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Research Article

PHYTOCHEMICAL COMPOSITION AND BIOLOGICAL EFFICIENCY OF *Capsicum annuum* L. AND *Strophantus hispidus* L.ORGANIC EXTRACTS AGAINST *Ceratitis cosyra* (WALKER), MANGO PEST INSECT, IN BURKINA FASO

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A B S T R A C T

A study of eight plant materials (*Capsicum annuum* L, *Cleome viscosa* L, *Mytragina inermis* (Wild) kuntze, *Strophantus hispidus* A.CD, *Ocimum basilicum* L., *Cassia nigricans* Vahl., *Cassia occidentalis* L.)organic extracts biological efficiencyagainst adults of *Ceratitis cosyra* (Walker) (Diptera: Tephritidae), which cause big damage to mango production in Burkina Faso, has been done in laboratory conditions. Extraction of the different substances has been done using solvents of increasing polarity (n-hexane, ethyl acetate and methanol) by Nair method (Kambou *et al*., 2008).The experimental design was a randomized Fisher block bioassays of 25 treatments in 5 replications. Phytochemical analysis of the active fraction of *C. annuum* and *S. hispidus* has been done using Ciulei' method (1982).The methanol extract of *M. inermis* provided the higher yield of 18.13%. Between the twenty-four extracts tested, the one of *C. annuum* ethyl acetate, followed by those of n-hexane and methanol of *C. annuum*, recorded a significant rate of mortality after 24 hours observation respectively 13.8% and 5%. After72 hours' observation, the ethyl acetate extract of *C. annuum* and methanol of *S. hispidus* gave the best mortality respectively 18% and 15%. Phytochemical analysis revealed that *C. annum* ethyl acetate extract fractions are abundant in sterols, triterpenes, alkaloids and flavonoids The methanol extract of *S. hispidus* contains many phytochemical groups recognized to be toxic and likely to have insecticidal properties. Only the extract of *S. hispidus* contains carotenoids. The purification of these most active fractions would make possible to identify active molecules and to build an integrated pest management program based on *C. annuum* and *S. hispidus* formulations against *C. cosyra.*

INTRODUCTION

In Burkina Faso, fruit and vegetable sector occupy 16% of agricultural production and contributes 4.5% of GDP and 2.8% of export income (IRSAT, 2014). In terms of produced quantity, mango is considered as "the first national fruit". Indeed, in 2017 the quantity of fresh mango exported on the international market was 4383,324 tons. It is estimated at 1274 tons in the sub-region's market. The delivered mangoes on the national market was 30,400 tones (APEMAB, 2017). However, this sector faces many constraints which limit its expansion. These constraints are biotic (diseases and insect pests) and abiotic. Between these biotic constraints, *Bactrocera dorsalis* (Hendel) and *Ceratitis cosyra* (Walker) are the two most harmful species for mango in West Africa. Tephritidae attack these fruits and destroy their nutritional and market qualities. Indeed, Tephritidae are among the redoubtable insects of the mango, including *C. cosyra*.

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It is a pest of many fruit crops still representing a serious threat of crop losses. The losses caused by fruit flies in Burkina Faso, can reach 80% of the production and depend to the period of mangoes production, to the cultivars and to production areas (Ouédraogo, 2011).

West African Countries as Burkina Faso, which have a large market in Europe, have been severely affected. Indeed, eight (8) interceptions and destruction of mangoes coming in European Union market, were noted in 2018 for Burkina Faso (COLEACP, 2018).

Several methods are used to control this pest. Between them, is the use of synthetic pesticides against C. cosyra fruit fly, which can develop resistance to these chemicals, making this control difficult (Ju-Chun Hsu *et al*.,2012). In addition, the massive use of synthetic pesticides is harmful to the environment and human health.

