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Phytochemical profiling and bacterioactivity of *Commiphora africana* oleoresin from Mauritanian origin

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ABSTRACT

After carrying out an ethnomedical survey which has showed an intensive use of Commiphora africana as traditional healing medicinal plant in Mauritania [1], we chose to investigate the oleoresin as the mostly used by Mauritanian healers. Oleoresin from identified plant has been collected in from 4 Mauritanian localities and extracted with the aid Soxhlet apparatus; and cyclohexane to give extracts MC, KC, RC and GC. Colorimetric test has suggested the presence of flavonoids and terpenoids in these extracts. In addition essential oils in oleoresin have been analyzed by modern techniques like Head Space, Clevenger and hydrodistillation. Furthermore cyclohexane extracts have been subjected to fractioning on a Si-Ge column and resulting fractions (MCF, KCF, RCF and GCF) were tested against Escherichia coli and Staphylococcus aureus strains and, in parallel, analyzed by HPLC_UVDAD and RP-UHPLC_ESItof. This bioguided profiling has permitted to establish for a first time a profiling of flavonoids by RP_HPLC_UVDAD in this plant part and also to present ad replication by RP-UHPLC_ESItof of bioactive compounds, like terpenoids, already identified in other commiphora oleoresins.

Keywords: *Commiphora africana*, oleoresin, bacterioactivity, HPLC_UVDAD, RP-UHPLC_ESItof.

INTRODUCTION

Commiphora africana is very important medicinal plant in West-Africa, particularly in Mauritania where its oleoresin is intensively used by local folk medicine. However, there are insufficient investigations of its biochemical properties, up to now [1, 2]. The goal of this study is to screen chemical and bacteriological properties of this oleoresin justifying its intensive use by local folk-medicine.

EXPERIMENTAL SECTION

Preparation, extraction and biotests of plant material have been carried out at UCME laboratory (University of Sciences, Technology and Medicine, Nouakchott Mauritania), whereas chemical analyses have been achieved at URCOM laboratory (Le Havre University France).

1.1 Collection and preparation of Plant material

First plant has been authenticated by PrBoumediana at Normal School of Higher Education Herbarium Mauritania, then after oleoresin was collected in 4 south-east regions of Mauritania(Magama, Kaedi, Rosso and Gleita). Conditions of collection for these 4 batches are presented on following table 1. First the plant trunk has been stressed by circular cutting with inoxydable knife, then after 2 weeks the oleoresin collected. Oleoresin samples were manually reduced to -2 mm in order to facilitate ultimate solvent extraction.

Table 1: Collection of *Commiphora africana* oleoresin

place of collection	abbreviation	GPS	date of collection	observation
Maghama	M	15.4051°N/13.9123W	january, 2010	young age
Kaedi	K	16.1330°N/13.1140W	january, 2009	middle age
Rosso	R	16.5128N/15.8050W	april, 2008	middle age
Gleita	G	17.0110°N/12.7851W	january, 2010	advanced age

1.2 Solvent extraction and column fractioning

Powdered oleoresin has been extracted with cyclohexane and Soxhlet apparatus during 8 hrs; and resulting cyclohexane extracts (MC, KC, RC and GC) fractionated on a chromatographic column Si-Ge type, with organic solvents of growing polarity. Resulting fractions (MCF, KCF, RCF and GCF) have been subjected to chemical analyses by HPLC-UVDAD and High Resolution UHPLC_ESItof and in parallel tested against *Escherchia coli* and *Staphyllococus aureus* strains.

1.3 Phytochemical screening

In order to screen chemical family potentially responsible for bioactivity of oleoresin as flavonoids, terpenoids/steroids, cyclohexane extract has been subjected to colorimetric screening according to literature [1, 3, 4, and 5].

1.4Bacterioactivity measurement

Bacterioactivity measurement was done using Disc Diffusion Method (Kirby-Bauer Method),according to literature [1, 6]. Optimally cultivated *Escherchia coli* and *Staphyllococus aureus* strains were spread on the surface of Muller-Hunton medium, and saturated with the tested fraction and witness, placed at the surface, then after immediately incubated during 24 Hrs at 37°C. Clear zone Diameter around diffusion disc corresponds to bacterioactivity.

