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Research Article

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Traditional Brewing Techniques for Production of Bioethanol from *Madhuca longifolia* Flowers using Isolated Strain VIMS 1, *Saccharomyces cerevisiae* ATCC-9763 and Study of Parameters

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Abstract

Traditionally mahua flowers are collected by the tribals and used for making liquor is practiced in many states in India different regions of Andhra Pradesh, Maharashtra, Chhattisgarh, and some tribal communities cultivate and harvest mahua flowers for alcoholic beverages. Yeast strain used for study was isolate VIMS 1 belonging to genus Saccharomyces spp. isolated from Soil sample and Mahua flowers was used for bioethanol production following optimization of various parameters. The fermentation process was optimized considering various factors influencing fermentation process and at optimized process the isolated strain VIMS 1 generated on an average of 10.88% (w/v) of bioethanol after 72 hours of fermentation which was almost similar to the standard reference strain *Saccharomyces cerevisiae* ATCC-9763. Which produced average of 15.64 % (w/v) bioethanol. The isolated strain can be engineered to utilize various substrates and production of bioethanol can be increased in a fermenter which can solve energy crisis and serve as a substitute to fossil fuels.

Keywords: Media optimization, Mahua flowers, Bioethanol, fermentation, Bioenergy, Brewing Techniques.

Introduction

Mahua is one of the most common trees available in the Maharashtra region and many tribal people earn their employment by collection, processing and shelling the flowers, fruits, and seeds of this plant.

It is a fast-growing tree that grows to ≈ 20 meters in height, possesses evergreen or semi-evergreen foliage, and belongs to the family Sapotaceae (Ramadan et al., 2006). Mahua is a forest tree abundant in tropical rainforests of the Asian and Australian continents (Bhagmol and Joshi, 2002). However, this species of tree was cultivated by tribal peoples of India and Pakistan for food (flowers), fodder (leaves and flowers), timber (timber), and alcoholic beverages fermented flower) locally called 'mahuli' in India (Swain, 2007). Mahua flowers are edible and tribal food (Priyanka et al., 2012), used to make medicinal purposes (Gopalkrishnan et al., 2012). The fermented to make the alcoholic drink Mahua. In India, different regions of Andhra Pradesh, Maharashtra, Chhattisgarh, and some tribal communities cultivate and harvest mahua flowers for alcoholic beverages. Maua is a staple drink for tribal men and women during celebrations (Kirtikar and Basu, 2001; Madhumita and Naik, 2010).

In many regions of Maharashtra tribal people were used to use the leaves and bark of Mahua (*Madhuca longifolia*) to treat their wounds (Sharma et al., 2013). They first crush fresh leaves and the active ingredients come out, apply this juice containing the extract to the wound. After using the extract regularly, they noticed good results with wounds beginning to heal, demonstrating Mahua's activity as an antibacterial agent**.** Several researchers have performed this work in the laboratory using aqueous, ethanolic and methanolic solutions, using the cup-plate method to extract plant components from leaves and bark parts of mahua (*Madhuca longifolia*). (Jayaweera et al., 2018). They have taken ampicillin as a standard drug and after comparison; they found satisfactory response against Staphylococcus and *Escherichia coli* as a sample bacteria.

The current study focuses on Traditional brewing techniques for production of bioethanol from *Madhuca longifolia* flowers using isolated strain VIMS 1, *Saccharomyces cerevisiae* ATCC-9763 and study of parameters**.**

Materials and Methods

Mahua flowers

Mahua flowers were collected and were dried for two days to reduce the moisture content to 15 % followed by sterilizing the flowers by moist heat sterilization in the autoclave at pressure 10 lb/inch2 for a period of 15 minutes (Benerji et al, 2010). The total sugar estimated using Anthrone method was found to be 55%.

