considered as a natural response of the body's immune system mainly orchestrated by immune cells via the secretion of key cytokines, interferons and chemokines to promote tissue repair and recovery[7]. However, in the pathological situation, a dysfunction of the immune system can lead to an excessive inflammatory response which can result in the development of chronic pathologies such as ulcer, tuberculosis, asthma and rheumatoid arthritis[8].

Plants have always played a central role in human history and the bioactive compounds found in *Centaurea* species such as flavonoids, saponins, terpenes, tannins and other phenolic compounds could explain their remarkable abilities to treat several illnesses related to oxidative stress, diabetes, inflammation, cancer, infections and neurological disorders[9-12]. This study is a continuation of the previous works in which demonstrated the antioxidant, anti-inflammatory, hepatoprotective, antidiabetic, haemostatic, neuroprotective, photoprotective and antimicrobial activities of *Centaurea tougourensis*[13-15].

The present study aimed to predict the physicochemical and biological properties of four identified compounds using *in silico* approach as well as to evaluate *in vitro* the anti-inflammatory effect of both extracts.

MATERIALS AND METHODS

Study area: The study was carried out in collaboration between the Laboratory of Biotechnology of Bioactive Molecules and Cellular Physiopathology (LBMBPC), University Batna 2, Algeria and the Department of Food Science and Nutrition, College of Sciences, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia from October, 2021 to January, 2022.

Plant collection: *Centaurea tougourensis* was collected from the commune of Fesdis (Algeria) (Lat 35.621975, long 6.241327) and conserved under a voucher specimen (CT/2019/LPTPCMB).

Plant extraction method: The plant was dried, covered away from sun's rays and then ground for future use. Maceration was carried out in triplicate with EtOH-H_2O mixture (70:30) at ambient temperature for 3 days then evaporated using solvents with different polarities.

In silico study

Generation of canonical smiles: For this approach, Open Babel (version 2.0.2)[16] and In Draw webserver (http://in.indraw.integle.com/) were used. Indeed, two compounds were selected from the n-BuOH extract and named, respectively, Silanediamine, 1,1-dimethyl-N, N'-diphenyl-(compound 1), Methanone, 1,3-dithian-2-ylphenyl- (compound 2) while two compounds were selected from the EA extract, named, respectively, 4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester) (compound 3), Propionic acid, 3-iodo-, tetradecyl ester (compound 4).

Physicochemical properties: SwissADME web-service (http://www.swissadme.ch/) was used for this context and six physicochemical properties were taken into account; lipophilicity, size, polarity, solubility, flexibility and saturation[17].

ADMET profile: The absorption, distribution, metabolism, excretion and toxicity of the four compounds was tested using admetSAR 2.0 (http://lmmd.ecust.edu.cn/admetsar2)[18].

Pharmacological properties: The bioactivity score of each compound was predicted using the online platform of PASS online (http://way2drug.com/passonline/)[19].

Cytotoxicity prediction: To estimate the possible cytotoxicity on human tumour cell lines, the online platform of CLC-Pred made it possible, based on the structural formula of each compound previously obtained[20].

Gene expression profiles: The *in silico* prediction of the possible influence of the four compounds on gene expression has been considered using DIGEP Pred (http://www.way2drug.com/ge/), which is primarily based on Upregulation and Downregulation processes[21,22].

Bovine albumin denaturation assay: The principle of this assay consists to evaluate the possible anti-inflammatory effect of our plant on bovine serum albumin (BSA) denaturation caused by heat (72°C). For this purpose, 1 mL of each concentration of extract or diclofenac sodium was added to 1 mL of BSA (0.2%) solution previously prepared in Tris-HCl buffer solution (pH = 6.6). The mixture was then incubated at ambient temperature for 15 min, then in a water bath at 72°C for 5 min. After cooling, the turbidity was measured at 660 nm[23]. In addition, the EC_{50} value was estimated and was calculated by this equation:

$$\text{Inhibiton } (\%) = \left[\frac{\left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)}{\text{Abs}_{\text{control}}}\right] \times 100$$

$Abs_{control}$ = Represent the absorbance of the control group
Abs_{sample} = Represent the absorbance of plant extract or standard drug

Statistical analysis: Data were expressed as Mean (±SD) for *in vitro* tests and ±SEM and the statistical analyses were performed by ANOVA test using GraphPad Prism version 8. Results were considered highly significant at $p<0.001$.

