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Phytochemical screening and cytotoxicity of juices and bark extracts of sugarcane (*Saccharum officinarum* Linn) in Benin

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Abstract

Sugarcane (*Saccharum officinarum* Linn) belongs to the Poaceae family and used widely for the preparation of traditional medicines in Benin. The present study was designed to evaluate phytochemical and cytotoxicity potential of seven sugarcane varieties. The precipitation and coloration method were used to determine phytochemical compounds whilst larval toxicity was performed using brine shrimp larvae (*Artemia salina* Leach). Results of the study revealed variation in phytochemical compounds in the different sugarcane varieties screened. Difference in phytochemical composition was observed also between the juice and bark of same sugarcane variety. Alkaloids, leuco-anthocyanins and reducing compounds were found in juice and bark from all sugarcane varieties used. The CL50 vary from 0.74 mg/mL to 2.66 mg/mL for sugarcane juice and from 0.28 mg/mL to 0.54 mg/mL for the bark extracts. Neither the juice nor bark of sugarcane has presented a risk of toxicity to consumers. The results of the study confirm the potency of sugar cane in preparation of indigenous food and medicine in Benin.

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Introduction

Plants are cheaper and safer sources of antimicrobials (Doughari, 2006). They produce secondary metabolites which constitute an important source of microbicides, pesticides and many other pharmaceutical drugs (Mahalingam *et al.,* 2011). Secondary metabolites from plant are known for their curative activity against several pathogens and this could explain the wide use of plants for the treatment of several human ailments (Adjatin *et al.*, 2013). Chemical compounds such as phenolic compounds, alkaloids, terpenoids, steroids, quinones and saponins, with complex structures have limited distribution than primary metabolites. They are non-nutritive for the plant that contains them but protect them against pest and also contribute to flavor and color of a plant (Zobra *et al.*, 2012; Molefe-Khamanga *et al.*, 2012). Their presence in this plant is an indication that the plant could yield drugs of pharmaceutical significance and have been known to be involved in ethnomedicine in the management of various ailments (Sharma and Paliwal, 2013).

Sugarcane (*Saccharum officinarum* Linn) is a common grass in tropical and subtropical regions with multiple uses (Souza *et al.*, 2010). It is grown for its sweet juice which is consumed directly or processed for sugar (Archimedes *et al.*, 2011; Abogo *et al.*, 2013). Sugarcane juice is highly nutritious with medicinal and pharmacological properties (Banerji *et al.*, 1997; Rajendran *et al.,* 2017) to fight sore throats, colds, influenza and has a low glycemic index that keeps the body healthy (Karthikeyan *et al.*, 2010). The roots and stems are used to treat urinary and bronchial tract infections, anemias, cough constipation, and regulate blood pressure (Mira *et al.*, 2011*)*.

In Benin, it is used in preparing some traditional medicines for the treatment of disorders such as miscarriage, sexual weakness and gonococcal disease (Ekpélikpézé *et al.*, 2016a). Agromorphological characterization of *S. officinarum* accessions has shown that national collection has a high degree of variability. Based on qualitative parameters, different morphotypes, based on stem colour were identified (Ekpélikpézé *et al.*, 2016b). Then, in these preparations of traditional medicines, varieties have been found to be more potent in treating different ailments (Ekpélikpézé *et al.*, 2016a). Despite variations in agromorphological, biochemical and ethnobotanical characteristics of sugarcane varieties, limited literature exists on the phytochemical composition and toxicity levels in the bark and juice of different sugarcane varieties in Benin. To promote the use of sugarcane in traditional medicine preparation, it is important to assess pharmacodynamic efficiency and toxicological risks associated with sugarcane juice and bark. The objectives of the study were to determine phytochemical properties in juice and bark of sugarcane and to evaluate cytotoxic potential of juice and bark obtained from different varieties of sugarcanes grown in Benin.

Materials and methods

Collection and preparation of sugarcane samples

Seven sugarcane varieties (Fig. 1) grown in Benin were collected from the experimental site of the Faculty of Sciences and Technology of Dassa (FAST/Dassa) of National University of Sciences Technologies Engineering and Mathematics situated at Central in Benin. These varieties were selected based on the agromorphological characterization of *S. officinarum* as reported by Ekpélikpézé *et al.* (2016b).

