



EXTRACTION OF BEETROOT FOR ALCOHOL PRODUCTION BY FERMENTATION METHOD: ALCOHOL CHARACTERIZATION BY GC-MS AND ITS BIOASSAY.

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Abstract:

The method reports the chief method to produce the alcohol from beetroots *Beta Vulgaris* via fermentation method. For the research purpose the fresh beetroots were collected from market, washed with water to remove soil dust and other impurities on the outer surface of beetroots. These beetroots then used for further research. For alcohol production by this method the fermentation method was adopted in which beetroots were cut down in the small pieces, then mixed up with the sugar and yeast, kept this mixture in vessel for days. The overall mixture produces the alcohol which was separated. The separated alcohol was tested chemically for alcohol formation. The extracted alcohol was analysed by GC-MS and microbial assay of beet extract was carried for some pathogenic organisms.

Keywords: Beet root, Alcohol, fermentation, Gas chromatography, Microbial assay.

1.0 Introduction:

There are several chemical methods are available for economical synthesis of alcohol. As the alcohol particularly ethyl alcohol is used as a solvent in large number of chemical process. As well as demand of alcohol for the beverage purpose is very in all around the world. Thus a large number of researchers and chemist are working on the production of alcohol by cost effective methods. Although the several important methods are reported by which alcohol can be produced for economical purpose but, there are several by-products associated with main product alcohol. Removal or separation of such impurities is again enhance the cost or budget to synthesis the alcohol.

Recently the several researchers are working on the production of alcohol from natural sources by fermentation method. This is the new and easy method where the alcohol production is possible at low economical budget. Although from ancient period the alcohol is being used for various purpose the most frequent application of alcohol is for beverage purpose. The general methods from the last 5 decades which are constantly used are fermentation of grapes, fruits and many plant extracts.

The plants extract are used for numerous application by researchers such as solely for the antimicrobial activity, some of the plants extract can also be utilized in combination with nanoparticles as antimicrobial agents. Some of the plants extract have excellent application for the antibacterial and anticancer agents such as *Madhuca Longifolia* and extract of Galls of rhus plant. In some cases the plant like *Jatropha* found to be very effective for the production of biodiesel. Now researchers are finding the alternatives ways for the fuel hence these types of research work like extraction of plant is can be a milestone in upcoming years.

In some cases the resercherers also reported that the plants extract and plant leaves, fruits have very important medicinal applications. Thus these plants can be utilized for ointments and external reagents for some medicinal -therapy. The work we are reporting the formation of extract of *Beta Vulgaris* for alcohol production and its application for the microbial activity.

2.0 Materials and Methods:

In this method the chemicals and materials required are Fresh beetroots, double distilled water, sugar, yeast and a sterile one litre glass vessel for fermentation process.

2.1 Collection of Beetroots:

The beetroots were collected from local market.

2.2 Sample Preparation for analysis.

The freshly obtained beetroots (1kg) were washed with distilled water, dried and then it was chopped into small pieces. These small pieces of beetroots then added to cleaned glass vessel of one litre. The glass vessel containing double distilled water (500 ml) to which small pieces of beetroots, sugar, and yeast (for fermentation) was added. The overall mixture was then allowed to settle down at room temperature. The appearance of this mixture is represented in Fig.3. This glass vessel along with overall composition was kept as it is for 8 days for the process of fermentation. After that overall mixture was filtered off from which alcohol was separated out. This isolated alcohol was sent up for GC-MS analysis and remaining was preserved for chemical analysis. The beet extract left after extraction of alcohol was kept for microbial assay.

2.3 Extract preparation for Microbial Assay

The fermented extract of *Beta Vulgaris* were ground on electric mixer for obtaining fine powder for extraction. Nearly 15g of fermented extract was mixed in conical flask containing 100 ml distilled water and allowed to boil at 80 °C for 120 minutes. After that, extract centrifuged at 2500 rpm for 15 min to remove sediment and supernatant was filtered with 0.45 µm filter. This extract was preserved in refrigerator for 4-5 days. Which was then used for antimicrobial activity against some pathogenic microorganisms.

2.4 Sample Preparation for GC-MS Analysis:

The fermented extract from which alcohol was isolated extracted in diethyl ether to aqueous and organic layer was separated by use of solvent extraction technique in separating funnel. Organic layer was stored in airtight glass vessel and used for GC-MS Analysis.