RESULTS AND DISCUSSION

In silico study

Canonical smiles generation: In order to estimate the physicochemical and biological properties of the tested compounds, the corresponding canonical smile of each compound was first generated as follow:

- **Compound 1:** (Silanediamine, 1, 1-dimethyl-N,N'-diphenyl-): C[Si](C)(NC1=CC=CC=C1)NC 2=CC=CC=C2
- **Compound 2:** (Methanone, 1, 3-dithian-2-ylphenyl-): C1CSC(SC1)C(=O)C2=CC=CC=C2
- **Compound 3:** (4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester)): CCOC(=O)C1=C(N=CN1C)F
- **Compound 4:** (Propionic acid, 3-iodo-, tetradecyl ester): CCCCCCCCCCCCCCOC(=O)CCI

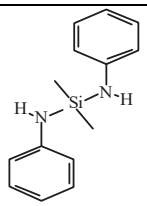
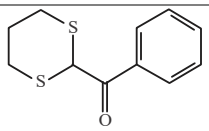
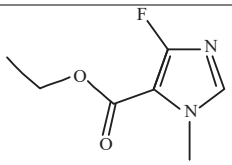
Table 1 provides detailed information about the molecular formula and structure of the tested compounds.

Physicochemical properties: The evaluation of the possible therapeutic effect of candidate compounds is directly linked to their physicochemical properties. This approach can help in the design process of new drugs to treat several diseases related to infections[24]. Indeed, scientists solved some physicochemical characteristics related to mucus, considered as a complex hydrogel biopolymer barrier which is found in several areas including airway epithelium, gastrointestinal tract or the ocular surface of eyes. Indeed, the mucus act by preventing direct access of microorganisms to these zones via its viscoelasticity property, which nowadays helped in the development of transmucosal drug delivery system[25,26].

Figure 1 shows the degree of unsaturation of compound 1 was considered the best, which could mean that this compound may be more reactive, since the degree of unsaturation is proportional to the number of carbons present in the molecule which contain generally double or triple covalent bonds between adjacent carbon atoms to add more hydrogens[27]. This information is very important since unsaturated compounds are giving very promising results in the treatment of human carcinogenicity[28]. In term of polarity, compound 2 showed the best results and the degree of unsaturation of this compound were also very important. Compound 4 exhibited the best flexibility, insolubility and lipophilicity properties and was considered remarkable. Lipophilicity and polarity are important parameters that allowed scientists to treat several infections affecting kidneys via the identification of physicochemical determinants of human renal clearance[29] and to develop more effective pesticides that are less harmful to human health and ecosystem[30]. A compound with high flexibility means an increase in the dynamics molecular binding process with respective receptors which will considerably strengthen the action of drugs on their biomolecular targets to generate the therapeutic effect[31]. Noting that, compound 3 showed also a non-negligible unsaturation property.

ADMET profile: Table 2 shows that, results clearly indicate that the four compounds may cross blood-brain barrier (BBB), Caco-2 cells but may also be absorbed by human intestine with a optimum oral bioavailability, except for compound 4, suggesting that most of these compounds may easily transit from the gastrointestinal tract into the bloodstream to reaches their site of action[32]. This process helps also in the development of more effective drugs with less risk of side effects and toxicity[33]. The majority of molecules with an important molecular weight are excluded from the central nervous system (CNS) via BBB membrane, which suggests that all compounds may be considered a suitable candidate for the elaboration of more effective neurotherapeutics and CNS drug delivery programs[34]. The data also revealed that these compounds may not be a P-glycoprotein (P-gp) substrates, excluding also a probable attenuation on the activity of P-gp, which suggest that these compounds will not interfere with the biotransformation and excretion of drugs[35].

Table 1: Generation of the canonical smiles of the four tested compounds

Extracts	Compounds	Molecular formula	Structure	Canonical smiles
nBuOH	Compound 1	$C_{14}H_{18}N_2Si$		C[Si](C)(NC1=CC=CC=C1)NC2=CC=CC=C2
	Compound 2	$C_{11}H_{12}OS_2$		C1CSC(SC1)C(=O)C2=CC=CC=C2
EA	Compound 3	$C_7H_9FN_2O_2$		CCOC(=O)C1=C(N=CN1C)F
	Compound 4	$C_{17}H_{33}IO_2$		CCCCCCCCCCCCCCCOC(=O)CCI