Stains and Culture Media

Yeast strain used for study was isolate VIMS 1 belonging to genus Saccharomyces spp. isolated from Soil sample by serial dilution pour plate and streak plate method on Sabouraud Dextrose Agar (Himedia- MH063-100G). Standard culture of *Saccharomyces cerevisiae*, ATCC-9763 was procured from Himedia. Yeast cultures were routinely subculture at an interval of 15 day and maintained Sabouraud Dextrose Agar.

Preparation of the seed culture

Fermentation slurry from Mahua: using distilled water (1:4) instead of glucose, malt extract 15 g/l, yeast extract 15 g/l and peptone 25 g/l at 121°C, 15 lbs was autoclaved for 30 minutes at a pressure of minute. The medium was cooled and 2-3 loops of the original maintained culture (Himedia) were added aseptically. Flasks of both VIMS 1 and ATCC-9763 isolates were incubated on a rotary shaker at 30°C, 80 rpm for 48 hours**.**

Fermentation process

The traditional brewing techniques of preparation of liquor from Mahua flowers at some of the tribal area of Palghar district, Maharashtra.

The investigation has used for the development of bioethanol production from Mahua flower extract by different types of fermentation process (batch and fed-batch) using separate VIMS 1 and yeast strain *S. cerevisiae* ATCC-9763 culture by inoculating. A mixture of bioethanol and hot water was separated using a simple distillation method at temperatures between 78 and 96 °C. In this method 85% of pure bioethanol is obtained, which rectified by using rectifier units to obtain 99.2% pure bioethanol. The alcohol percentage of the non-distilled product was determined by titration of the samples against sodium thiosulfate solution using dichromate oxidation method and expressed in percentage volume by volume (Wang et al, 2003). The distilled bioethanol concentration is estimated by potassium dichromate oxidation method and followed by spectrophotometric method using UV-spectrophotometer- Model LMSP UV1000B (Stanbury, et al 1997).

Effect of various parameters on bioethanol production

Effect of substrate concentration

The substrate concentration was varied from 5% to 35%, other parameters such as pH were kept at 5, inoculum age was 72 h, agitation was 80 rpm, temperature was RT and urea concentration was constant at 0.04%. kept.

Effect of pH

The effect of pH was studied by varying the pH of the fermentation medium from 2 to 10 using 0.1 M Sodium hydroxide and 0.1 M Hydrochloric acid.

Effect of temperature

The temperature affects ethanol production due to the volatility of ethanol. The temperature was varied from 25°C to 50°C.

Effect of inoculum age

The process of fermentation was carried out for a period of 7 days and after every 24 hours the ethanol yield was calculated.

Effect of inoculum level

Inoculum levels were examined to determine the percentage of inoculum in the medium for optimum fermentation. The range chosen was between 0.5 % to 3%.