According to this, the need of alternative method is now required by the use of environmentally friendly, inexpensive natural plant-based substances as a way of pest management. Some work on the efficacy of organic plant extracts against

Ceratitis genus has been successfully conducted. These are those of Maria Miguel *et al.,* (2010) who worked on the toxic effects of three essential oils against *C. capitata* in southern Portugal. Silva,Márcio A *et al.,* (2011) investigated the toxic effect of neem seed cake on the prepupal stage of the Mediterranean fruit fly *C. capitata* (Diptera: Tephritidae). Giovanni Benelli *et al.,* (2013) worked on the bio-toxicity of *Melaleuca alternifolia* aerial parts extracts, grown in Italy, against *C. capitata* (Diptera: Tephritidae) adults and its parasitoid, *Psyttalia concolor* (Hymenoptera: Braconidae). Andrea Oviedo *et al.,* (2017) studied the effects of biopesticides on the pupae and adults mortality of *Anastrepha fraterculus* and *C. capitata* (Diptera: Tephritidae), two of the fruit production main pests in South America. Stella *et al.,*(2017) have studied the toxicity and hormonal effects of three components (limonene, linalool and α-pinene) of essential oils on Mediterranean citrus flies (*Ceratitis capitata*) adults. Mabrouka Ghabbari *et al.,* (2018) worked on the insecticidal effects of four plant species leaf extracts: (*Ruta graveolens, Eriobotrya japonica, Rubus ulmifolius* and *Ficus carica*) altering the behavior of adults of *C. capitata* (Wiedemann) (DipteraTephritidae).So, Very little work with plant extracts has been conducted to fight against *C. cosyra*.This is why it became necessary to study the biological efficiency of eight plant materials(*Capsicum annuum* L, *Cleome viscosa* L, *Mytragina inermis* (Wild) kuntze, *Strophantus hispidus* A.CD, *Ocimum basilicum* L., *Cassia nigricans* Vahl., *Cassia occidentalis* L.) organic extracts against adults of *C. cosyra*(Walker) (Diptera: Tephritidae) with the aim to find new actives ingredients and to produce some formulations.

MATERIAL AND METHODS

MATERIAL

The fresh and green leaves of the plants (*M. inermis, P. kotschyi, S. hispidus, O. basilicum, C. nigricans, C. viscosa, C. occidentalis,* and ripe fruits of *C. annuum*) have been collected from the south-west region of Burkina Faso and have been identified by the plant taxonomy experts of Forest Production Department. After the time they have been dried in the shade, they were crushed and reduced to powder. The plant material was powdered leaves of *M. inermis, P. kotschyi, S. hispidus, O. basilicum, C. nigricans, C. viscosa, C. occidentalis* and ripefruitsof *C. annuum*.

The animal material consisted of *C. cosyra* adults. Adults of *C. cosyra* were obtained from the incubation of *Sclerocaria birrea* and *Sarcocephalus latifolius* fruits collected in Kénédougou and Comoé provinces.

METHODS

Vegetable Powders Extraction

Different extracts were obtained from the plant powders. The extractions were carried out according to Nair's method (Kambou *et al.,* 2008).The principle of this method is based on the ability of bioactive substances of plant origin to be carried out by solvents of different polarities.

For the present study, a sample of each vegetable powder (400 g) was macerated with 1200 ml for 24 hours, three times, at room temperature of the laboratory, and then extracted by successive percolation with solvents of increasing polarity, until completely exhaustion. The successive extracting solvents used were n-hexane, ethyl acetate and methanol (VWR Prolabo Chemicals, France).

The obtained extracts were then concentrated under reduced pressure using a rotavapor Büchi R 110 with a marry-regulated bath at an appropriate temperature of each extracting solvent. The concentrated extracts were then dried in a Cole Parmer Instrument brand ventilated oven set at 45 ° C. The mass of dry extracts obtained was determined by weighing using a Radwag precision analytical balance Model AS 110.R1.

Biological Efficiency of Organic Plant Extracts Against C. cosyra.

