



## UTILIZATION OF EICHHORNIA CRASSIPES, IN BIOFUEL PRODUCTION AND EXPLORING ITS FEASIBILITY AS A POTENT HYPOGLYCEMIC DRUG

### Biochemistry

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### ABSTRACT

Bioethanol is being considered as a potential liquid fuel due to limited amount of natural resources. Bioethanol produced from renewable biomass (lignocellulosic material) is one of the best eco-friendly method employed to eradicate the notorious aquatic weed permanently. This alternative approach also meets the increasing energy demands within the transport sector, therefore decreasing hazardous gas emissions. The medicinal properties of this plant have also been explored to certain extent. The purpose of present work is to study the production of ethanol from water hyacinth, used as substrate, using isolated microbial strain and to explore its antidiabetic property. The substrate concentration  $1.1 \times 10^{-2}$  M is efficient for bioethanol production. The ethanol yield estimated by potassium dichromate method is found to be 5.2ml. The plant exhibits the hypoglycemic effect which is confirmed by  $\alpha$ -Amylase inhibitory assay.

### KEYWORDS

Bioethanol, hypoglycemic, aquatic weed, utilization.

### 1. INTRODUCTION

The transport sector is considered as one of the largest energy consumers as well as environmental pollutant. It accounts for about 60% of the world's total oil consumption[1]. It is responsible for about one-fifth of CO<sub>2</sub> emission on a global scale[2]. Motor vehicles account for more than 70% of global CO emission and 19% of global CO<sub>2</sub> emissions. This could be alleviated by using biofuels and it is expected that by 2030, the energy need can be achieved by converting biomass into biofuel[3]. Fuel ethanol production from lignocellulosic biomass is emerging as one of the most important technologies for sustainable production of renewable transportation fuels. Ethanol can be blended with petrol or used as alcohol in dedicated engines, taking advantage of the higher octane number, low cetane number and higher heat of vaporization, and also it is an excellent fuel for future advanced flexi-fuel hybrid vehicles. In addition, the ethanol is an oxygenated fuel containing 35% oxygen, which reduces particulate and NO<sub>2</sub> emissions from combustion, it is biodegradable and contributes to sustainability [4].

Water hyacinth, the notorious weed therefore, have a great potential if seen as raw material for industries or if incorporated into agricultural practice. Its fast growth is a feature valued in crops grown by man[5][6]. Aquatic plants have many advantages such as growing on and in bodies of water without competing against most grains and vegetables for arable land; they are also used for water purification to extract nutrients and heavy metals. Aquatic plants have waxy coatings on their surface (which are complex alcohols), which are modified fatty acid deposition and helps in increasing the yield of biofuels[7]. It was estimated that the presence of lignin in aquatic plants is less than 10%. This would enable them to be processed efficiently into biofuels[6]. The present study is to yield alcohol efficiently using yeast which is cost effective method that requires less labour and time.

Almost all plants have some medicinal as well as nutritional value to offer, so does water hyacinth. The medicinal properties of this plant have also been explored to certain extent. The known hazards of eating this plant includes itching, prickling sensation as it contains HCN, alkaloids and triterpenoids. Plants sprayed with 2,4-D may accumulate lethal doses of nitrates. However, young leaves and petioles are sometimes cooked and consumed. The leaf petioles can be used in the treatment of diarrhoea. An infusion of the inflated petioles is used in a bath to treat fever. It is used to treat blood disorders, emaciation and weakness and also used in the treatment of goiter, cholera, heals sore throat, treats snake bites, sexually transmitted diseases, controls cholesterol, enhances digestion, also works as anti-inflammatory. Some potent benefits of hyacinth are: 1) It treats eczema 2) Makes skin healthy 3) Offers great fragrance to hair.

One of the major outbreak was the discovery of anticancer property of

the plant. The connection between man and his search for drugs in nature dates from far past. Phytochemical studies carried out revealed the presence of flavanoids and other metabolite in the plant extract. It also possess antimicrobial and antifungal activity. The antidiabetic property of the plant has not been reported yet. Thus, in our study we also explore the antidiabetic activity of the plant[8][9].

### 2. MATERIALS AND METHODS

#### 2.1. Fermentation

Cellulose in water hyacinth is hydrolysed to reducing sugars which are then subjected to fermentation by yeast to yield alcohol. *Saccharomyces cerevisiae* was used for the production of ethanol. Glucose yeast extract agar medium was used to maintain *S. cerevisiae*. The production medium from previous stage was autoclaved at 121°C for 20 min and inoculated with yeast. The samples were incubated at room temperature for 3 days. The alcohol content of the reaction mixture was estimated by dichromate oxidation method[12].

#### Potassium dichromate assay

For the estimation of ethanol by dichromate method, we can take 1ml of filtrate and then make final volume 5ml with distilled water and then add 10ml potassium dichromate method (same way prepare blank but instead of filtrate take distilled water) and put flask in dark for 30min and then after incubation take out flasks and then add 4ml 20% KI solution then immediately titrate against 0.1N sodium thiosulphate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>) till pale yellow colour appear after that add 2-3 drops of 1% Starch as indicator and again titrate against sodium thiosulphate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>) till blue colour disappear and note the amount of sodium thiosulphate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>) required to bring colour changes, then using blank, calculate (B-E) and using standard graph find out the concentration of our filtrate.