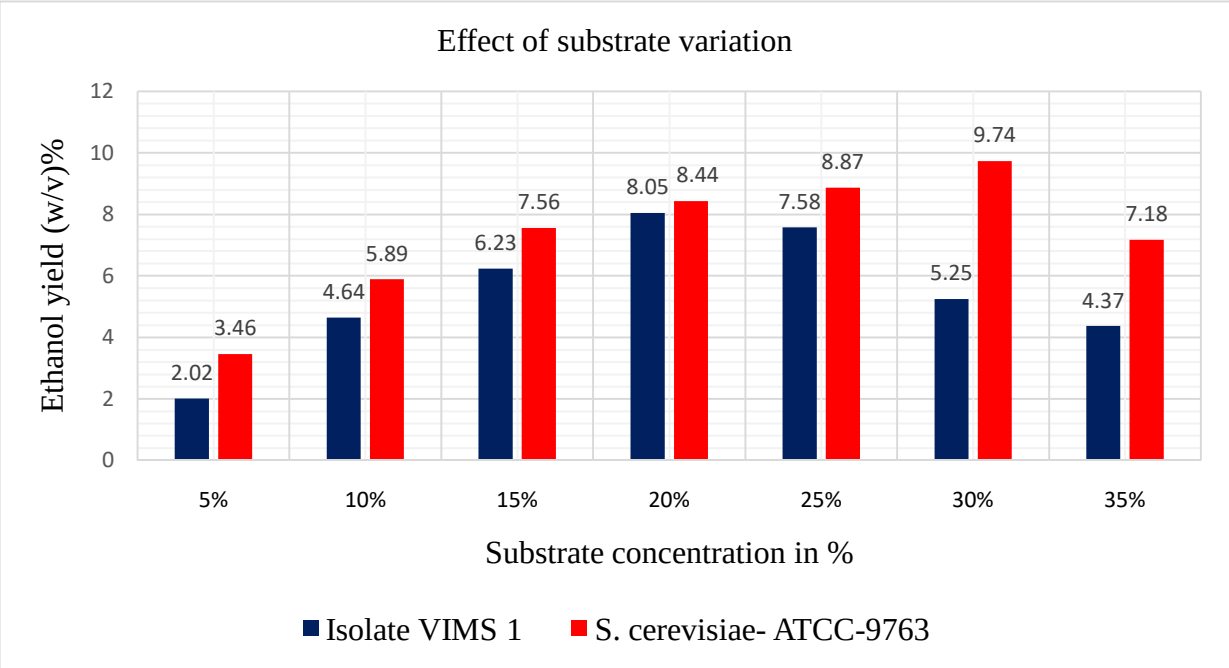
All the above parameters were studied and the production was carried out using the optimized parameters the percentage yield was carried for isolate VIMS 1 and compared with the standard reference strain *Saccharomyces cerevisiae* ATCC-9763.

Results and Discussion

Effect of substrate concentration

The substrate concentration was varied between 5 % to 35 % and the results were obtained by running three sets in different time span. It was found that 20% substrate concentration was best suited for VIMS 1 and 30% substrate concentration was best suited for *S. cerevisiae* ATCC-9763. The minimum fermentation time was 72 hours for both the strains after which even though the substrate concentration increased the yield decreased. Thus the optimum yield was 8.05 % (w/v) at 20 % utilization of substrate for VIMS 1 and 8.44 % (w/v) at 20 % utilization of 20 % substrate *S. cerevisiae* ATCC-9763 however the yield was 9.74 (w/v) for 30 % substrate utilization for the standard strain. To compare the effect of changes of various parameters on production of bioethanol the substrate concentration 20% was kept constant and other parameters were studied. The results obtained are reported in figure 1

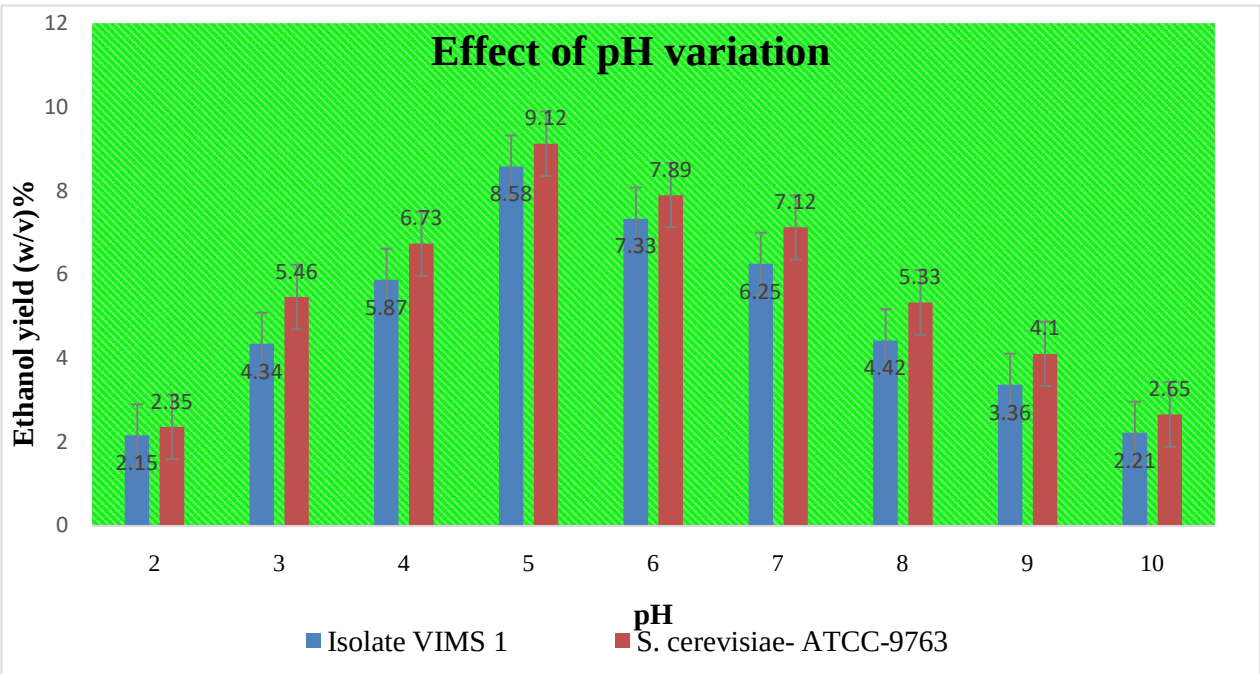
Figure 1: Effect of substrate concentration on bioethanol production