In the laboratory, for the test of eight (8) organic plant extracts on *C. cosyra* adults, 100 mg of extracts from each sample were weighed and placed in a micro tube, in which 1 ml of acetone was added. The mixture was dispersed using a vortex. The organic extract and acetone mixture of each microtube was then poured into 99 ml of water. In addition, 1.50 ml of a syringe have been taken and placed in a vial containing previously 1 g of sugar to which 0.25 g of cotton was added. Each vial was covered with a muslin cloth, and then 20 insects, 3 days aged were aspirated with a mouth aspirator and placed inside the sampling vial. Each vial has been maintained by elastics. This operation has been repeated 5 times for each of the eight organic extracts. Each flask containing the fruit flies was placed on shelves and kept in the flies breeding room. The mortality rate of *C. cosyra* has been evaluated after 24h and 72h.

Bioactive Extract Fractionation

A test portion of 5.00 g of the *C. annum* ethyl acetate extract which was the most active was dissolved in 50 ml of extracting solvent. The extract solution was mixed with 50 g of silica gel for column chromatography (Silica Gel 60, 0.063-0.20 mm, Merck) in ratios 1/10; m / m. The mixture of silica gel and extract was homogenized using a spatula, and then placed in a ventilated oven at a temperature of 45 ° C to remove the extracting solvent. The silica mixture and dried extract was transferred into an Erlenmeyer flask and a volume of 250 ml of analytical acetone was added thereto. The extracted mixture and solvent was macerated for 1 h and then was transferred to a percolator in the form of a glass column. After leaching percolate with a total volume of 750 ml of acetone, the silica gel and residual extract mixture was macerated and drilled successively until exhaustion with 750 ml of methanol, ethyl acetate and 1-butanol analytic saturated with distilled water.

The fractions of obtained extracts with acetone; methanol; ethyl acetate and 1- butanol were concentrated under reduced pressure using a Buchi-type rotary evaporator and then dried in a ventilated oven set at 45 ° C. The mass of the dry fractions was determined using the Radwag brand analytical balance and the extraction yields by the percentage expression of the dry extracts obtained with respect to the test portion of the initial total extract.

Bioactive Extract Fraction Chemical Profile

The chemical profile of the ethyl acetate extract fractions of the most efficient *C. annum* vegetable powder was determined by thin layer chromatography. The goal was to find groups of chemical substances which could be isolated, purified and identified.

A volume of 5 µl of the extract solution of each fraction was deposited on a stationary phase of silica gel (Silicagel, G-60, F254). The deposited extract fractions were migrated into a solvent system composed of chloroform; glacial acetic acid; of methanol and distilled water in the respective proportions of: 64; 32; 12 and 8; v / v / v / v. After migration, the chromatoplates were dried with a hair dryer, and developed spots were observed in daylight and under ultraviolet radiation (254 nm and 366 nm).

Phytochemical Screening of Bioactive Extracts

For the present study, the most active identified ethyl acetate extracts were screened according to the method described by Ciulei I. (1982). A portion of the unhydrolyzed ethyl acetate extracts of each plant material was used to find polyphenolic compounds (tanins), saponosides, reducing compounds and alkaloid salts. Another portion of the ethyl acetate extracts was hydrolysed in an acid medium to extract the total genomes for sterol and triterpene glycosides, flavonoids, anthracenosides, coumarins and derivatives, anthocyanosides.

Statistical Analysis and Data Processing

The data collected during these different studies were processed with the Microsoft Office 2013 Excel 2013 software. An analysis of variance and a means comparison according to the Newman - keuls test at 5% probability threshold have been done with GenStat Edition 11 software.

RESULTS

Plant Extracts Yields

The extraction of vegetable powders with n-hexane gave some yields which varied from 0.66% to 5.76%. The best yield has been obtained with the vegetable powder of *M. inermis* and gave the lower yield with the vegetable powder of *O. basillicum* (Table 2).