Each collected sugarcane stem was washed under running water, hand-peeled and bark cut into pieces. Peeled sugarcane barkswere dried in on oven at 55°C. After oven drying, peels of each variety was blended into powder using an electrical blender. Sugarcane juice was extracted using power operated sugarcane crusher machine. The extracted sugarcane juice was filtered using double sieve and muslin cloth to remove the extraneous matter. The filtrate was used for the phytochemical and cytotoxicity analysis.

Phytochemical analysis

Qualitative phytochemical analysis on the filtrate and bark powder was done using standard methods based on colouring and precipitation reactions as described

by Houghton and Raman (1998) and used by Agbankpé *et al.* (2015) and Rajendran *et al.* (2017).

Phytochemical compounds for included and their identification method are: Alkaloids (Mayer Reagent), Cathetic Tannins (Stiasny Reagent), Gallic Tannins (Ferric Chloride and saturation with sodium acetate), Flavonoids (Shinoda test with magnesium powder) , Anthocyanins (50% hydrochloric acid and ammonia), Leuco-anthocyanins (Shinoda reaction), Quinone derivatives (Born-trager reagent), Saponins (Foam index test), Triterpenoids (Liebermann-Buchard reaction), Steroids (Kedde Reaction), Cyanogenic derivatives (Picric Acid Test), Mucilages (Absolute Alcohol Test), Coumarins (Test with Ether and Ammonia), Reducing compounds (Fehling Liquor Test), Free Anthracene derivatives (Test with chloroform and ammonia), combined anthracene derivatives O-heterosides (Hydrochloric acid Test with chloroform and ammonia), combined anthracene derivatives C-heterosides (Test of FeCl3 with chloroform and ammonia), Heterosides cardiotonics (Raymond-Marthoud Reagent).

Evaluation of larval toxicity

Cytotoxic activity of sugarcane juice and bark was determined using brine shrimp larvae (*Artemia Salina* Leach) as described by Ahouansinkpo *et al.* (2016) and Djengue *et al.*, 2017. *A. salina*'s eggs were grown in Erlenmeyer dish containing brine water.

The *A. salina*'s eggs- brine water suspension was agitated for 48 hours until eggs hatched into larvae. 1g bark powder from each sugarcane variety was mix in 50 mL of distilled water to obtain a stock concentration of 50 mg/mL solution.

The stock solution was further diluted to obtain 25 mg/mL; 12.5 mg/mL; 6.25 mg/mL; 3.125 mg/mL; 1.582 mg/mL; 0.781 mg/mL; 0.391 mg/mL; 0.195 mg/mL; 0.098 mg/mL; 0.049 mg/mL. Using a cone micropipette, a colony of 16 live larvae was transferred into the various concentrations. Distilled water was used as control. The set up were under agitated and number of dead larvae counted 24 hours after incubation.

Data analysis

Counting the number of surviving larvae in each solution allowed assessment of the solution toxicity. Mortalities in control media were corrected by the formula: % death = [(test – control)/control)] x 100. For each extract or sample, the lethal concentration that induces 50% larval death (CL_{50}) was calculated at 95% confidence interval by linear regression analysis and also by using the Probit analysis method following Adjatin *et al.* (2013) and Djengue *et al.* (2017). A regression line equation was derived for each extract with the mortality data obtained and used to calculate the LC50 value. The detailed mathematical steps used to derive the regression line equation are reported in the literature (Vincent, 2012). The toxicity of these samples was evaluated as described by Mousseux (1995) (Table1) and used by Adjatin *et al.* (2013) and Djengue *et al.* (2017).

Results

Phytochemical screening of juice and bark extracts from sugarcane varieties

Sugarcane juice and bark evaluated contained several phytochemical compounds (Table 2). Phytochemical compounds obtained consisted of alkaloids, cathetic and gallic tannins, flavonoids, anthocyanins, leuco-anthocyanes, quinone derivatives, mucilages, coumarins, reducing compounds, combined anthracene O-heterosides and combined anthracene C-heterosides. It was observed that phytochemical compounds identified in sugarcane juice varied from each sugarcane variety used. Similarly, phytochemicals identified differed between juice and bark.

In general, alkaloids, leuco-anthocyanes and reducing compounds were present in the juice of the various varieties. Juice from black stem sugarcane and yellow stem sugarcane contained the phytochemicals (alkaloids, leuco-anthocyanins, mucilage, reducing compounds) which was low compared to juice from red wine stem sugarcane, green stem sugarcane and dark green stem sugarcane which recorded higher

number of phytochemicals (alkaloids, gallic tannins, cathetic tannins, flavonoides, leuco-anthocyanins, anthocyanins, quinone derivatives, steroids, mucilage, coumarins, reducing compounds combined anthracene O-heterosides and combined anthracene C-heterosides).Alkaloids, leuco-anthocyanes, reducing compounds, gallic and cathetic tannins, flavonoids, anthocyanins and quinone derivatives were present in the bark of all sugarcane varieties used.