3.0 The diagrammatic representation of the overall procedure is represented as in Fig 1, 2,3,4,5.



Fig.1 Freshly Collected Beetroots



Fig.2 Beetroots chopped to small pieces



Fig.3 Beetroots Extract along with

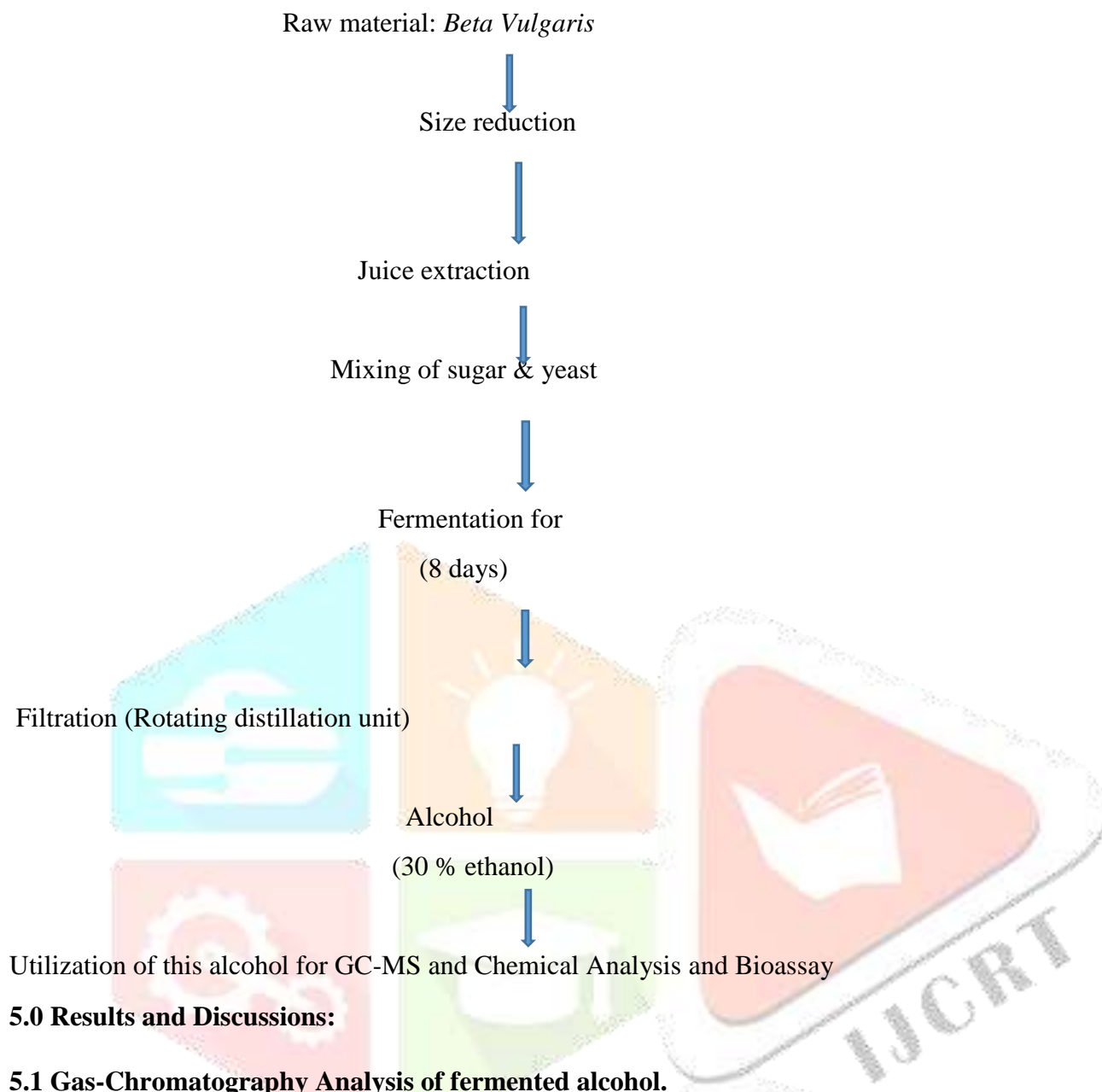


Fig.4 Beetroot Beer sample



Fig.5 Alcohol obtained from Beetroots by Fermentation

4.0 Flow chart for alcohol Extraction:



5.0 Results and Discussions:

5.1 Gas-Chromatography Analysis of fermented alcohol.

The GC-MS technique is generally utilized for getting the mass fragmentation data which confirms the possible fragments of our compounds that are detected in GC-MS. It facilitates the possible compound present in the particular compound. The confirmation of this unknown compound is can be known by m/z obtained from spectrum analysis. The gas chromatography has multiple uses for chemical analysis such as isotopic abundance calculation, molecular weight investigation, and Impurities detection by mass in drinking water, waste water, from air and other gases present in atmosphere. As well as GC-MS has wide range of scope for the analysis such as Structural elucidation of organic compounds, study of fragmentation process, Molar mass and structural analysis of small biomolecules, Environmental, Flavours, Fragrances, Forensic, Pharmaceuticals, Organic, Chemical, Petrochemicals samples etc.

