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Original Research Article

A Simple Method for Production of Nutraceutical Wine from Flowers of *Madhuca longifolia* (mahua)

Goswami R[1], Senapati SK[2*]

[1]Department of Biotechnology, Guru Ghasidas Vishwavidyalaya, Bilaspur, C.G, India
[2]R. C. M. Sc. College, Khallikote, Ganjam, Odisha, India

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***Corresponding author:** Senapati SK
R. C. M. Sc. College, Khallikote, Ganjam, Odisha, India

Abstract

In the world alcoholic beverages, such as whiskey, rum, beer etc were taken daily by about 2 billion people (WHO report). About 12% people consumed wine in their party, occasion, festival but the more alcohol containing wine are harmful for human. Some of local and cheap alcoholic beverage contain high amount of ethanol or contaminated by methanol, arsenic and other microorganism that are harmful for human being. In this present work, the nutraceutical wine was prepared using *Madhuca longifolia* (mahua) flower. The results of this investigation shown that the nutraceutical wine produced from mahua flower was highly nutritious containing 41% polyphenol, 28% ascorbic acid and a high content of protein and low concentration of ethanol and carbohydrate, which signifies the potential benefit of the wine.

Keywords: Nutraceutical wine, *Madhuca longifolia*, Ethanol, Polyphenol.

1. INTRODUCTION

Wine is an "alcoholic beverage" prepared from fermented grapes, berries, apple and other fruits with the help of yeast since thousands of years. Wine is widely consumed throughout the globe from ancient time in the name of Madeira, Sula etc. Wine have potentially beneficial effects on human health, especially due to the presence of polyphenols and resveratrol (Kalt, 2010; Karlsen *et al*., 2010; Kaplan, 2011; Quideau *et al*., 2011; Soyollkham *et al*., 2011; Wood, 2011; Xie *et al*., 2011; Weingerl, 2012). The wine prepared traditionally through anaerobic fermentation by using Grape and other fruits as substrates contain about 10-14% alcohol in 100 ml and have many problems in their taste, purity and quality. The local wine companies were not well conscious regarding removal of contamination of chemicals, excess content of ethanol, sugar etc. because of which stuck fermentation starts again after the wine is sold out in the market. Sometimes arsenic compound and yeast cells are present in it due to bad filtration or clarification of wine. High content of alcohol in wine is harmful to our health and also didn't have minerals or vitamins. The hybrid of nutritional and pharmaceutical termed as Nutraceutical was first coined by Dr. DeFelice in year 1989 are playing significant role in modifying and maintaining the normal physiological function that maintains good health of human being. The nutritional product prepared from food source have their basic nutritional value like dietary fibers, vitamins and minerals etc along with polyphenols, resveratols, flavonoids, anthocyanins. Due to presence of Number of secondary metabolites these products have antioxidant properties, and help in prevention from diseases and maintain the physiological functions. USA is a largest nutraceutical market about 50.4 million USA dollar. Nutraceautical wine contain nutrional ingredients, vitamins, pigment and natural chemicals. Nutraceautical wine prepared by anaerobic fermentation using very costly fruits, soft berries and supplemented with nutritional ingredients, vitamins, pigment and natural chemicals. It is generally made by *Vitis venifera* by using some yeast strain in anaerobic fermenter with subsequent aging process because grapes already have some nutraceutical benefits. Nutraceutical wine contain polyphenol, resveratol, anthocyanins, flavonoids and vitamins that increase the quality of wine and also use as medicinal purpose. Nutraceutical wine has low alcohol content and costly than normal wine. *Madhuca longifolia* are Indian tropical trees found all over India. The flowers of mahua are used for making alcoholic beverage in local region but they do not clarify it which is harmful for us but it has several herbal tonics and vitamins. Production of wine from grapes is well known

but, limited information is available on the production of wine from other plants particularly from mahua. So, preparation of wine using flower of Mahua with different concentration of sugar syrup on the quality of wine was proposed in the current investigation with the following objectives.

1. Standardization of the protocol for preparation of wine from Mahua through Yeast mediated fermentation.
2. Qualitative analysis of different chemicals present in the Neutraceutical Wine

2. MATERIAL AND METHODS

In this investigation *Madhuca longifolia* commonly known as mahua was used as the plant substrate for preparing the wine. *Madhuca* is an Indian tropical tree largely found in the forests of central and north India. This fast-growing semi-evergreen foliage tree of Sapotaceae and grows up-to 20 meters in height (Fig-1). The flowers are being consumed by tribal people as a food item, make syrup for medicinal purposes and also fermented to produce the alcoholic drink *mahua*, a country liquor. Tribals of Bastar in Chhattisgarh and peoples of Western Orissa, Santhals of Santhal Paraganas (Jharkhand), Koya tribals of North-East Andhra Pradesh, Bhil tribal in western Madhya Pradesh and tribals of North Maharashtra consider *Mahua* as an essential drink for tribal men and women during celebrations.

2. Method of Wine production

The Production of Mahua wine was divided in to two phase i.e.