Table 1. Correspondence between CL_{50} and toxicity.

CL_{50}	Toxicity
$CL_{50} \geq$ 0.1mg/Ml	(Non-toxic)
0.1mg/mL>$CL_{50} \geq$ 0.050 mg/mL	< (Low toxicity)
0.050mg/mL>$CL_{50} \geq$0.01mg/mL	++ (Average toxicity)
CL_{50}<0.01mg/mL	+ + + + (High toxicity)
Source: Mousseux (1995)	

Among the varieties studied, the bark of sugarcane varieties with black stem, yellow stem, red wine stem, green stem and reddish yellow stem had same phytochemical profile, whilst the bark of varieties red and dark green varieties differed in anthracene derivatives and steroids and anthracene derivatives respectively. Thus, extracts from the bark of dark green stem varieties were richer in chemical compounds than other varieties. Alkaloids, leuco-anthocyanins and reducing compounds were found in juice and bark from all sugarcane varieties used.

Table 2. Qualitative evaluation of phytochemical compounds in juice and residue extracts of sugarcane varieties.

Chemical compounds	Black S		Yellow S		Red S		Red wine S		Green S		Dark green S		Reddish yellow S	
	J	B	J	B	J	B	J	B	J	B	J	B	J	B
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyphenolic compounds														
Gallic tannins	-	+	-	+	-	+	+	+	+	+	+	+	-	+
Catechical tannins	-	+	-	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	-	+	-	+	-	+	+	+	+	+	+	+	+	+
Anthocyanins	-	+	-	+	-	+	+	+	+	+	+	+	-	+
Leuco-anthocyanes	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Quinonic Derivatives	-	+	-	+	-	+	+	+	+	+	+	+	-	+
Saponines	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Triterpenoids	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Steroids	-	-	-	-	-	-	+	-	+	-	+	+	-	-
Cyanogenic derivatives	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mucilage	+	-	+	-	-	-	+	-	+	-	+	-	+	-
Coumarins	-	-	-	-	-	-	+	-	+	-	+	-	+	-
Reducing compounds	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anthracenic derivatives														
Free anthracenic derivatives	-	-	-	-	-	+	-	-	-	-	-	+	-	-
Combined anthracenics O-heterosides	-	+	-	+	-	+	+	+	+	+	+	+	+	+
Combined anthracenics C-heterosides	-	+	-	+	-	+	+	+	+	+	+	+	+	+
Cardiotonic glycosides	-	-	-	-	-	-	-	-	-	-	-	-	-	-

S: Stem, J: Juice, B: Bark.

Toxicity degree of Saccharum officinarum

Sensitivity curves for the cytotoxicity of sugar cane juice (Fig. 2) and bark powder (Fig. 3) showed that larval mortality increased with concentration and this sensitivity follows the dose response relationship. Variation in lethal rate of *Artemia salina* was

observed to be variety dependent. For sugarcane juice, the highest CL_{50} was recorded with the yellow stem (2.66 mg/mL) variety whilst the lowest (0.74 mg/mL) was observed in variety with green stem. With respect to the bark, the highest CL_{50} (0.54 mg/mL) was recorded in the red wine stem with the red stem variety recording the lowest (0.28 mg/mL). In addition, black stem sugarcane and reddish yellow stem varieties recorded the same CL_{50} for juice (0.93 mg/mL) and bark (0.45 mg/mL).

Table 3. CL_{50} values of juice extracts of different sugarcane varieties.

Sugar cane juice	Logarithmic regressions	R^2	CL_{50} (mg/mL)
Black stem	y = 3.086ln(x) + 8.192	0.947	0.93
Yellow stem	y = 2.614ln(x) + 5.439	0.940	2.66
Red stem	y = 3.130ln(x) + 7.287	0.941	1.25
Red wine stem	y = 3.112ln(x) + 7.689	0.941	1.11
Green stem	y = 3.077ln(x) + 8.893	0.934	0.74
Dark green stem	y = 3.139ln(x) + 8.187	0.928	0.94
Yellow reddish stem	y = 3.086ln(x) + 8.192	0.947	0.93

Discussion

Phytochemical analysis of sugarcane juice and bark from different varieties revealed the presence of alkaloids, cathetic and gallic tannins, flavonoids, anthocyanins, leuco-anthocyanins, quinone derivatives, mucilages, coumarin, reducing compounds, combined anthracene O-heterosides and combined anthracene C-heterosides. In a similar study, Rajendran *et al.* (2017) identified saponosides in sugarcane juice and Uchenna *et al.* (2015) identified them in the bark of sugarcane. However, in the present study, saponosides were not identified in juice and bark of sugar cane varieties used.