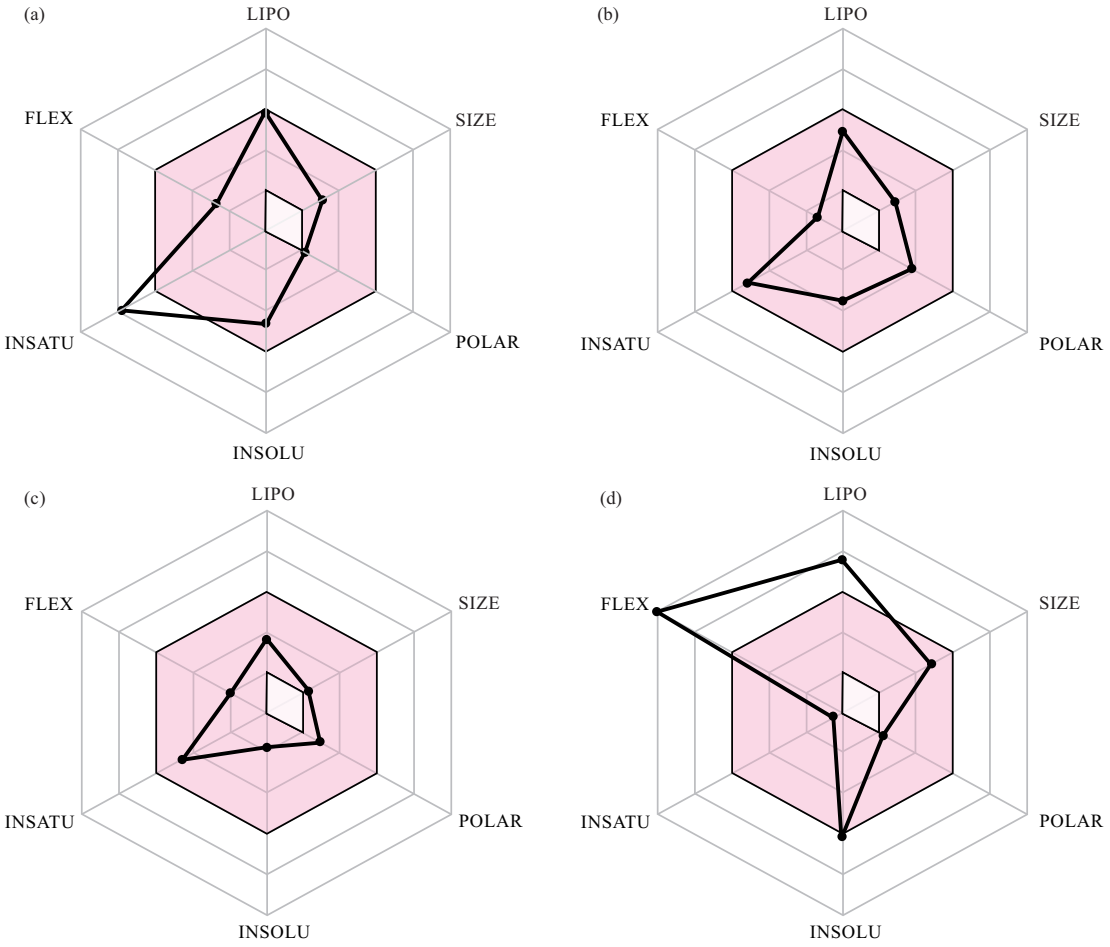


Fig. 1: Prediction of the physicochemical properties of the 4 tested compounds, (a) Compound 1, (b) Compound 2, (c) Compound 3 and (d) Compound 4

LIPO: Lipophilicity, SIZE, POLAR: Polarity, INSOLU: Insolubility, INSATU: Unsaturation and FLEX: Flexibility

