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ASSESSMENT OF FRUITS OF SOME EXOTIC PLANTS IN THE PRODUCTION OF BIOETHANOL USING *Saccharomyces cerevisiae*

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ABSTRACT
The fruits of exotic plants have high sugar content and are not usually edible to humans; thus, wasted every season. This study determined the potentials of some selected fruits from the exotic plants in the production of bioethanol using Saccharomyces cerevisiea. Golden newdrop (Duranta repens), scarlet jungle flame (Ixora coccinea) and malmo (Syzygium guineense) were considered for this purpose. The fruit samples were collected from Bayero University Kano, pretreated accordingly in conical flasks and autoclaved; the concentrations of reducing sugars were determined using standard procedures. Pretreated samples were aseptically inoculated with suspension of the pure culture of S. cerevisiae and incubated for three weeks for fermentation to take place. After incubation, the concentrations of bioethanol produced were also determined. The highest reducing sugars yield of 28.6% was found in I. coccinea with significant difference ($p < 0.05$) when compared to S. guineense (with 23.2%) and D. repens (with 5.3%). However, there was no significant difference in bioethanol yield between I. coccinea and S. guineense ($p > 0.05$), but there was significant difference when compared with D. repens. The production of bioethanol from the fruits of exotic plants should be optimized and commercialized to address energy issues and environmental impact associated with them.
Keywords: Bioethanol, fruits, exotic plants, fermentation, Saccharomyces cerevisiae.

INTRODUCTION

Plants play a significant role in our planet. They are the basis of life upon which all other creatures depend on. Some plants are naturally pleasing, and are used for beautification purposes; they are usually exotic and ornamental in nature (Isah *et al*., 2019). Exotic plants are those non-native species that are introduced to an environment intentionally or unintentionally through human or other animals and birds. These plants grow faster when compared with indigenous ones, and beautify lanscapes, thus, their faster propagation (Ariharan *et al*., 2012). In Kano, Northern Nigeria, golden newdrop (*Duranta repens*), scarlet jungle flame (*Ixora coccinea*) and Malmo (*Syzygium guineense*), among others, are the most common exotic plants, and are found in residential areas, institutions, government agencies, commercial and religious places (Cordero *et al*., 2013).

Ixora coccinea is an evergreen perennial shrub belonging to the genus of flowering plants the family *Rubiaceae*. The plant is native to the tropical and non-tropical areas throughout the world, with brightly coloured flowers, thus used in decorating landscapes (Figure 1). It produces round and dark purple to black berries that are rich in sugars.

Figure 1: (a) *I. coccinea* used for landscaping at Senate Building, Bayero University Kano (b) Fruits of *I. coccinea*, photo taking from the same place.

Duranta repens is an evergreen species of flowering shrub with fast-growing, multi-stemmed and pendulous branches native to Mexico to South America and the Caribbean. The plant belongs to the family *Verbenaceae*, with deep blue flowers that are held at the ends of weeping branches (Akanji *et al.*, 2018). In tropical and subtropical areas, *D. repens* is widely planted as an ornamental plant. It produces clusters of bright orange-yellow berries that follow the flowers, often causing the slender branches to droop gracefully (Figure 2).

Figure 2: (a) *D. repens* used for landscaping at Bayero University Kano (b) Fruits of *D. repens*, photo copied from www.cabidigitallibrary.org.

Syzygium guineense, locally known as water berry, is an evergreen, fast-growing, medium-sized plant with a dense, heavy, rounded crown. It can grow 30 meters tall or more, and can be branchless for up to 15 meters in large trees, up to 150cm in diameter (Okhale *et al.*, 2018). This plant is found in a wide range of habitats, and produces fruits that are oval berries containing single seed and look red to dark purple when ripe, which could be brewed into a drink (Figure 3).

Figure 3: (a) *S. guineense* used for landscaping at Bayero University Kano (b) Fruits of *S. guineense*, photo copied from the twitter account of Bahirdar Photography.