Table 4. CL_{50} values of bark extracts from different sugarcane varieties.

Bark of sugarcane	Logarithmic regressions	R^2	CL_{50} (mg/mL)
Black stem	2.640ln(x) + 10.13	0.920	0.45
Yellow stem	y = 3.182ln(x) + 9.282	0.879	0.67
Red stem	y = 2.544ln(x) + 11.24	0.810	0.28
Red wine stem	y = 2.815ln(x) + 9.719	0.897	0.54
Green stem	y = 2.526ln(x) + 10.44	0.903	0.38
Dark green stem	y = 2.649ln(x) + 9.835	0.930	0.5
Yellow reddish stem	y = 2.640ln(x) + 10.13	0.920	0.45

According to Makemba Nkounkou *et al.* (2017), the absence of saponosides in sugarcane varieties is attributed to soil quality, seasons and environmental factors. Phytochemicals identified in the sugarcane varieties, justify their use in traditional medicines preparation (Adjatin *et al.*, 2013; Agbankpé *et al.*, 2015; Djengue *et al.*, 2017) to treat several diseases such as malaria, miscarriages, painful rules, gonococcal disease and sexual weakness (Ekpélikpézé *et al.*, 2016). Alkaloids are known for their antibiotic and antiplasmodiale properties (Okwu *et al.*, 2007; Djengue *et al.*, 2017). The presence of alkaloids in all morphotypes could justify the use of sugarcanes in the treatment of malaria. According to Boua *et al.*, (2013), the use of *Turraea heterophylla,* favours blood circulation due to the presence of alkaloids. The use of sugarcane juice in medicine preparation for the treatment of sexual weakness in Benin could be due to abundance of alkaloids as observed in sugar varieties screened.

Fig. 1. Different varieties of sugarcane.

Apart from alkaloids, phenolic compounds have been found to possess anti-inflammatory, oestrogenic and antimicrobial properties (Okwu, 2004). The identification of these compounds in sugar cane juice and bark confirms the findings of Uchenna *et al.* (2015) that the bark extract of sugar cane has bactericidal activity and could be used in the control and treatment of bacterial infection. Edeoga *et al.* (2006) showed the importance of *Hyptis suaveolens* and *Ocimum gratissimum* in the pharmaceutical industry due to their steroid composition which has been found to have a relationship with compounds linked to sex hormones. The identification of steroids in the sugarcane varieties could justify its use. According to Makemba Nkounkou *et al.* (2017), alkaloids, flavonoids, terpenes and steroids contained in extracts of *Anchomanes difformis* significantly decreased frequency of spontaneous uterine contractions in guinea pigs. These phytochemicals have varying properties and therefore synergistically (Allangba *et al.*, 2016); their presence in sugarcane juices and bark extracts could justify its use in in traditional medicine preparations for to treat painful rules, miscarriages and sexually transmitted diseases such as gonococcal disease as reported by Ekpélikpézé *et al.* (2016).

According to Ekpélikpézé *et al.* (2016), all varieties are used for the treatment of sexual weakness whereas it is only red wine color variety which is mainly used by populations to treat painful rules, miscarriages and sexually transmitted diseases such as gonococcal disease.

Considering the chemical composition of the studied varieties, the samples of juice from red wine stem, green and dark green stem on the one hand and bark extracts from red stem and dark green stem on the other contain more phytochemical elements and could be identified as the most effective varieties in the treatment of mentioned diseases above. These results clearly show that the local communities surveyed have a good knowledge of the genetic resources of sugarcane they use and that, in terms of identification and use, their knowledge would be extremely useful to scientists as reported on leafy vegetable (Adjatin *et al.*, 2012) and yam (Dansi *et al.*, 2000).