Table 2: Prediction of the pharmacokinetic characteristics of the 4 compounds

	n-BuOH extract		EA extract	
Parameters/compounds	Compound 1	Compound 2	Compound 3	Compound 4
Absorption				
Human oral bioavailability	HOB+	HOB+	HOB+	HOBG
Human intestinal absorption	HIA+	HIA+	HIA+	HIA+
Blood brain barrier	BBB+	BBB+	BBB+	BBB+
Caco-2 permeability	Caco2+	Caco2+	Caco2+	Caco2+
P-glycoprotein substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate
P-glycoprotein inhibitor				
Distribution and metabolism	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
Subcellular localization	Mitochondria	Mitochondria	Mitochondria	Mitochondria
CYP450 3A4 substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 2C9 substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 2D6 substrate	Non-substrate	Non-substrate	Non-substrate	Non-substrate
CYP450 3A4 inhibition	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2C9 inhibition	Inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 2C19 inhibition	Inhibitor	Inhibitor	Inhibitor	Non-inhibitor
CYP450 2D6 inhibition	Non-inhibitor	Non-inhibitor	Non-inhibitor	Non-inhibitor
CYP450 1A2 inhibition				
Excretion and toxicity	Inhibitor	Inhibitor	Inhibitor	Inhibitor
Acute oral toxicity	Class III	Class III	Class III	Class III
Hepatotoxicity	Non-hepatotoxic	Non-hepatotoxic	Hepatotoxic	Non-hepatotoxic
Carcinogenicity	Carcinogens	Non-carcinogens	Non-carcinogens	Non-carcinogens
Ames mutagenesis	Non-mutagenic	Non-mutagenic	Non-mutagenic	Non-mutagenic
Eye corrosion	Non-corrosive	Non-corrosive	Non-corrosive	Corrosive
Eye irritation	Irritant	Irritant	Irritant	Irritant
Honey bee toxicity	Non-toxic	Toxic	Non-toxic	Toxic
Crustacea aquatic toxicity	Toxic	Non-toxic	Non-toxic	Toxic
Fish aquatic toxicity	Toxic	Non-toxic	Non-toxic	Toxic
Estrogen receptor binding	Binding	Non-binding	Non-binding	Non-binding
Androgen receptor binding	Non-binding	Non-binding	Non-binding	Non-binding
Thyroid receptor binding	Non-binding	Non-binding	Non-binding	Non-binding
Glucocorticoid receptor binding	Non-binding	Non-binding	Non-binding	Non-binding
Biodegradation				
ADMET predicted profile (regression)	Non-biodegradable	Biodegradable	Non-biodegradable	Biodegradable
Water solubility (log S)	-3.416	-2.396	-4.247	-3.491
Plasma protein binding (%)	1.115	0.977	0.44	0.759
Acute oral toxicity (kg mol^{-1})	2.88	2.303	2.211	2.816
Tetrahymena pyriformis (pIGC50, µg L^{-1})	0.517	1.522	0.56	3.007

Concerning distribution, the mitochondrion seems to be the targeted organelle for all tested compounds which suggest that these compounds could modulate mitochondrial functions since this organelle is considered the main source of cellular energy via the production of adenosine triphosphate (ATP) by oxidative phosphorylation process[36], which contribute to the maintenance of optimal tissue homeostasis. It is also important to note that dysfunction in mitochondrial energy balance could contribute to the emergence of several pathologies associated with diabetes, immunity and aging[37,38]. In term of metabolism, the four compounds may be classified as non-substrates for CYP450 3A4, 2C9 and 2D6 isoforms. It was also observed that the compounds 2, 3 and 4 can be considered as non-inhibitors of CYP450 3A4, 2C9 and 2D6 isoforms while the four compounds could be considered potential inhibitors of CYP450 1A2 isoform. This information is very important since Cytochrome P450 enzymes are essential for the metabolism of many medications but also to avoid the formation of a variety of toxic products[39].

Concerning the Ames mutagenicity, the results suggest that all compounds could be considered non-mutagenic and only compound 1 may be carcinogenic. These non-mutagenic compounds could be considered as potential antitumoral agents since the development of more effective anticancer drugs is crucial in the actual era[40]. The acute oral toxicity for all tested compounds was very high (class III) and excepting the compound 3, the other compounds seem to be non-toxic for the liver. The compounds appear to be irritant to the eyes, however, only compound 4 could corrosive. The toxicity prediction revealed that compounds 1 and 4 could express high toxicity on fish and Crustacea, while compounds 2 and 3 could be non-toxic. Concerning the toxicity to the honey bee, compounds 1 and 3 could be non-toxic.

Table 3: Bioactivity prediction of the 4 compounds

Extracts	Compounds	Pa	Pi	Biological activities
n-BuOH	Compound 1	0.749	0.012	Fragilysin inhibitor
		0.742	0.030	Antiseborrheic
		0.562	0.028	Antiviral (Picornavirus)
	Compound 2	0.669	0.020	Anti-inflammatory
		0.594	0.044	Kidney function stimulant
		0.512	0.047	Oxygen scavenger
EA	Compound 3	0.723	0.005	Cognition disorders treatment
		0.698	0.007	Antiobesity
		0.614	0.011	Antidiabetic
	Compound 4	0.800	0.003	Leukopoiesis stimulant
		0.729	0.010	Macrophage colony-stimulating
		0.734	0.035	Factor agonist antieczematic

Pa: Probability of activity, Pi: Probability of inactivity

The data also revealed that the four compounds may not interact with androgen, triiodothyronine (T3), thyroxine (T4) and glucocorticoid receptors. However, concerning estrogen receptors, data showed that compound 1 could interact with this receptor suggesting that compound 1 may mimic the activity of estrogen. This information is very important, since this steroid hormone plays a key role in mammalian reproduction, regulate uterus function and contribute to the development of women's secondary sex characteristics[41]. This hormone is also involved in the treatment of breast cancer and cardiovascular disease[42,43].