1.5 Chemical analysis by HPLC-UVDAD and RP-UHPLC_ESItof

In order to present a profiling of subclasses of flavonoids possibly linked to the activities of fractions, we have analyzed them withHPLC_UVDAD and High Resolution UHPLC_ESItof, using optimized concentrations.

1.6 Reagents and equipment

All reagents used for extraction, fractionating and chemical analysis were HPLC grade. They were from Accros Organics, except for methanol ultrapure (mass spectrometry quality) which was from PANREAC France. Muller-Hunton and culture media were from Biokar Diagnostics-Espagne whereas6 mm Diffusion disc was from Whatman France. Positif witness (fosfomycine from Mast-France 50 µg) and bacterial strains (isolated from patients) were donated by Nouakchott Hospital Center (Mauritania). To detect essential oils in cyclohexane extracts 2 GC systems have been used: GC 2000 ThermoFinnigan coupled to Automass III spectrometer equipped with BPX5% column and GC Agilent 6890N, equipped with CARBOWAX 20 column and FID detector. SPME fiber type DVB/Car/PDMS-2 was from Supelco Ltd-France. Analysis by HPLC-UVDAD has been achieved with Agilent Système(HPLC_UVDAD HP-1100 equipped with column C18 Eclipse XDB, 4.6 x150 mm, 5µm) controlled by Agilent Chemstation software, whereas UHPLC-ESI analysis have been carried out with the aid of UHPLC Agilent Technology modèle 1290 equipped with short column (SB-C18 Zorbax 2.1 X 3 cm 1,8µm) coupled to analyzer type Q-TOF 6530 and ESI inizer type JetStream.

RESULTS AND DISCUSSION

Oleoresin from identified *C africana* has been collected from 4 localities in South-East of Mauritania (§ 1.1). Collected samples have been put into polyethylene bags and conserved at ambient temperature and protected against light (figure 1).

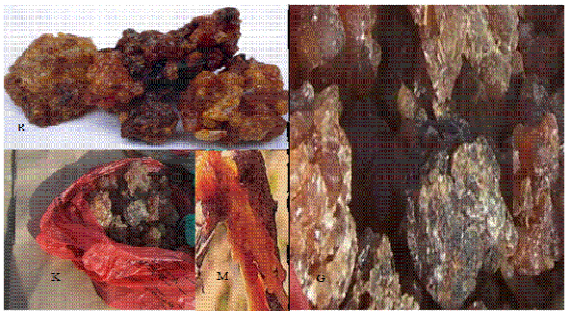

Figure1: Collected oleoresins from batches M, K, R and G

2.1 Colorimetric screening for flavonoids and terpenoids

Cyclohexane extracts have been screened with colorimetric analysis (§1.3) for the presence of flavonoids and terpenoids. Results of this colorimetric detection are presented on table 3.

Tableau 3: Phytochemical screening of extracts for the presence of flavonoids and terpenoids

chemical class Products	flavonoïds by Shibata test			terpenoids by Salkowski test		
	Color		Detected compound	Couleur		Detected compound
	Before indicator	After indicator		Before indicator	After indicator	
MC	beige	Yellow	flavonoïd	Yellowish	red	terpenoids
KC	incolored	yellowish	flavonoïd	Colorless	red	terpenoids
GC	beige	Yellow	flavonoïd	Yellow	red	terpenoids

To the best of our knowledge, flavonoids have not yet been investigated in C africana oleoresin; whereas terpenoids have been revealed as monoterpenoids by G Provan et al [7]. Revelation of flavonoids by chemical screening is limited [8]. Hoewever,sesquiterpenoids have been determined in *C myrrha, C holtziana, C kua and C guidottii* oleoresin [1, 9] and as triterpenoids in *C wightii, C incisa, C holtziana, C confusa, C kua*and *C glandilosa*oleoresin [10]. In conclusion revelation of terpenoids in this oleoresin is in perfect agreement with results obtained for other Commiphora species whereas flavonoids detection is revealed in *C africana* oleoresin for the first time.

2.2 Bacteriological activity of cyclohexane extracts and fractions

Bacteriactivitiy of cyclohexane extracts and fractions of 4 batches from its column fractioning MCF; RCF; KCF and GCF (§1.2) were investigated with disc diffusion method against *E coli*et*S aureus* (§I.3).