#### 2.2 Antidiabetic activity

Amylase and amylase inhibitor activity assays were based on Bernfeld's method[10]. Amylase inhibitor extracts (0.5-2.5 ml) were mixed with amylase and incubated for 30 min at 37°C. The reaction was started by adding extract-enzyme mixture to test tubes containing buffered starch solution (2 mg starch in 20 mM phosphate buffer of pH 6.9 containing 0.4 mM NaCl) and was incubated for 20 min. This reaction was terminated by adding 3,5- dinitrosalicylic acid (DNS) reagent to the assay mixture. The assay tubes were kept in a boiling water bath for 5 min, cooled under tap water and the colour formed by maltose oxidation was measured at 540 nm. Controls without inhibitor were run simultaneously. One amylase unit is defined as the amount of enzyme that will liberate 1  $\mu$ mol of maltose from starch under the assay conditions (pH 6.9, 37°C, 5 min). Inhibitory activity is expressed as the percentage of inhibited enzyme activity out of the total enzyme activity used in the assay.

$$\% \text{ of Inhibition} = (\text{Abs of control} - \text{Abs of sample}) / \text{Abs of control} * 100$$

**3. RESULTS AND INTERPRETATION**

The substrate concentration  $1.1 \times 10^{-2} M$  which was evaluated using DNS method[11] is found to be efficient for bioethanol production. The results are shown below:

**Table 1: Alcohol fermentation from fermentable sugars obtained by hydrolysis of Water hyacinth**

	Ethanol production(ml)
Control	10.6
Sample	15.8

From the above data recorded, the total ethanol yield at pH 4 and temperature 29°C [12] from biomass is 5.2ml (sample- control).

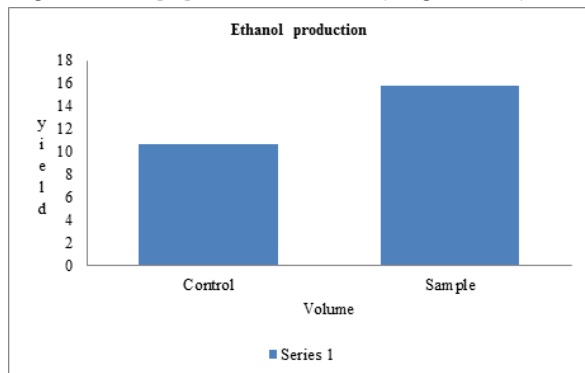


Fig 1: Biofuel Production

**ANTIDIABETIC ACTIVITY**

An attempt has been made to know the invitro antidiabetic activity of the plant extract by using  $\alpha$ -Amylase inhibition assay. This enzyme activity in the body is responsible for increased postprandial hyperglycaemia by breakdown of dietary carbohydrates into glucose. Hence if water hyacinth plant extract possess the inhibitory effect on this enzyme. It may lead to reduction in postprandial hyperglycaemia in diabetic condition.

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**Table 2: DNS method for measuring  $\alpha$  Amylase Inhibition activity**

Volume of plant extract(ml)	(%) Activity
0.5	43.4
1.0	42.3
1.5	26.5
2.0	13.6
2.5	3.1

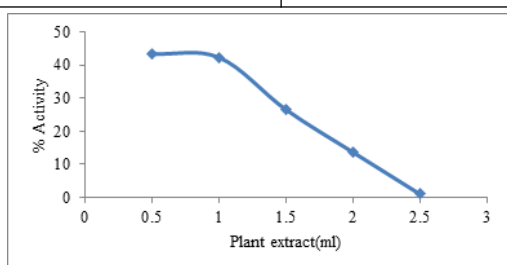


Fig 2: Inhibitory effect of the plant extract on the activity of Amylase

From the above data it can be inferred that, as the concentration of sample is increased the inhibitory activity of the plant also increases. Medicinal property (anti-diabetic activity) has not been reported yet thus it is a new approach to explore the medicinal value of the plant. The present study intends to screen novel alpha amylase inhibitors from natural sources like plants in order to minimize the toxicity and side effects of the inhibitors currently used to control hyperglycemia [13].

**4.CONCLUSION**

By potassium dichromate assay, we achieved 5.2 ml ethanol per 100g of the plant extract under optimum conditions. Maximum alcohol production was found to be at temperature of 29°C and pH Invitro  $\alpha$  Amylase inhibitory assay reveals that Eichhornia crassipes possess antidiabetic activity to certain extent. It is an initial step towards exploring its hypoglycemic activity. Therefore, it requires further advance research to explore its antidiabetic property.

It is the time to look for new material sources of biofuels, which are naturally amenable to processing during extraction of biofuel, thus reducing costs drastically and substituting fossil fuels in all aspects. Water hyacinth has long been seen as an invasive species all over the globe and considerable amount of resources have been spent for their control. However, they have certain qualities which can be utilized to produce biofuels (both bioethanol to power vehicles and motors, biogas to generate electricity) as the plants are low in lignin content and have rapid growth rate. There are so many alternative uses of these plant species and time has come to look at the plant from a different viewpoint and utilize their potential as much as possible. This tends to generate ample employment particularly in developing world. And the growth rate is so tremendous that there will be no dearth of biomass in the long run. Further, they do not compete with arable crops in term of land resources and needs little to no care to grow them.

**5.APPENDIX**

- No. of Tables: 2
- No. of Figures: 2
- No. of Charts: 0
- No. of Words: 1953
- No. of SOP followed: Nil
- No. of SOP Prepared: Nil
- No. of Chemical Safety Date Sheet referred: Nil

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