The ethyl acetate extracts gave some yields which varied from 1.64% to 5.91%. The vegetable powder of *O. basillicum* gave the highest yield while the one of *C. occidentalis* gave the lowest yield. The yields with methanol varied from 1.45% to 18.13%. The most important yield has been obtained with *M. inermis* vegetable powder and the least with the one of *C. occidentalis*.

In general, the lowest yields of plant extracts were observed with n-hexane while methanol gave the highest yields. The vegetable powder of *M. inermis* was important in apolar substances, extractable by n-hexane and polar by methanol.

Table 2 Yields of the different vegetable powders extraction.

Powder vegetable	Test sample (g)	n-Hexane		Ethyl-acetate		Méthanol	
		Extract mass (g)	Yield (%)	Extract mass (g)	Yield (%)	Extract mass (g)	Yield (%)
C. viscosa	400.00	3.54	0.93	8.91	2.35	17.51	4.63
C. annuum	400.00	18.92	5.23	7.48	2.07	34.16	9.44
P. kotschyi	400.00	15.55	4.08	20.00	5.25	57.60	15.11
C. nigricans	400.00	3.61	0.93	18.83	4.87	22.94	5.93
C.occidentalis	400.00	10.48	2.73	6.31	1.64	5.56	1.45
S. hispidus	400.00	6.27	1.64	8.72	2.29	11.69	3.07
O. basilicum	400.00	2.49	0.66	22.47	5.91	11.28	2.97
M. inermis	400.00	21.71	5.76	13.11	3.48	68.37	18.13

Biological Efficiency of the Different Organic Extracts

After 24h, statistical analysis has shown that there is a very high significant difference (P ≤ 0.001 at the 0.05 threshold) between the different organic extracts (Figure 1). The average effect of the different organic extracts(2.65%)is an increase of 100% mortality in comparison with the untreated control. The n-hexane, ethyl acetate and methanol extract of *C. nigricans* showed zero deaths in 24 hours of observation. Between the *C. occidentalis* methanol extract and the untreated control there is not a significance difference. The highest mortality rate after 24 hours' observation was obtained with ethyl acetate *C. annuum* extract (13.8%). The n-hexane, methanol extract of *C. annuum* (5%), n-hexane of *P. kotschyi* (5%) and *O. basilicum*-ethyl acetate (5%) which are not different between them, were followed.

After 72 hours, the statistical analysis showed that there is a very significant difference (P ≤ 0.001 at the 0.05 threshold) between the different plant extracts. The average effect of the different plant extracts (6.04%) against *C. cosyra* adults is an increase of 202.08% mortality in comparison with the untreated control. The highest mortality rate after 72h has been observed with the ethyl acetate extract of *C. annuum* (18%) followed by the methanol extract of *S.hispidus* (15%), of*C. annuum* (13%) and the hexane extract of *P. kotschyi* (11%) (Figure 2).

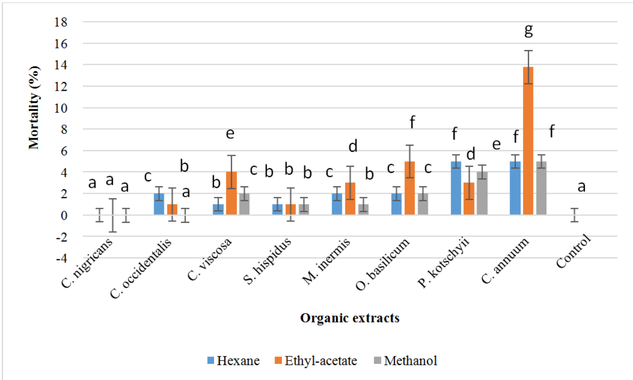

Figure 1: Biological efficiency of plant extracts against *C.cosyra* adults after 24h.