Despite their high sugar content, the fruits from these exotic plants are wasted every season since they are not usually consume by our people. These fruits constitute a valuable source of sugars and other nutrients (Isla *et al.*, 2022) that can be used as feedstock for bioethanol production. Bioethanol is liquid biofuel, which is produced from several different biomass using various conversion technologies. It has been reported as an attractive alternative fuel because of its non-toxic and renewable nature with potential to reduce the emission of greenhouse gases into the atmosphere (Guo *et al.*, 2015). Bioethanol is one of the promising future energy alternatives that could contribute to the reduction of negative environmental impacts generated by the use of fossil fuels. It has been produced from a variety of raw materials containing fermentable sugars (Voloshin *et al.*, 2016). This study therefore assessed the production of bioethanol from the fruits of some selected exotic plants using *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Sample collection

All the samples of the three selected exotic fruits, i.e. *I. coccinea, D. repens* and *S. guineense* (Figure 4), were collected from Bayero University Kano New Campus in clean polythene bags. Samples were washed with water to remove any dirt.

Figure 4: Samples of the fruits from three selected exotic plants from Bayero University Kano.

Pretreatment of the Samples

The samples were blended to paste to accelerate microbial digestion by increasing the surface area for biochemical reaction. A quantity of 50g of the paste sample placed in 500ml-capacity conical flasks and diluted with 250ml of distilled water to produce a slurry with appropriate total and volatile solids suitable for microbial digestion. This was covered appropriately with cotton wool and aluminum foil, and then autoclaved (STV-I, Bioevopeak, China) at a pressurized temperature of 121^{O}C for 15 minutes. This was carried out on all the samples and in triplicate; pretreatment was not carried out on control samples, which were collected from each fruit, and was also prepared in triplicate.

Determination of Reducing Sugars

UV-visible spectrophotometry was used to determine the reducing sugars in the samples after autoclaving, as described in Leonald-Emeka *et al*. (2020). Standard glucose concentrations of 2.5, 5, 7.5, 10 and 12.5 mmol/L were prepared from a stock solution of 20 mmol/L and 5ml of each was put in a test tube. Two milliliter (2ml) of Benedict's reagent was added to each tube and boiled on water bath for 5 minutes. Absorptions were taken from each tube at 477nm. The readings were used to plot a graph of absorbance against concentrations. The blank, which was 10ml distilled water and 2ml Benedict's reagent, was used to zero the spectrophotometer.

Reducing sugar (glucose) concentration in the samples were determined by adding 2ml Benedict's reagent in 5ml of each filtrate, and the mixture was placed in a boiling water bath for 5 minutes. Absorption of a portion of each mixture was then taken using spectrophotometer at 477nm, and the glucose concentration was calculated from the slope of the standard curve. Similar procedure was also done for the control samples.

Bioethanol Production

Bioethanol production from the pretreated samples of exotic fruits was carried out using the method described in Mishra *et al.* (2012). The autoclaved samples were allowed to cool and aseptically inoculated with 25ml of pure culture broth of *Saccharomyces cerevisiea*. The flasks were incubated in shaking incubator (SI – 200, Major Science Co. Ltd., Singapore) at 45^{o}C for fermentation to take place, as fermentation at this temperature was reported to be very effective (Egbere *et al*., 2008); the flasks were allowed for twenty-one (21) days to ensure absolute utilization of the nutrients in the medium (Ziemlewska *et al*., 2021), and bioethanol produced was recovered from the fermentation media using distillation.