2.2.1 Bacterioactivity of cyclohexane extracts

Bacterioactivity for cyclohexane extracts have been measured against *E coli* and *S aureus* (§1.4), for only 3 batches MC, KC and GC (table 4).Unfortunately results were not exploitable for RC batch. This table shows varying moderate bacterioactivity.

Tableau 4: Bacterioactivity of cyclohexane extracts MC, KC and GC

parameter / extract	Concentration ≈mg/ml	activity (diameter of inhibition) mm		observation
		E coli	*S aureus*	
MC	6.5	7	6,5	diffuse
KC	7.3	8	7	diffuse
GC	6.7	8	9	regular

2.2.1 Bacterioactivity of fractions

Cyclohexane extracts have been subjected to column fractioning to give products MCF; RCF; KCF and GCF (§1.2). Bacterioactivity of these fractions have been measured against *E coli* and *S aureus* (§1.4). Results of fractions bacterioactivity measurement are presented on figure 2.

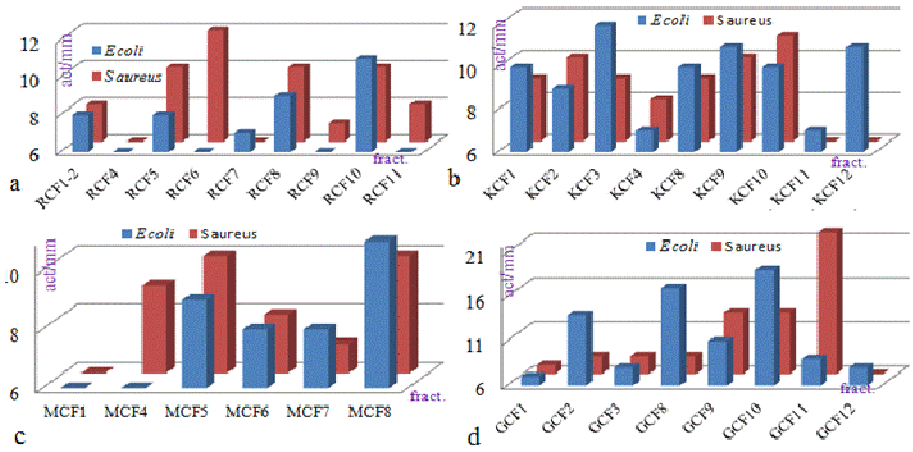

Figure2: Bioactivity of fractions, a) RCF ; b) KCF ; c) MCF ; d) GCF

As shown figure 2 the batch GCF (aged plant) corresponds to the most active fractions exceptionally reaching 19 mm and 21 mm against *E coli* and *S aureus*, respectively. Table 5 recapitulates the most bacterioactive fractions.

Tableau 5: most active fractions recapitulation

Fractions/ strain	RCF	KCF	MCF	GCF
E coli	6-11mm	10-12mm	9-11mm	9-19mm
S aureus	10-12mm	6-11mm	9-10mm	8-22mm
most active fractions	RCF5, RCF6, RCF8, RCF10	KCF3, KCF9, KCF10, KCF12	MCF5, MCF8	GCF8, GCF9, GCF10, GCF11

This table shows that active fractions (12 mm, 11 mm, 10 mm et 22 mm for RCF, KCF, MCF et GCF; respectively) largely exceeds bacterioactivity measurements of *Commiphora molmol* oleoresin extract reported by Abdallah et al, namely 6-10.8mm [11]. In order to elucidate compounds possibly responsible for these activities we have analyzed the active fractions by coupled methods: GC_MS, HPLC_UVDAD and UHP LC_ESItof.

2.3 Chemical analysis

Fractions MCF, KCF, RCF and GCF have been analysed in order to detect essential oils, flavonoids families and for dereplication of bioactive compounds already identified in resins of other commiphora species.

2.3.1 Essential oils

Our analyses (Head Space concentration, Clevenger hydrodistillation and GC_EIms) shows there is no measurable essential oils in *C africana* oleoresin originated from Mauritania; in contrast with *C africana* from Kenyan origin reported by G Provan[7]. This result may be explained by differences in climate (sahelo_sahelian for Mauritania, hot and humid for Kenya) [12].

2.3.2 Flavonoïds

The compounds with 2 benzenerings A and B attached to aheterocyclepyrane or pyroneC (figure 3) are named flavonoids. They are reputed for their bioactivity [3].