In the present analysis we carried out the GC-MS analysis of fermented alcohol with by instrument Thermo Scientific TSQ 8000 Gas Chromatograph - Mass Spectrometer with Ion Source Type: EI source programmable to 350 °C, Mass Range: 2.1100 amu. The GC-MS spectrum of fermented alcohol is as depicted in

Fig.6. In which fermented alcohol peak is can be seen at retention time 0.6263 minutes. The sharp single peak in Fig.6 represent the alcohol fragment.

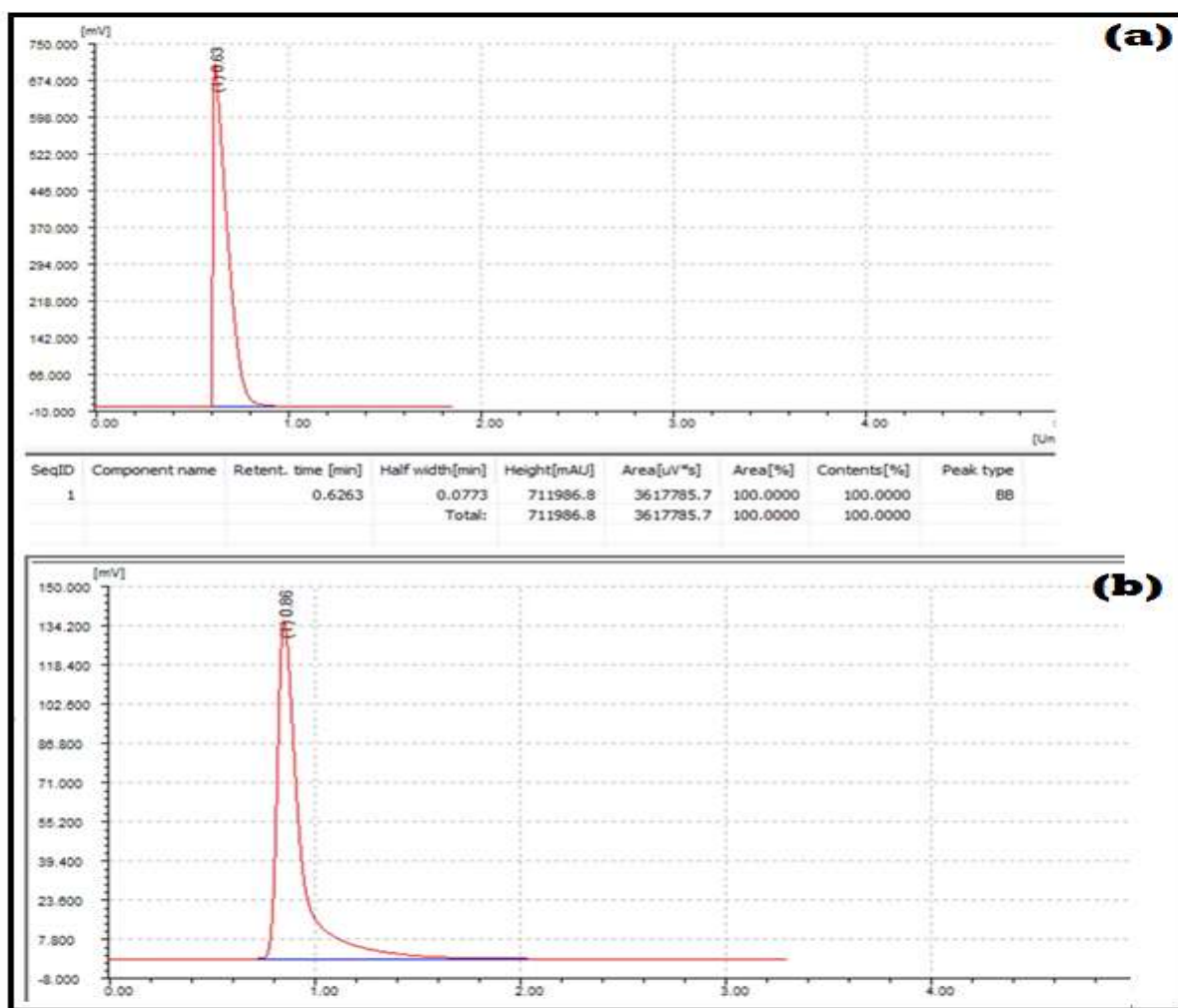


Fig.6 a-b GC-MS Spectrum of fermented alcohol obtained from Beetroots.