Concentrations of Bioethanol Produced

Determination of the concentrations of bioethanol produced was carried out using UV-Spectrophotometry as described by Leonald-Emeka *et al*. (2020). One milliliter of the standard ethanol was diluted with 100 ml of distilled water to produce 1%, and each of the 2, 4, 6, 8 and 10 ml of this was diluted to 10 ml with distilled water to produce 0.2, 0.4, 0.6, 0.8 and 1.0% of the ethanol. To each of these concentrations, 2ml of dichromate reagent was added and allowed to stand for one hour for colour development. The absorbance of each concentration was taken at 588nm using UV-Visible spectrophotometer. Standard ethanol curve was developed using absorbance against concentrations.

Four milliliter of each bioethanol sample was placed in a test tube and 2ml of dichromate reagent was added. The mixture was left for one hour and absorbance were taken at 588nm. The concentrations of the bioethanol produced was calculated using the slope of the standard ethanol curve.

Statistical Analysis

One-way analysis of variance (Anova) was carried out to determine whether there is significant difference in reducing sugars and bioethanol yield between the fruits samples and control.

RESULTS AND DISCUSSION

Figure 5 below shows the results of the reducing sugars concentrations after physico-thermal pretreatment. The highest reducing sugars concentration (28.6%) was found in *I. coccinea*, with significant difference ($p < 0.05$) when compared with *S. guineese* (23.2%) and *D. repens* (with 5.3%).

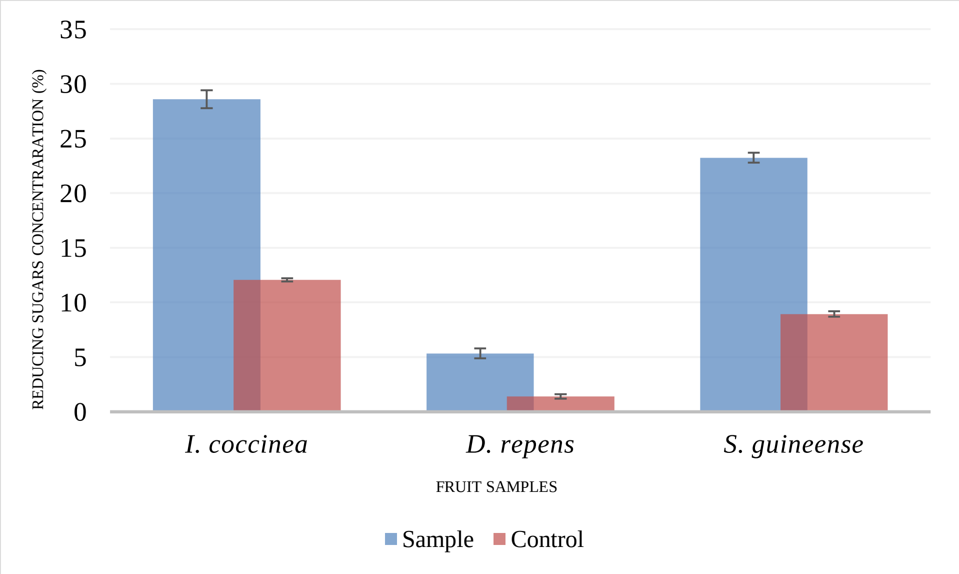

Figure 5: Result of the reducing sugars concentrations in the fruit samples and Controls

The results of the bioethanol yield is presented in Figure 6 below. No significant difference was observed in bioethanol yield among *I. coccinea* and *S. guineense* ($p < 0.05$), but there was significant difference when compared with *D. repens* ($p > 0.05$).