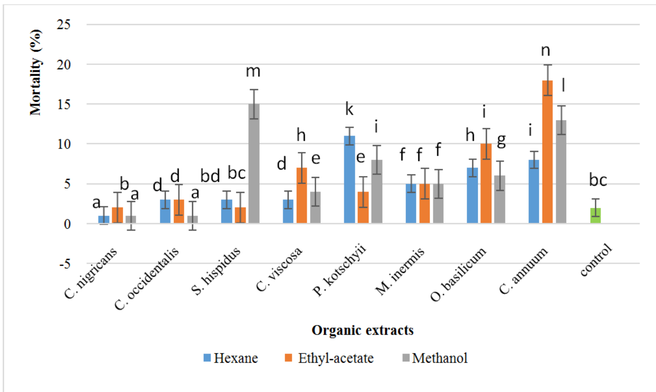

Figure 2: Biological efficiency of plant extracts against *C. cosyra* adults after 72 h.

Fractionation of Active extract

The fractionation of the most active (ethyl acetate) extract of *C. annum* yielded some fractions which varied from 0.57% to 66.43%. The highest yield of the extracted fraction has been obtained with acetone and the lowest with ethyl acetate and 1-butanol (Table 3)

Table 3 yields of *C.annuum* ethyl acetate extract fractions.

Fractions	Extracts mass (g)	Yield (%)
Acétone	3.74	66.43
Méthanol	0.36	6.38
Ethyl-acetate	0.033	0.58
1-butanol	0.032	0.57

Biological Efficacy of Active Extract Fractions

The statistical analysis showed that there is a very significant difference ($P \leq 0.001$ at the 0.05 threshold) between the *C.annuum* ethyl acetate extract fractions. The average effect of all the fractions (1.32%) against *C. cosyra* is an increase of 100% mortality in comparison with the untreated control. The high mortality rate after 24 h has been obtained with the *C. annuum* acetone fraction (1.99%), followed by the 1-butanol fraction (1.05%) and the *C.annuum* methanol (0.91%) (Figure3). After 72 hours, statistical analysis showed very significant differences ($P \leq 0.001$ at the 0.05 threshold) between the *C.annuum* ethyl acetate extract fractions. The average effect of these fractions(1.32%), is an increase of 304.87% mortality compared to the untreated control.. The high mortality rate was observed with the 1-butanol fraction and the methanol (20%) and acetone (9.8%) fraction of *C. annuum* (Figure 4).

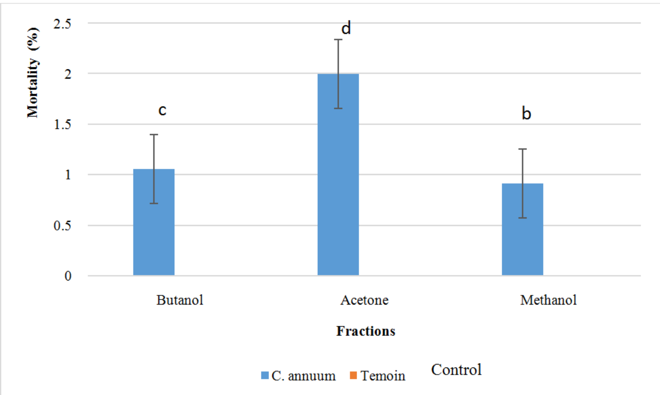

Figure 3 Efficacy of the different fractions against *C. cosyra* adults after 24 h.

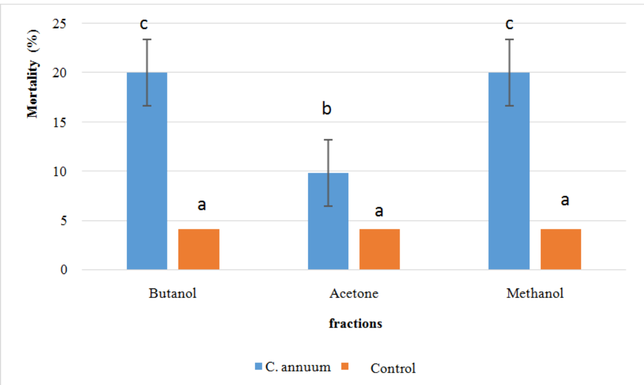

Figure 4 Efficacy of the different fractions against *C. cosyra* adults after 72 h.