Compound 3 exhibited the lowest value of water solubility (-4.247) while compound 1 showed the highest affinity for plasma proteins (1.115%) and also the most important acute oral toxicity (2.88 kg moL^{-1}). Finally, compound 4 was the most active on *Tetrahymena pyriformis* by expressing a median population growth of (3.007 μg L^{-1}).

Pharmacological properties: Table 3 shows that, compound 1 could be useful as fragilysin inhibitor as well as a potent antiseborrheic agent with corresponding values (Pa = 0.749, Pa = 0.742). Fragilysin is an enterotoxin produced by pathogenic microbial strains including *Bacteroides fragilis* that is responsible for diarrhoeal disease in humans[44]. It is well known that an excess of sebum could generate scaly patches, stubborn dandruff and red skin[45]. Compound 4 may also exert a significant effect against eczema (Pa = 0.734), which suggests that compounds 1 and 4 could be considered good candidates for the treatment of seborrheic dermatitis and other conditions affecting the skin.

Data also revealed that compound 2 may exert a moderate anti-inflammatory effect (Pa = 0.669), but also a non-negligible stimulation on kidney function and oxygen scavenging process with corresponding values (Pa = 0.594, Pa = 0.512). This information is very important since the deregulation of the inflammatory response could break body homeostasis and generate long-term severe pathologies[46,47].

It is also interesting to underline that compound 3 could be a potential candidate to treat cognitive disorders (Pa = 0.723), which can be temporary or progressive disorders[48]. A decrease in cognitive performance will have a bad repercussion on intellectual function including reasoning, memory and speech but also on spatiotemporal perception which will directly affect motor skills since these elements are interconnected[49]. The compound 3 showed also moderate antiobesity and antidiabetic effects with respective values (Pa = 0.698, Pa = 0.614). There is an important relationship since obesity increases the risk of developing type 2 diabetes[50], but also cardiovascular diseases and certain types of cancers[51]. This information suggests that compound 3 could be useful to treat metabolic disorders related to diabetes and obesity[52].

Compound 4 showed a remarkable stimulation on leukopoiesis process (Pa = 0.800) but also on the expression of a cytokine called macrophage colony-stimulating factor (Pa = 0.729) which suggest that this compound could boost the hematopoiesis phenomenon to accelerate the production and differentiation of immune system cells[53], especially macrophage, lymphocytes, granulocytes and natural killer to fight infections especially intercellular viral infections[54].

Cytotoxicity prediction: Table 4 shows that, compounds 1 and 4 are active on brain tissue. Indeed, these two compounds exhibited a moderate cytotoxic activity in which compound 1 was active on the oligodendroglioma (Hs 683) cell line (Pa = 0.734), while compound 2 was on glioblastoma (SF-539) cell line (Pa = 0.507). This information is very important since neurodegenerative diseases are rapidly rising in prevalence[55] and the actual treatment especially antibiotic used to treat

Table 4: Probable cytotoxic activities of the 4 compounds on some tumour cell lines

Extracts	Compounds	Cell-line	Cell-line full name	Tissue	Tumour type	Pa	Pi
n-BuOH	Compound 1	Hs 683	Oligodendroglioma	Brain	Glioma	0.734	0.007
		MDA-MB-468	Breast adenocarcinoma	Breast	Adenocarcinoma	0.563	0.009
		HOP-18	Non-small cell lung carcinoma	Lung	Carcinoma	0.454	0.018
	Compound 2	HeLa	Cervical adenocarcinoma	Cervix	Adenocarcinoma	0.669	0.007
		Hs 683	Oligodendroglioma	Brain	Glioma	0.537	0.044
		HOP-18	Non-small cell lung carcinoma	Lung	Carcinoma	0.311	0.069
EA	Compound 3	NCI-H838	Non-small cell lung cancer stage 3	Lung	Carcinoma	0.648	0.013
		DMS-114	Lung carcinoma	Lung	Carcinoma	0.566	0.015
		SK-MES-1	Squamous cell lung carcinoma	Lung	Carcinoma	0.541	0.004
	Compound 4	SF-539	Glioblastoma	Brain	Glioblastoma	0.507	0.012
		SN12C	Renal carcinoma	Kidney	Carcinoma	0.505	0.013
		NCI-H838	Non-small cell lung cancer stage 3	Lung	Carcinoma	0.430	0.103