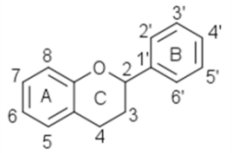

Figure 3: Basic structure of flavonoids

Flavonoids absorb at two bands: I (300-550 nm), corresponding to A ring and II(240-295 nm),corresponding to B ring. After optimization, the waves 254 nm, 272 nm, 330 nm et 372 nm have been selected to detect the presence of flavonoïds (classical bands I and II). Compiling of λ_{max} from UV spectra may be used to reveal absence or presence of flavonoid sub-classes(flavanones, flavanonols, flavones, flavonols, anthocyanines and isoflavones) and eventually their primary identification [13, 14]. Analyzing the HPLC-UVDAD spectra from 4 batches fractions RCF; KCF; MCF and GCF, the flavonoids subclasses have been assigned comparing λ_{max} with literature [3,13, and 14]. Detection of flavone in fraction GCF7 (aged plant), depicted on figure 4, presents an illustration of this analysis.Table 6 presents the profiling results of flavonoids subclasses with HPLC-UVDAD analysis. This table shows the absence of flavones in fractions MCF and KCF. Both RCF_{14} and KCF_9 fractions contain one flavan-4-ol compound for every fraction. GCF fractions (aged plant) are the richest in flavonoids with the presence of 6 flavan-4-ols (GCF_2, GCF_4, GCF_5, GCF_{10}) ; 3 flavones (GCF_3, GCF_5, GCF_7) and 1 flavanonol (GCF_7), whereas MCF fractions (young plant) are the poorest in flavonoids. Fractions from KCF and RCF (middle aged plant) are intermediate. However Barioactivity is observed for fractions from 4 batches, it is remarkable that most active fractions of 4 batches contain flavones, flavan-3-ols and flavanonols. This is an indication that bacterioactivity is at least partially linked to presence of flavonoids, and also this bacterioactivity and flavonoids content are potentially linked to the plant age. Flavonoids in commiphora oleoresins have been insufficiently investigated [15, 16]. In addition colorimetric screening is limited to *C myrrha*and *C mukul* [17, 18]. Furthermore only one flavonoid compound (petunidin 3-rhamnoglucoside) has been isolated in *C angolensis* oleoresin [19]. To our best knowledge flavonoids profiling by UHPLC_UVDAD on authenticated material has been achieved for these oleoresins for the first time.