5.2 Microbial Assay:

Microbial Assay is an important parameter for the conclusions regarding the microbial response to the prepared materials.

Agar well diffusion method

The pathogenic bacteria culture was obtained from the department of Pharmacology, MGV's college of Pharmacy, Panchavati, Nashik. Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*Bacillus cereus*) were analysed in this study. The antibacterial activity of *Beta Vulgaris* were perform by agar well diffusion method. [8]. 100 μl bacterial suspensions was dropped on agar plate and spreaded uniformly with L-shaped sterile glass spreader. Sterile 8 mm cork-borer was used to prepare wells on agar plate and 100 μl of synthesized *Beta Vulgaris* extract after fermentation with a concentration 100 μg/ml were dropped on each well; *Beta Vulgaris* stock solutions were prepared in sterile distilled water. Later, plates were incubated at 37 °C for 24 h. After completion of incubation plates with clear zone diameter around each well called zone of inhibition (ZOI) indicates inhibition of bacterial growth, measured in mm. Experiment performed in triplicates. Fig.7 and 8

indicated Zone of inhibition obtained for bacteria *Escherichia coli*, *Bacillus cereus* against *Bacillus cereus* fermentation extract.



Fig.7 Zone of Inhibition (ZOI) for *Escherichia coli* for *Beta Vulgaris* fermented Extract

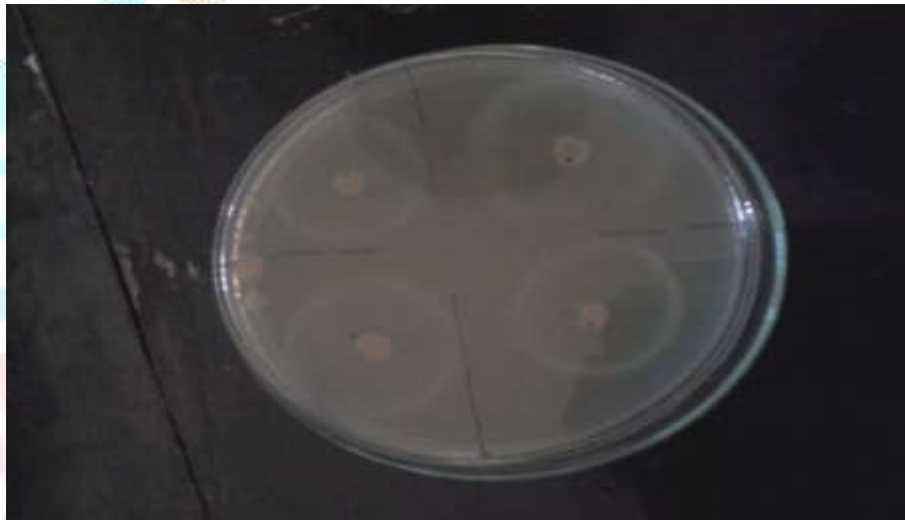


Fig.8 Zone of Inhibition (ZOI) for *Bacillus cereus* for *Beta Vulgaris* fermented Extract

SR.NO	Bacterial Strain	Gram Class	Zone of inhibition
1	<i>Escherichia coli</i>	Gram Negative	8 mm
2	<i>Bacillus cereus</i>	Gram Positive	16 mm

Table.1 Evaluation of antibacterial activity of Fermentation extract of Beta Vulgaris against the pathogenic microorganisms *Escherichia coli*, *Bacillus cereus* against the

6.0 Coclusions:

The present research deals with the Extraction of Beetroot for alcohol production by fermentation method a natural method. By this method we successful to extract the pure alcohol up to 30 % from the total extract of Beta Vulgaris. Represented in Fig.5. The main object of this research was to extract alcohol by fermentation method and the microbial assay of Beta Vulgaris extract. GC-MS data shows the retention time of alcohol in Fig.6 as well as the typical curve is also confirms the presence of alcohol. The microbial assay as represented in Table 1, clearly shows that the Beta Vulgaris extract inhibits the growth of microorganism and the extract can be utilized as antimicrobial agent for *Escherichia coli*, *Bacillus cereus*. In overall we can conclude the

alcohol is can be produced at minimum economical budget with the help of Beta Vulgaris extract and the material is can also be utilized to suppress the growth of several microorganisms such as *Escherichia coli*, *Bacillus cereus*.

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