Pa: Probability of activity, Pi: Probability of inactivity

Table 5: Possible effect of the 4 compounds on mRNA expression level of some genes

Extracts	Compounds	Genes (upregulation)	Pa	Pi	Genes (downregulation)	Pa	Pi
n-BuOH	Compound 1	SAT	0.887	0.014	MYO5C	0.862	0.014
		POR	0.878	0.028	IFIH1	0.852	0.037
		HTATIP2	0.866	0.009	TEP1	0.848	0.011
	Compound 2	TNNT1	0.724	0.030	IFI27	0.663	0.079
		GAS6	0.687	0.056	TFAM	0.616	0.131
		SLC2A4	0.629	0.088	TEP1	0.582	0.124
EA	Compound 3	LST1	0.866	0.036	ALDH18A1	0.897	0.010
		SQLE	0.747	0.061	SLC15A1	0.876	0.014
		SFRP1	0.610	0.095	SLC2A1	0.505	0.023
	Compound 4	TNNT1	0.776	0.023	ELAVL1	0.628	0.037
		FTL	0.763	0.070	RARB	0.583	0.101
		FECH	0.706	0.067	SPAST	0.501	0.092

Pa: Probability of activity, Pi: Probability of inactivity

pathologies related to the nervous system presents a lot of side effects[56], which explain the urgent situation to elaborate more effective drugs. Compound 1 also showed a moderate cytotoxic effect (Pa = 0.563) on breast adenocarcinoma (MDA-MB-468) cell line. Noting that this type of cancer is in increase now-a-days, in 2020, records reported that 2.3 M women diagnosed with breast cancer[57].

Data also revealed that compound 2 may exert a noticeable cytotoxic effect on the cervical adenocarcinoma (HeLa) cell line (Pa = 0.669). Noting that, the statistics established in 2020 for 185 countries, reported that this type of cancer is ranked fourth most common cancer in women and represents 6.5% of all female cancers58. It is also very interesting to note that all tested compounds were active on lung tissue, especially compound 3 which showed a non-negligible cytotoxic effect on non-small cell lung cancer stage 3 (NCI-H838) cell line (Pa = 0.648), which is important since lung cancer is considered the leading cause of death among both men and women, representing almost 25% of all cancer deaths[58].

Compound 4 demonstrated also a modest cytotoxic effect on renal carcinoma (SN12C) cell line (Pa = 0.505), which is important since kidneys ensure vital functions, especially the regulation of extracellular fluid volume and the filtration and excretion of waste products and toxins out of the blood via urine[59].

Gene expression analysis: Data indicated that compound 1 may remarkably upregulate the mRNA expression of SAT, POR and HTATIP2 genes with corresponding values of (Pa = 0.887, Pa = 0.878, Pa = 0.866) (Table 5). These genes play key roles, indeed, a study revealed that SAT gene may suppress tumor growth in xenograft tumor models[60] and may also play a significant role in the cell survival process by acting as a rate-limiting enzyme in the pathway of polyamine metabolism[61]. The POR gene is crucial for the biosynthesis of cytochrome P450 enzymes[62], which means that this gene could be a precursor for the synthesis of steroid hormones[63]. Another study suggested that the HTATIP2 gene may act as a metastasis suppressor via an antiangiogenic effect to prevent the formation of new blood vessels to block the blood supply into the tumor zone[64].

Concerning the downregulation process, compound 1 showed also a great decrease in the mRNA expression of MYO5C (Pa = 0.862), IFIH1 (Pa = 0.852) and TEP1 (Pa = 0.848) genes and this information is crucial since the emergence of

several illnesses is linked to the overexpression or mutation of these genes; in which MYO5C is associated with an abnormal spreading of neurofibrillary tangles in Alzheimer's disease[65], while IFIH1 may generate neuro-immunological disorders caused by an abnormal elevation of interferon due to excessive inflammatory reactions[66], also TEP1 gene activity could be associated with an infectious disease called campylobacteriosis and prostate cancer[67]. Noting that compound 2 exerted a moderate downregulation on this TEP1 gene (Pa = 0.582).

Among the four tested compounds, results revealed that compound 2 could possibly increase the mRNA expression of TNNT1 (Pa = 0.724), GAS6 (Pa = 0.687) and SLC2A4 (Pa = 0.629) genes.