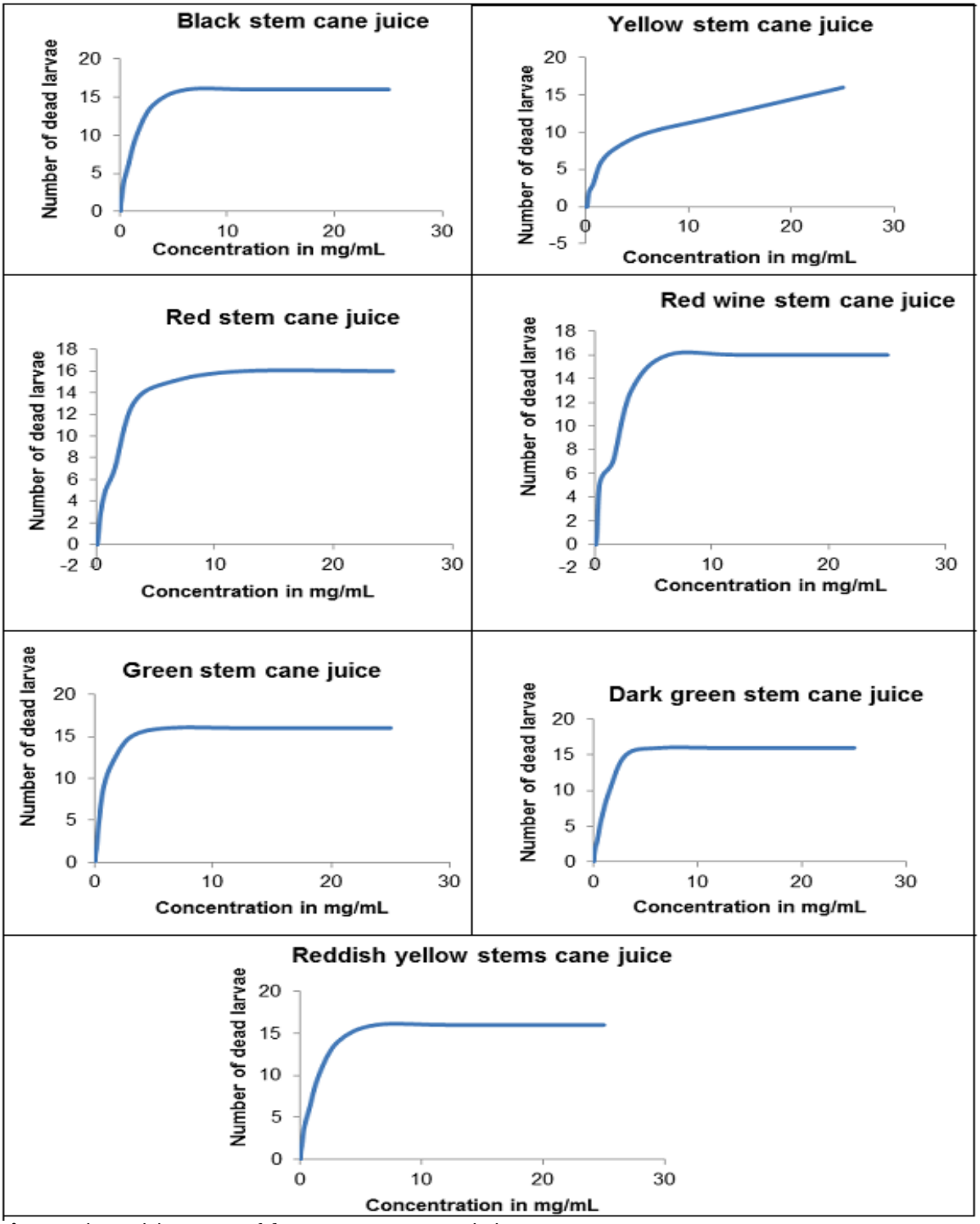

Fig. 2. Juice toxicity curves of the seven sugarcane varieties.

Several studies have demonstrated the relevance of the larval toxicity test in preliminary toxicity studies (Quignard *et al.,* 2003; Ahouansinkpo *et al.*, 2016; Djengue *et al.*, 2017*)*. However, results of the present study reveals no adverse effect of sugar cane juice and bark on animal or human health (Pelka *et al.*, 2000; Carballo *et al,*, 2002) and can therefore be consumed without the risk of short and medium term toxicity.

This study ranks sugarcane in the non-cytotoxic crop group as *Amaranthus hybridus* and *Crassocephalum crepidioides*, which are vegetables consumed in Kenya (Orech *et al.*, 2005) and Benin (Adjatin *et al.*, 2013).