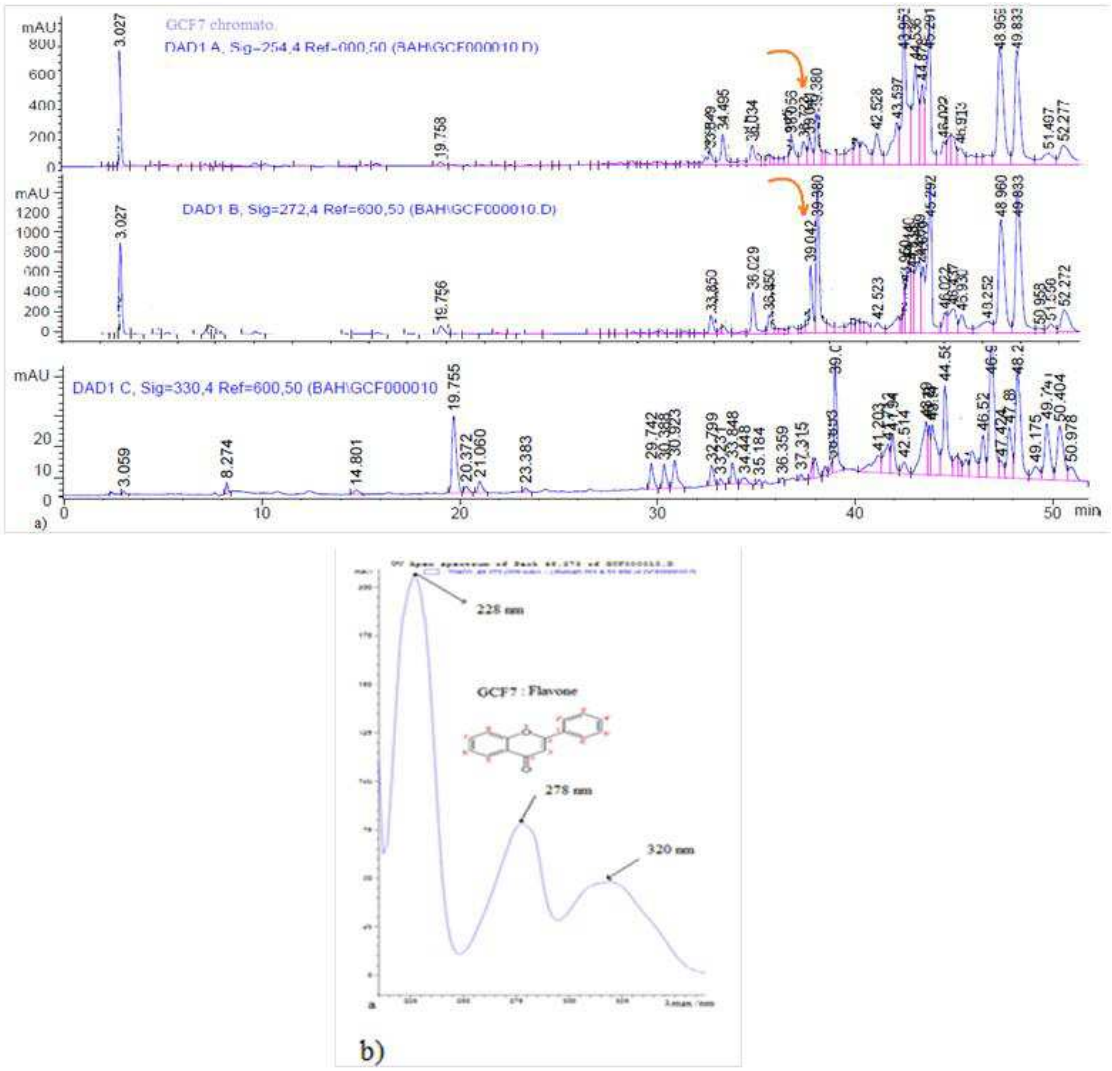

Figure 4: Analysis of fraction GCF_7 par RP-HPLC-UVDAD

a) chromatogramm b) UVDAD spectraof peak 39.0 min

Table6: Profiling of flavonoids subclasses by HPLC_UVDAD

Fraction	TR/min	λ_{max} /nm	Flavonoids
RCF5	8.4	262/294	Flavone
RCF14	33.5	282	flavan-3-ol
KCF2	16.5	230/280	Flavanol
	33.2	255sh/292	Flavanonol
KCF9	16.4	230/276	Flavanone
	37.6	288	flavanone/flavan-3-ol
MCF7	16.6	228/276	Flavanone
GCF2	6.2	284	flavanone/1 flavan-3-ol
GCF3	10.5	248/312/348	Flavone
GCF4	1(14.0); 2(16.2)	1(280); 2(284)	1flavan-3-ol, 2 flavanone
GCF5	1(8.1) ; 2(11.5)	1(254/328); 2(280)	1(flavone); 2(flavan-3-ol)
GCF_7	1(48.3) ; 2(32.8)	1(228/278/320); 2(274sh, 312)	1(flavone) ; 2(flavanone)
GCF10	1(3.7); 2(4.9)	1(280); 2(286)	1 flavan-3-ol; 2 flavane
GCF11	1(4.8) ; 2(16.8)	1(250/285); 2(230/280)	1 flavanone, 2 flavan-3-ol

2.3.3 Bioactive compounds dereplication by RP-UHPLC_ESItof

Fractions analysis byUHPLC_UVDAD has showed the presence of compounds absorbing at 240-290 nm, probably corresponding to phenolic compounds, indicating the possibility of its analysis by RP-UHPLC_ESItof[1, 17, and 20]. This technique has been used to dereplicate bioactive compounds already identified in other Commiphora oleoresins, based on extraction of signals corresponding to the exact mass and basic chemical formula. As an

illustration of this analysis, figure 5presents a dereplication of these compounds in fractions GCF_{10} and RCF_9.All detected compounds are of the types di(-tri)terpénoïde, phytosteroid or ferulic ester. Two triterpenoids (myrrhanol A et myrrhanone A), known for their anti-inflammatory potential and which have been isolated from C myrrha oleoresin, have been detected in MCF, RCF and GCF fractions. Another poly-podane type compound ODHMPD[16] has been detected in all 4 batches fractions. This phytocompound is a natural analog to ferulic ones, used in cosmetic industry to treat wrinkles and skin ageing[16]. The presence of the 3 above mentioned phytocompounds may be justification of *C africana* oleoresin use by Mauritanian folk medicine as an anti-inflammatory agent and as a treatment against infected tumors [1]. Indeed the isolated ferulic esters from C myrrhaoleoresin were tested active against malign cellules MCF-7[16].