Human physiology depends a lot on these genes, the TNNT1 gene plays a key role in the regulation of actin thin filament function in skeletal muscle[68], while the GAS6 gene may enhance hemostatic response by enhancing platelet aggregates and clot formation processes[69]. The expression of the SLC2A4 gene could significantly stimulate glucose uptake by adipose tissue and skeletal muscle due to an important increase in the expression of glucose transporter protein type-4 (GLUT4) mediated by SLC2A4 gene[70]. Noting that compound 4 showed a better result in the expression of the TNNT1 gene (Pa= 0.776). The results also indicated that compound 2 may act with a moderate way to down-regulate the gene expression of IFI27 (Pa = 0.663) and TFAM (Pa = 0.616). A recent study made by Zhao *et al.*[71] revealed that the IFI27 gene could be closely associated with Hepatitis C and Oral Leukoplakia while an alteration in the TFAM gene may increase the risk of developing Parkinson's disease due to a mitochondrial DNA depletion syndrome generated by the excessive activity of this gene[72].

Its also important to underline that compound 3 may considerably upregulate the mRNA expression level of the LST1 gene (Pa = 0.866), since the activity of this gene may regulate leukocyte abundance in lymphoid organs but also the inflammatory response in the gut[73]. This compound may also increase the mRNA expression of SQLE (Pa = 0.747) and SFRP1 (Pa = 0.610) genes, respectively. Recently, a study showed that the SQLE gene could be used as a potential prognostic biomarker for the identification of head and neck squamous cell carcinoma[74], while SFRP1 may prevent renal damage in a mouse model through the non-canonical Wnt/planar cell polarity pathway[75]. On the other side, compound 3 showed a considerable decrease in the mRNA expression of ALDH18A1 (Pa = 0.897) and SLC15A1 (Pa = 0.876) respectively. Some clinical and genetic investigations reported that a mutation in the ALDH18A1 gene could generate cerebellar ataxia and cognitive impairment[76], while SLC15A1 polymorphisms were associated with dyslipidemia, considered as an important risk factor for stroke and coronary heart disease[77]. A modest don regulation process was also exerted by this compound on the SLC2A1 gene (Pa = 0.505), noting that a mutation in this gene could lead to a deficiency in glucose transporter type 1 (GLUT1), which will generate severe metabolic disorders[78].

Results also revealed that compound 4 may be useful to increase the mRNA expression process of FTL and FECH genes with corresponding values (Pa = 0.763, Pa = 0.706). Some studies revealed that the FTL gene may act as an iron detoxifier agent since, during reactions involving iron such as electron transfer, there is an important generation of free, highly toxic radicals, that's why the elimination of iron excess by this gene is vital[79]. The FECH gene is vital for the synthesis of ferrochelatase, a key enzyme mandatory for the production of heme[80]. This information is fundamental since heme is an important component of hemoglobin that ensures the fixation of oxygen (O_2) in red blood cells and allows the transport of O_2 from the lungs to the rest of the body[81]. In contrast, compound 4 showed a modest down-regulation process on the mRNA expression level of ELAVL1 gene (Pa = 0.628). It was reported that this gene is the central oncogenic driver for several malignant peripheral nerve sheath tumors[82]. A non-negligible down-regulation process was also exerted by this compound on RARB and SPAST genes with a respective value (Pa = 0.583, Pa = 0.501). This information is very important since a mutation in the RARB gene may cause microphthalmia or diaphragmatic hernia[83], while a mutation in the SPAST gene may cause a rare inherited disorder called hereditary spastic paraplegia which is characterized by weakness and stiffness in leg muscles[84].

***In vitro* anti-inflammatory assay (BSA):** The test of the ability of *C. tougourensis* to inhibit bovine albumin denaturation revealed a moderate effect for both extracts. Thus, the n-BuOH extract showed a maximum inhibitory activity of (68.94±0.01%) at the maximum tested dose of (800 µg mL^{-1}) while (63.47±0.01%) for EA extract. Those data were considered highly significant ($p<0.001$) when compared to standard diclofenac sodium which showed an inhibition percentage of (90.23±0.81%) at the same tested concentration in Fig. 2. It is also interesting to see that the anti-inflammatory effect of the two tested extracts was almost the same at the tested concentrations of 200 and 400 µg mL^{-1}.