Phytochemical Screening of Active Extracts

The phytochemical screening of the *C. annuum* ethyl acetate extract and*S. hispidus* plant powder revealed an abundance of anthraquinones, sterols and triterpenes as well as a significant presence of volatile oils. In the present study, phyto-chemical analysis of the methanol extract revealed more chemical constituents including sterols and triterpenes, coumarins and derivatives, anthraquinones, alkaloids, flavonoids and anthocyanosides.

The chemical phyto-chemical analysis of the fractions of *C. annuum* and *S. hispidus* active extracts (n-hexane, ethyl acetate, methanol) give the possibility to highlight groups of chemical compounds which are potentially bioactive: sterols and triterpenes, coumarins and derivatives, anthraquinones, alkaloids, flavonoids, tannins and cardenolides (Table 4).

Table 4 Phyto-chemical composition of *C. annuum* and *S.hispidus* active fractions.

Chemical groups	Fractions of extracts					
	n-Hexane		Ethyl-acetate		Méthanol	
	1	2	1	2	1	2
Stérols/triterpenes	+	+	+	+	+	+
Anthraquinones	+/-	(-)	+/-	+	+	+
Coumarins and dérivatives	+	(-)	+/-	+/-	+	+
Carotenoids	+	+	+/-	+/-	(-)	(-)
Cardenolides	+/-	+	+/-	+	+/-	+
Alkaloids	+/-	+/-	+	+	+	+
Tannins	(-)	(-)	(-)	+	(-)	+
Flavonoids	+	(-)	+	+	+	+
Saponosids	(-)	(-)	(-)	(-)	+/-	+
Anthocyanosides	(-)	(-)	+/-	+	+	+
Reducing compounds	(-)	(-)	(-)	+/-	+	+

Legend: 1 = *C. annuum*; 2 = *S.hispidus* +/- = traces; + = presence; (-) = absence

DISCUSSION

The biological efficiency of the different organics extracts of the natural substances depend on the structure of their active ingredients, the way they act, their apply rate, their action persistence, the length of exposure to the organism. It depends too, on exogenous factors as temperature, insect anatomy and morphology, cell permeability for the product. During this experimentation the efficacy of natural substances has been determined by all these factors. During these tests, biological efficiency of these organic extracts was weak perhaps in relationship with the apply rate. However, the obtained results allow saying that it depends on one part, to the active ingredients nature in these organic extracts but in other part to the capacity of *C. cosyra* enzyme to degrade some of ingredients, to the aim to not affect the different biochemistries process going inside.

The biological efficiency of the various plant materials organic extracts against *C. cosyra* is related to the nature of phytochemicals groups they contain. Indeed, the sterols, terpenoids, alkaloids, anthraquinones and phenolic compounds highlighted in the various extracts could justify the varied activity of the extracts tested. Thus, the fractions of the *C. annuum* methanol and n-butanol extracts which contain steroidal and triterpenic glycosides, flavonoids, anthraquinones, coumarins and alkaloids have been shown to be more efficient, confirming the results obtained by Kambou *et al.*, (2011, 2015) demonstrating the insecticidal and insect repellent properties of *C. annuum* against insects of green beans and tomatoes (*Bemisia tabaci, Caliothrips impurus, Caliothrips impurus* and *Helicoverpa armigera*). However, the aqueous extracts were not efficient against coleopteran (*Nisotra* spp.) because of their anatomy. In that work, chemical analysis have shown an important quantity of anthraquinons, sterols, tri-terpens and a significant presence of volatiles oils. In this work, phytochemical analysis of the methanolic fraction showed more chemicals constituents particularly sterols, triterpens, anthraquinons, but derivated coumarins, alkaloids, flavonoïds and desanthocyanosides.