In future these bioactive compounds must be isolated with aid of preparative HPLC and bioguided analysis and extend the tests to cytologic investigations

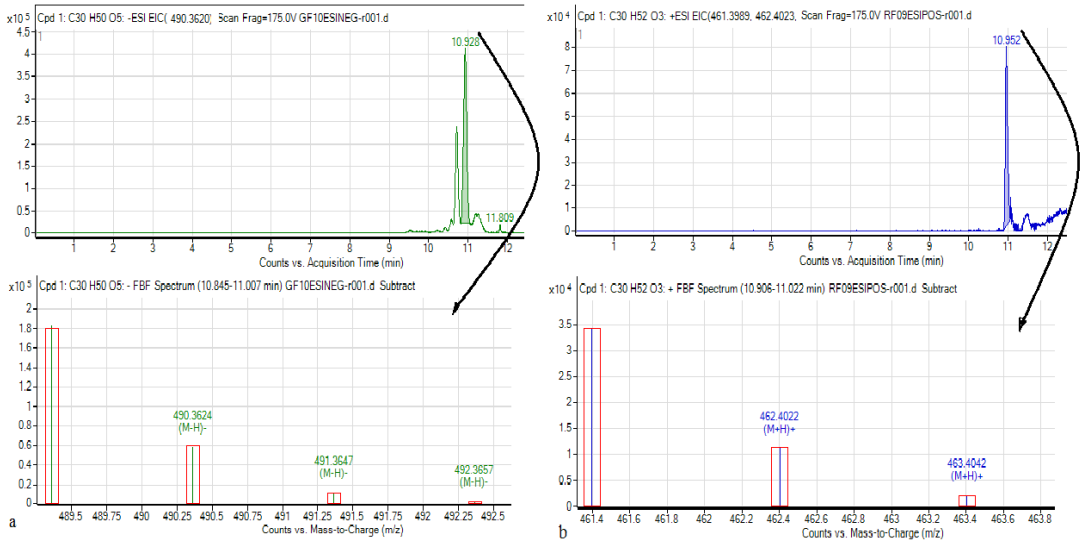

Figure 5: Déréplication des phytocomposés bioactifs par UHPLC_ESItof a) Myrrhanone A dans GCF GCF_{10}, b) Myrrhanol A dans RCF_9

CONCLUSION

Although Commiphora *africana oleoresin*is intensively used to heal numerous illnesses in Mauritania, it is insufficiently investigated by modern studies. Cyclohexane extracts from 4 batches collected from authenticated plant from Mauritania have been fractionated and fractions analyzed by HPLC-UVDAD and UHPLC-ESItof in order to present a profiling of flavonoids subclasses and to dereplicate bioactive compounds already identified in other commiphora oleoresins. In the same time bacterioactivity of these fractions has been measured against Escherechia coli and Staphyllococus aureus strains. First analysis by HPLC-UVDAD has permitted to assess the presence of flavan-4-ol in KCF, RCF and and GCF fractions; flavones and flavanonol in GCF whereas MCF ractions (young plant) seems to be the poorest in flavonoids. This study shows that bacterioactivity is linked to the presence of flavonoids and that bobacterioactivity and flavonoid content are linked to the age of plants with young plant oleoresin being poorest in flavonoids and less bacterioactive, middle aged intermediate bacterioactivity and the aged having most bacterioactive fractions and greatest flavonoid content. Also analysis by UHPLC-ESItof has permitted to dereplicatetwotriterpenoids (myrrhanolA et myrrhanone) in addition ferulic esters, which are known for bioactivity. This study must be continued towards isolate bioactive compounds and, extending the tests to cytologic investigations.

In future we will apply further detailed bioguided analysis conjugated with semi-preparative chromatography and extend biotests to cytological investigation in order to isolate and characterize new bioactive compounds.

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