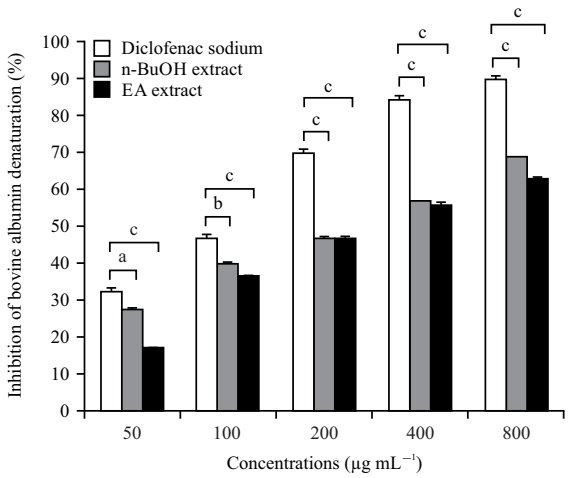


Fig. 2: Anti-inflammatory activity (Bovine albumin denaturation assay) of *C. tougourensis* extracts
All values are expressed as Mean±SD (n = 3). One-way ANOVA followed by multiple Dunnett' s test. Level of significance ap<0.05, bp<0.01 and cp<0.001 is statistically significant with comparison to diclofenac sodium

Table 6: EC_{50} values of standard and *C. tougourensis* extracts from the bovine serum albumin denaturation assay

Extracts/standard	EC_{50} (µg mL^{-1})
n-BuOH extract	335.00±0.03
EA extract	338.00±0.07
Diclofenac sodium	115.76±0.19

The median effective concentration (EC) of each sample is represented in Table 6 and its important to note that comparable EC_{50} values were also recorded for the two tested extracts and were (335±0.03 µg mL^{-1}) for n-BuOH and (338±0.07 µg mL^{-1}) for EA extract while (115.76±0.19 µg mL^{-1}) for diclofenac sodium.

Albumin, the most abundant circulating protein (35-50 g L^{-1})[85], ensures vital functions like the transport of hormones, vitamins and fatty acids[86]. It is also crucial to maintain normal osmotic pressure in blood vessels by stabilizing extracellular fluid volume via contribution by its pression known as oncotic pressure[87]. Hypoalbuminemia may have serious repercussions on kidneys and liver functions[88].

The results are linked with Banerjee *et al.*[89] and Marrassini *et al.*[90], in which they clearly showed the ability of their plant to inhibit albumin denaturation but also proteinases activities and associated that with the richness of their species in flavonoids, tannins and alkaloids compounds. Noting that, these classes of secondary metabolites are very important at both nutritional and pharmacological levels[91] which were identified in previous work[92]. This information is very important since protein denaturation may lead to certain pathological manifestations such as rheumatic arthritis, stroke and even cancer[93] due to abnormal and excessive production of autoantigens. Another study showed that the actual bio compounds of plants, especially flavonoids can maintain the dynamic structure of proteins by limiting the oxidative stress exerted on these molecules through reactive oxygen species (ROS)[94,95].

In the previous study[14], two key compounds were identified, vanillin and rutin, respectively. A study made by Siddiqui *et al.*[96] reported that vanillin may inhibit protein denaturation by inhibiting the activity of lipopolysaccharides (LPS), which can alter mRNA expression of bovine albumin and ovalbumin genes, using the mouse as the experimental model. Another study also revealed that vanillin could inhibit the inflammatory responses induced by LPS in BV-2 microglial cells[97]. This protective effect was explained by these researchers by the ability of vanillin to regulate changes in the early glycation process and amyloid-like aggregation of albumin, thus preventing any change in its spatial conformation.

Rutin may also be a good anti-inflammatory candidate by slowing down the secretion of Interleukin 6 (IL-6) induced by lipopolysaccharides (LPS) as well as the expression of NF-κB[98] since the inhibition of these two pro-inflammatory mediators can alter the structure of proteins.

CONCLUSION

In conclusion, the anti-inflammatory assay by bovine serum albumin (BSA) denaturation test revealed that both extracts have a moderate inhibition effect on protein denaturation with almost the same effect. The various *in silico* tests made in the present study, also showed that some phyto-compounds of this plant could be potent candidate to treat metabolic disorders linked to diabetes and obesity, but also inflammation and skin pathologies. These compounds could also modulate the expression of some genes which could be helpful for the prevention and treatment of some illnesses linked to immunity, metabolism and the nervous system. These compounds could also exert significant cytotoxicity against various human tumor cell lines. In addition, the absorption, distribution, metabolism, excretion and toxicity of these tested compounds were also investigated as well as their physicochemical properties.

SIGNIFICANCE STATEMENT

This preliminary work brings novel information about the pharmacological potential of new phytocompounds that could be very useful for the scientific community but also for pharmaceutical industries to develop new effective drugs, more natural and respectful of human health.

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