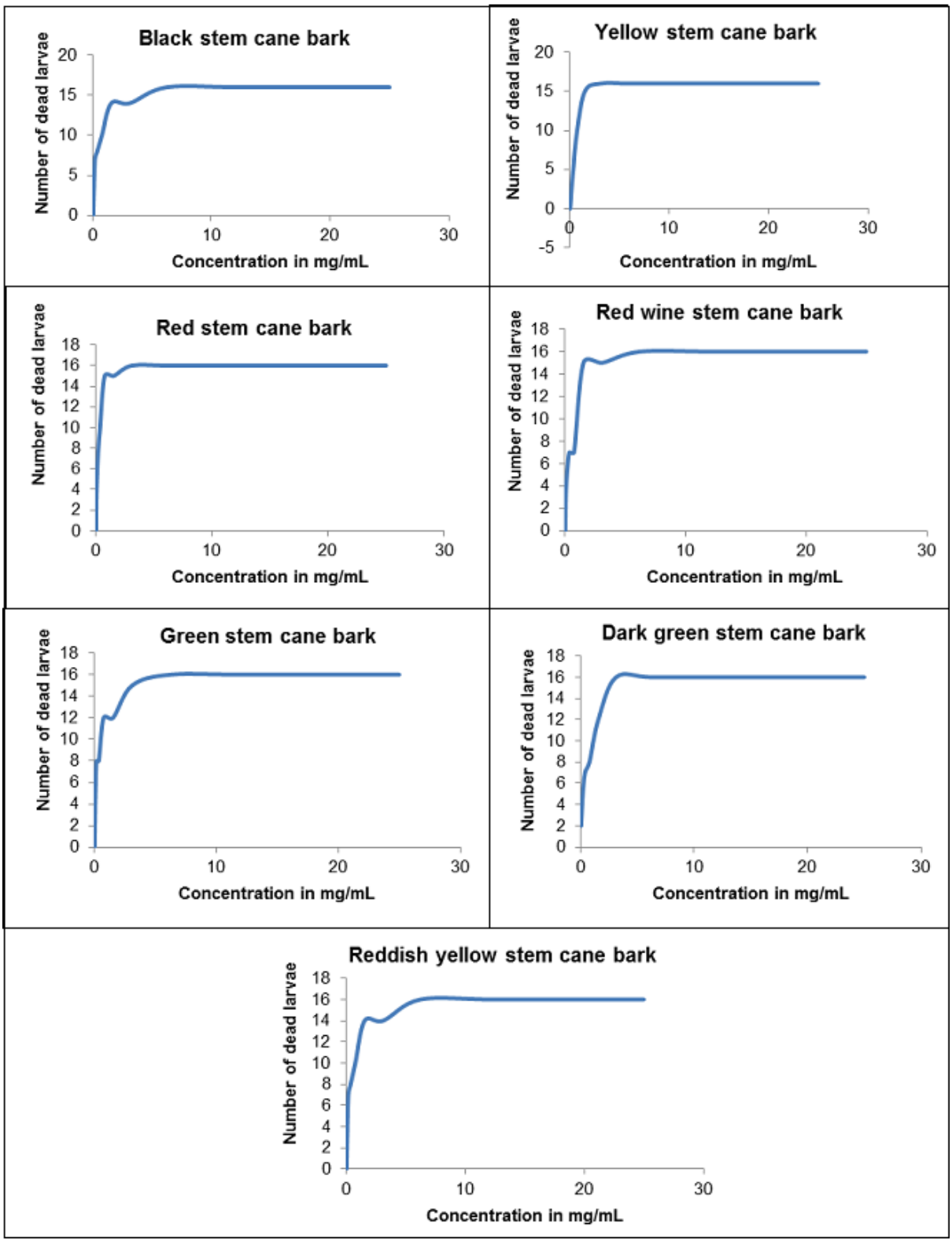

Fig. 3. Toxicity curves of bark extracts from seven sugarcane varieties.

Conclusion

This study carried out on the phytochemical screening and larval toxicity of the juices and bark extracts of sugarcane. The qualitative phytochemical screening revealed the presence of many chemical compounds that are known to have medicinal properties justifying the use of the plant in traditional medicine and reinforce the species in category of nutraceutical. While combining phytochemical compounds and stem color, the most promising

varieties were those with red wine, green and dark green stem. Therefore, the stem color can be used to identify effective varieties in the treatment of reported diseases.

The toxicity study reveals that neither juice nor bark extracts are toxic and therefore be consumed without risk. These varieties that have been shown to be effective in this study could be valorised by large-scale intensive cultivation and consisting of raw materials in both food and pharmaceutical industries.

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