Effect of pH

The effect of pH was studied by varying the pH from 2 to 10. The ethanol yield was lower in acidic pH as well at highly alkaline pH. The optimum pH was 5 which showed 8.58 % (w/v) for isolate VIMS 1 and 9.12 % (w/v) for *S. cerevisiae* ATCC-9763. The results obtained are reported in figure 2.

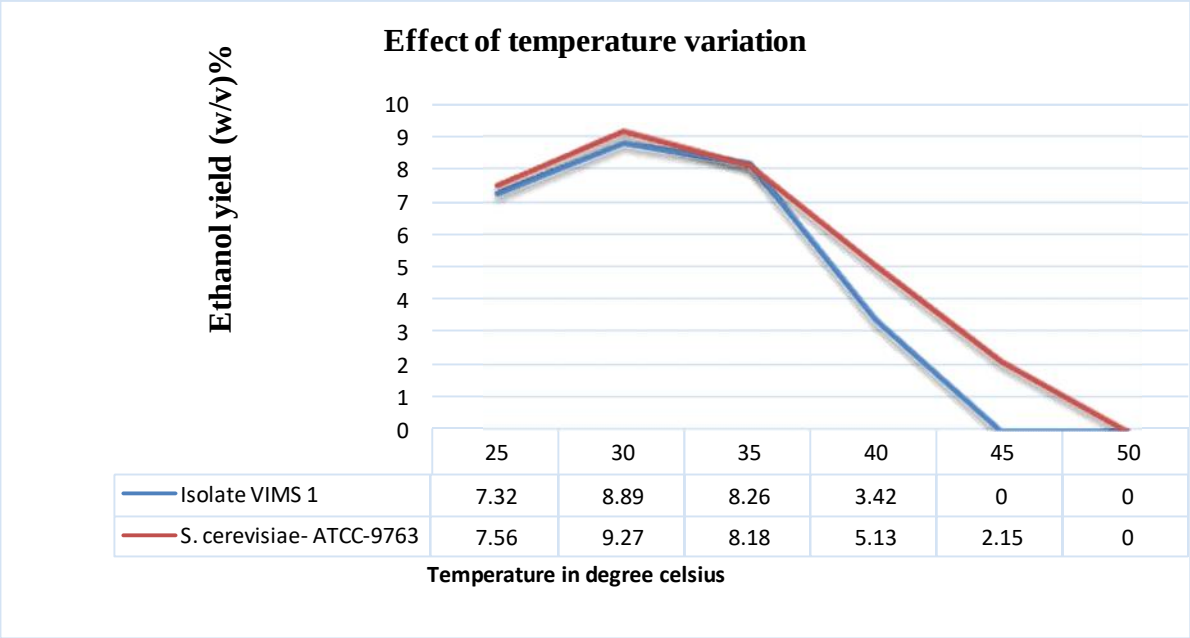
Figure 2: Effect of pH variations on bioethanol production