Indeed, terpenes are hydrocarbons of plant origin which have bacterial properties. Their insecticidal activities are not excluded as well, since insects generally do not have the capacity to turn tri-terpens into sterols. Terpinoids are therefore often very toxic like anthraquinons. Anthraquinons which belong to the family of aromatic polycyclic hydrocarburs are recognized to be ingredients of pesticides. Phenolic compounds are known to be toxic to insects because they inactivate their digestive enzymes. Alkaloids are known for their wide range of biological and especially toxic activities. Only deep analysis would allow to define the compound of the biological activity.However, many authors have detected active ingredients as those found in the extracts studied during this experimentation.

Thus, Qin *et al.,* (2004) showed that capsaicin was the most important compound of the volatile oil of *C. frustescens* and the total phenolic compounds or pungent principles. Tchiegang *et al*., (1939). Wesolowska *et al.,* (2011) showed that *C. annuum* contained alkaloids called capsaicinoids which, extracted with acetone and hexane, revealed that the major compounds are capsaicin, dehydrocapsaicin and nonivamide (pelargomic acid vanilleylamide) and also ascorbic acid (9, 5-11.8%) (Ikuomola and Emiola., 2015).

In *S. hispidus*, Paris *et al.,* (1976) found flavonoids in the leaves but not in the other organs of the specie. Cardenolides were present in the seeds and weakly and at decreasing concentrations in the roots, in leaves, in pericarp and stems. Keller and Tamm (1959) noted the presence of 5 other cardenolides in *S.hispidus* seeds following by a long conservation period due to oxidation.

The present experimentation showed the presence of cardenolides in the extracts of *S. hispidus* and some traces in those of *Capsicum annuum* (Table 4). For the present experimentation against *C. Cosyra*, the fractions of butanol and methanol extracts were the most efficient. This shows that, in addition to capsaicin, other molecules could be responsible for this observed biological activity.

The biological efficiency of *S. hispidus* has been shown only after 72 hours, at the opposite of the one of *C. annum* after 24h. It suggested that, despite the very toxic nature of cardenolides, the active ingredients contained in *S. hispidus* were slow to alter the vital organs of *C. cosyra*. Despite the presence of cardelonides in *S. hispidus* tested extract, it showed a slow action compared to that of *C. annuum*. This is one of the reasons that the extract of *S. hipidus* was not fractionated. Extracts of *S. hispidus* would probably be safe for human health when applied topically. The presence of important bioactive compounds in the various extracts tested, did not allow attributing the biological efficiency observed to a molecule or to a group of phytochemical compounds. For this, only advanced analysis with a Nuclear Magnetic Resonance supply will allow in the future to define the compound or group of compounds responsible for the observed biological efficacy against *C. cosyra* adults.

CONCLUSION

The vegetable powder of *M. inermis* was important in apolar substances, extracted by n-hexane and polar by methanol. The highest yield of *C. annuum* extracted fraction has been obtained with acetone and the lowest with ethyl acetate and 1-butanol. Ethyl acetate extracts of *C. annuum* and methanol extracts from *S. hispidus* showed strongest toxic activities against *C. cosyra* adults, in comparison with the other types of extracts. Phytochemical analysis of the methanol fractions revealed more chemical constituents including sterols and tri-terpenes, coumarins and derivatives, anthraquinones, alkaloids, flavonoids, anthocyanosides but more cardenolides in so-called toxic *S. hispidus*. Thus, further study of *C. annuum* and *S. hispidus* methanol extract fractions, will allow identifying the molecular structures of compounds which could be use for formulations and for an application in an integrated pest management program against *C. cosyra* of mango.

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