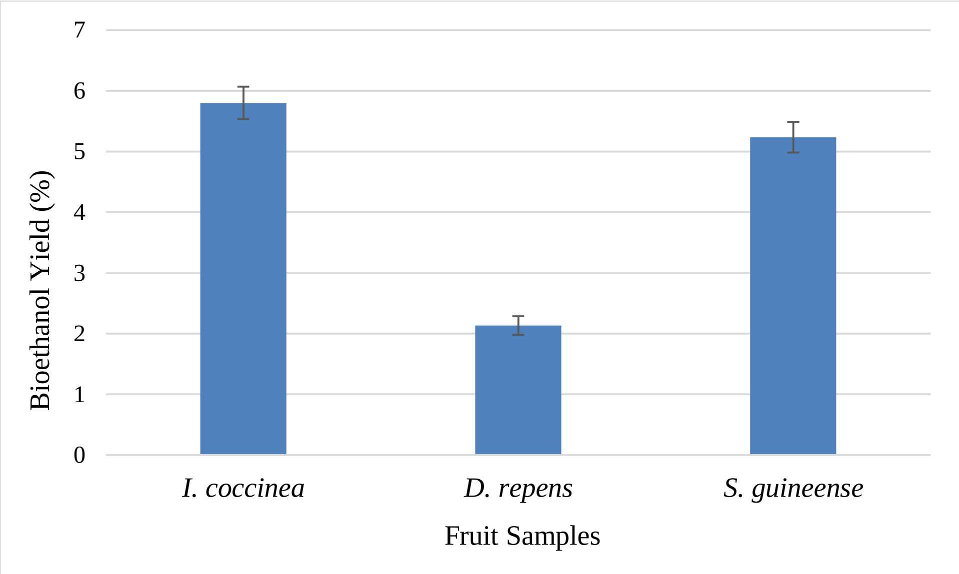

Figure 6: Result of the bioethanol yield from the fruit samples

DISCUSSION

Fruits from exotic plants have great potential as candidates for bioethanol production using *S. cerevisiae*. The proliferation of exotic plant, in Kano in particular, is on the rise, so also their fruits. The fruits from *I. coccinea*, *D. repens* and *S. guineense* used in the present study have high sugar content mostly in form of glucose, fructose and sucrose (Jordaan and Downs, 2012). The concentrations of reducing sugars in the control samples presented in Figure 5 above therefore come presumably from glucose and fructose contents, which are monosaccharaides sugars requiring no any pretreatment.

With regards to the determination of reducing sugars in the samples after pretreatment by autoclaving, there was rise in the concentration of *I. coccinea* from 12.1% to 28.6%, indicating the hydrolytic effect of the method on the fruit sample. The rise was from 8.9% to 23.2% in *S. guineense*, and from 1.4% to 5.3% in *D. repens*. The reducing sugars yield was highest in *I. coccinea*, followed by *S. guineense* and it was lowest in *D. repens*. This finding was in line with the work of Damayanti *et al.* (2012), where hydrothermal pretreatment was found to have significant effect on the reducing sugars yield in fruit juices as observed in the present study. Zaafouri *et al.* (2016) achieved pretreatment using hydrothermal approach at 100^{o}C for 60 minutes, indicating similarities with present study. The use of thermal pretreatment such as autoclaving has been employed across the globe in the hydrolytic process and bioethanol production (Scherzinger *et al.*, 2020; Debiagi *et al.*, 2020).

After three weeks of fermentation of the pretreated fruit samples using *S. cerevisiea*, *I.* coccinea was found to have the highest bioethanol yield.

This had to do with higher available fermentable sugars utilizable by *S. cerevisiea*. Bioethanol yield was also high in *S. guineense*, but low in *D. repens*. The yield of bioethanol from these sampled fruits was proportional to the availability of reducing sugars; the higher the sugar content, the more the bioethanol is produced. This finding tallies with that of Ogboru *et al*. (2015).

CONCLUSION

By virtue of their higher sugar content, fruits from exotic plant have a great potentials in bioethanol production. In particular, *I. coccinea* and *S. guineense* are good candidates for commercial production of bioethanol with significant difference when compared with other fruits such as *D. repens* when *S. cerevisiea* is used as fermenting organism. The fruits of *I. coccinea* and *S. guineense* should not, therefore, be wasted; the fruits should be used for bioethanol production. However, there is need to optimize the production processes, including the selection of the most efficient pretreatment methods and selection fermenting organisms.

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