Effect of temperature

The effect of temperature was studied on the ethanol production. The temperature was varied from 25°C to 50°C. The optimum temperature was 30°C as yielded 8.89 % (w/v) for isolate VIMS 1 and 9.27 % (w/v) for *S. cerevisiae* ATCC-9763. The isolate VIMS 1 could not tolerate 45°C and 50°C hence no fermentation was obtained in the flask. The results obtained was reported in figure 3

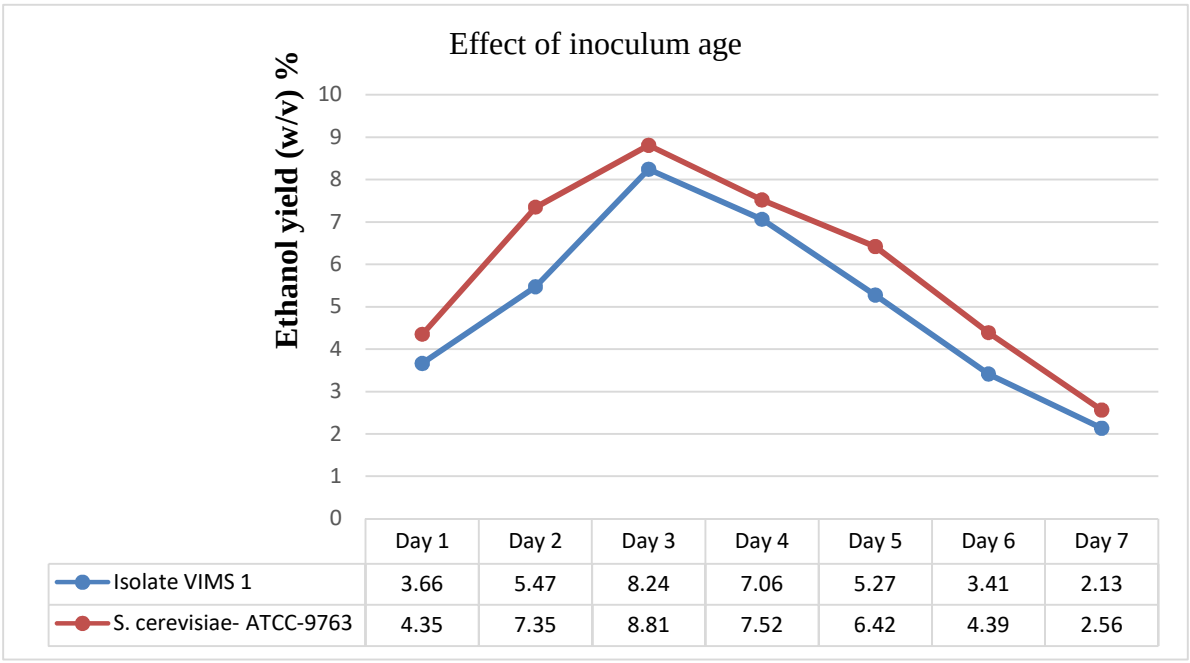
Figure 3: Effect of temperature variation on bioethanol Production



Effect of inoculum age

The process of fermentation was carried out for a period of 7 days and after every 24 hours the ethanol yield was calculated. It was found that the optimum yield of 8.24% (w/v) for isolate VIMS 1 and 8.81% (w/v) for *S. cerevisiae* ATCC-9763 was found after 72 hours. The fermentation continued for all seven days but dropped down drastically to 2.13% (w/v) and 2.56 % (w/v) for VIMS 1 and *S. cerevisiae* ATCC-9763 respectively. The results obtained are reported in figure 4.

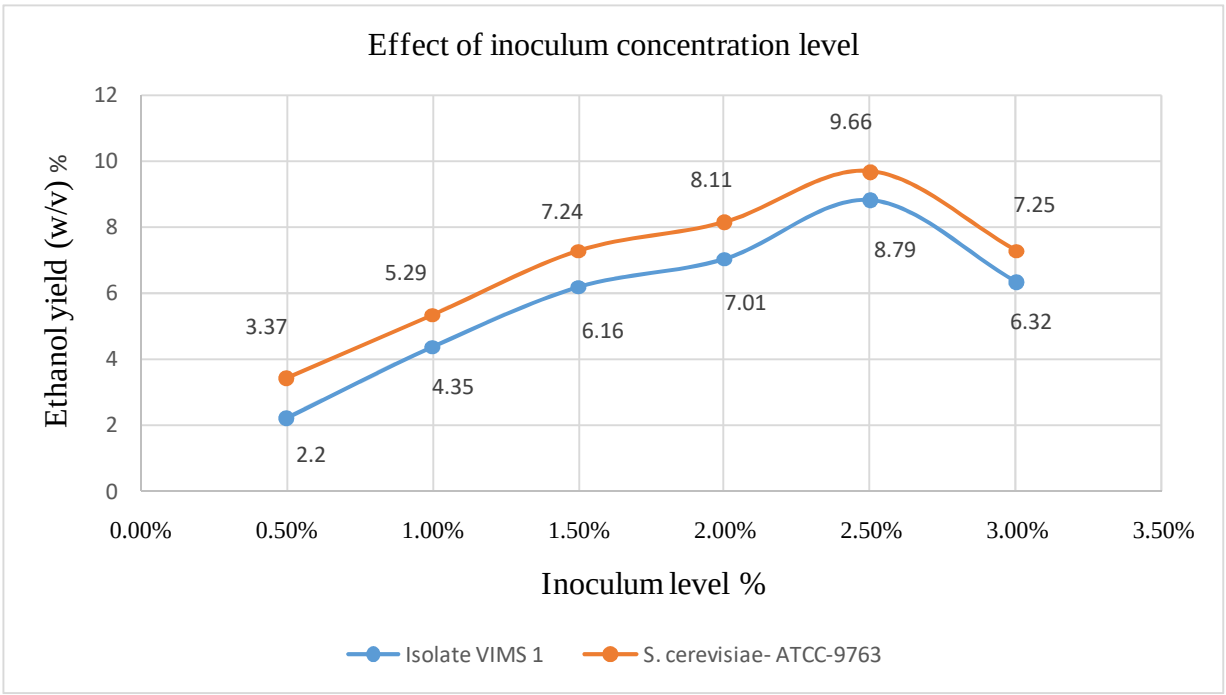
Figure 4: Effect of inoculum age on bioethanol production



Effect of inoculum level

Inoculum levels were examined to the percentage of inoculum in the medium for optimum fermentation. The range selected was from 0.5 % to 3%. It was found that 2.5% of inoculum was effective to yield 8.79 % (w/v) for isolate VIMS 1 and 9.66 % (w/v) for *S. cerevisiae* ATCC-9763. The results obtained are reported in figure 5.

Figure 5: Effect of inoculum concentration level on bioethanol production



Optimization of fermentation process

From the results obtained of the above parameters the optimum conditions for the fermentation process were set for isolate VIMS 1 and standard reference strain *S. cerevisiae* ATCC-9763. Which is summarized in table 1.

Mandal P and Kathale N. (2012) reported 302 ml of bioethanol production using *S. cerevisiae* 3044.

Table 1: Optimized conditions for fermentation process using mahua substrate and strains Isolate VIMS 1 and *S. cerevisiae* ATCC-9763.

Parameter	Value (w/v)%	
	VIMS 1	*S. cerevisiae* ATCC-9763
Substrate concentration (20%)	8.44	9.74
pH (5)	8.58	9.12
Temperature (30^0C)	8.89	9.27
Inoculum age (72 hr)	8.24	8.81
Inoculum concentration level (2.5%)	8.79	9.66

Table 2: Production of bioethanol (% w/v) under optimized conditions for fermentation

Strain/ Days	Day 1	Day 2	Day 3	Day 4	Day 5
Isolate VIMS 1	9.05	10.42	**10.88**	9.35	8.15
S. cerevisiae- ATCC-9763	10.45	13.12	**15.64**	12.43	10.34

Conclusion

Out of the isolated strains, VIMS 1 strain was better ethanol tolerant, thermo- tolerant, osmo-tolerant strain. The VIMS 1 strain was selected to ferment mahua flowers as a substrate and was compared with standard reference *Saccharomyces cerevisiae* ATCC-9763. The fermentation process was optimized considering various factors influencing fermentation process and at optimized process the isolated strain VIMS 1 generated on an average of 10.88% (w/v) of bioethanol after 72 hours of fermentation which was almost similar to the standard reference strain *Saccharomyces cerevisiae* ATCC-9763. Which produced average of 15.64 % (w/v) bioethanol. The isolated strain can be engineered to utilize various substrates and production of bioethanol can be increased in a fermenter which can solve energy crisis and serve as a substitute to fossil fuels.

References

Benerji, D. Rajini, K., Rao, B. Banerjee, D. Rani, K. Rajkumar, G and Ayyanna, C. 2010. Studies on physico-chemical and nutritional parameters for the production of ethanol from mahua flower (*Madhuca indica*) using *Saccharomyces cerevisiae* - 3090 through submerged fermentation (smf). J. Microbial Biochem. Technol. 2: 46-50

Bhagmol and Joshi V (2002) Underutilized plant resources. (Accessed 2. 03. 2005). http://www.Ipgri.Cgiah.org/

Gopalkrishnan B and Shraddha N. 2012. Pharmcognostical studies on stem bark of *Madhuca longifolia* (Koen.). Indian J. Nat. Prod. Resour. 3: 232-236.

Kirtikar, K. R and Basu, B.D.2001. Indian Medicinal Plants. Vol VII. Oriental enterprises. pp 2058-2061.

Madhumita P and Naik SN .2010. Flowers of *Madhuca indica* J. F. Gmel.: Present status and future perspectives. Indian J. Natl. Prod. Resources.1: 438-443

Mandal, P and Kathale N. 2012 Production Of Ethanol From Mahua Flower (*Madhuca latifolia* L.) using *Saccharomyces cerevisiae* - 3044 and Study Of Parameters While Fermentation Abhinav National Monthly Refereed Journal Of Reasearch In Science & Technology .(1)9.

Priyanka, Y., Anurabha M, Nayak S .2011. Microscopic studies of *Madhuca longifolia.* Scholars Res Lib.1: 66-72.

Ramadan, M., Sharanabasappa, G. Paramjyothi, S. Seshagirim M and Moersel J. 2006. Profile and levels of fatty acids and bioactive constituents in mahua butter from fruit-seeds of buttercup tree *Madhuca longifolia* (Koenig). Eur Food Res Technol. 710-718. DOI: 10.1007/s00217-005-0155-2

Sharma SK, Reddy AS, Akhil M, Sankar S. 2013.Phytochemical and antimicrobial activity of whole plant of *Madhuca indica*. IJRPC. 3(1):15-9.

Stanbury, P.F., Whitaker, A. and Hall, S.J., 1997. "Principles of fermentation technology", New Delhi (India): Aditya Books; 1.

Swain, M., Kar, S. Sahoo AK and Ray RC. 2007. Ethanol fermentation of mahula (*Madhuca latifolia* L.) flowers using free and immobilized yeast *Saccharomyces cerevisiae*. Microbiological Research 162: 93-98. DOI: 10.1016/j.micres.2006.01.009

Wang, M.L., Choong, Y.M., Su, N.W. and Lee, M.H. 2003. A Rapid Method for Determination of Ethanol in Alcoholic Beverages Using Capillary Gas Chromatography", J of Food and Drug Analysis. 11:33-40.

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