A. Upstream Processing

B. Downstream processing.

2.1. Upstream Processing

Step-1

Preparation of Sample and designing of low cost fermenter for fermentation: A total of 750 gm of mahua flower (Fig 2A) was collected and washed with water followed by antifungal agent, then it was cleaned with distilled water and the unwanted waste materials were removed. The cleaned material was stored in a beaker and 5 gm of sugar was added in it and left for overnight. Extraction of juices from mahua was done with the help of juicer and all the juice was collected and mixed in 1:1 ratio with distilled water and stored aseptically for further use.

The anaerobic fermenter was designed for fermentation by taking a plastic jar covered with cap. A hole was made in the cap and a tycoon pipe was fitted at one end of the closed jar. The other end was suspended in water for preventing inlet of O_2 and facilitating evacuation of CO_2 from the fermenter. The sample port was designed for collection of sample for testing. A stirrer is attached with motor for agitation and mixing (Fig-2B). The Sugar level was also maintained upto 20 Brix through refractometer and the pH was maintained up to 3.5 by adding of tartaric acid or Sodium bicarbonate. The Initial specific gravity of sample was checked after addition of sugar and the final specific gravity was checked after secondary fermentation with the help of hydrometer.

Step-2: Fermentation

Fermentation is a process in which sugar molecules are broken down by yeast to produce ethanol which comprises of two stage process i.e. Primary fermentation and Secondary fermentation.

In Primary fermentation, 5% Yeast (*Saccharomyces cerevisae*) were grown in a 5% sugar solution and warmed up to 40°C and incubated for 3 hours at 28°C. Then 5% activated yeasts were inoculated in sample along with it, 0.2 gm of sodium meta bisulphite and were mixed well for prevention of growth of other microorganisms in the sample. The sample was put in fermenter for primary fermentation for 10 days. The yeast viability, production of alcohol, sugar content and pH of sample was checked regularly. After 10 days, the presence of alcohol was checked and then the sample was set for secondary fermentation.

In the Secondary fermentation sample was collected and its specific gravity was checked after primary fermentation and dead cells of yeast were removed from the sample and fermenter was cleaned and sterilized it. The sample was then in fermenter for secondary fermentation for 15 days.

Step-3: Addition of Nutraceutical compound

Nutraceutical compound from grapes skin (Fig-2C) was added in the sample (100 ml) and mixed well and put for 3 days.

Step-4: Checking of Yeast viability

To check the viability of the yeast, one drop of wine sample was taken on a glass slide and one drop of (0.1%) methylene blues was added to it and observed under microscope. The white cells observed under microscope indicated that the viable yeast cells.

2.2. Downstream Processing

Step 1: Siphoning and clarification

Racking and filtering of the sample is carried out by filter paper, cheese cloth followed by centrifugation. In this step clarification of the wine was done by adding enzyme such as pectinase, chemical fining agent sodium benzoate (0.5g), bentonite clay, gelatin and charcoal. Then filtration was done using filter paper (Fig-2D) and the other particles removed by centrifugation.

Step 2: Qqualitative and Quantitative analysis of the wine

Qualitative analyses of different phytochemicals were made through different methods. The details of different types of the phytochemicals

analyzed and methods adopted were reflected in Table-1.

Quantitative analysis of the wine is perform to find out the total content of alcohol, protein, carbohydrate, ascorbic acid and polyphenol. Alcohol content was measured from the specific gravity (measured through hydrometer) using the following formula

$$\% \text{ Alcohol} = \frac{(SG1-SG2) \times 1000}{7.36}$$

Protein analysis was made through by Lowry method (Lowry et.al), carbohydrate analysis was conducted through Anthrone reagent (Hedge, J.E. and Hofreiter, BT), ascorbic acid analysis was carried out through calorimetric method and content of polyphenol was measured through folin-ciocalteau reagent method.

Step 3: Bottling of sample

Bottling was made in a clean sterilized glass bottle of 650ml capacity and sealed with cork stopper.

3. RESULTS

Nutraceutical wine should have balanced quantities of alcohol; essential nutrients like sugar, ascorbic acid and tannins; nutritional salt for growth of yeast and water to produce a naturally stable and drinkable wine, which was mainly determined the growth of yeast and rate of fermentation. Hence the concentration of sugar and pH should be optimum during the fermentation to provide better environment for growth of yeast. In this investigation it was observed that sugar level of 20brix and pH of 3.4 provides a healthy environment for yeast fermentation of the Mahua substrate inside the fermenter. One of the important parameter which determine the quality of the wine is the microbial contamination particularly yeast contamination. In the viability test of yeast it was observed that the yeast viability inside the fermenter reduced with time (Table-2) and at the end of the secondary fermentation no traces of yeast or any microorganisms were found (Fig2 E).

The most important factor which determines the quality of the wine is the type of alcohol like ethanol, methanol; chemical nutrients like carbohydrate, protein, phenols, flavinoids, alkaloids, terpenoids etc and their concentration present in the wine. The qualitative analysis of the wine reveled that it contains ethanol, carbohydrate protein, phenols, flavinoids, alkaloids, terpenoids (Table-3, Fig-4). Quantitative analysis of the nutraceutical wine produced shown that it contain a maximum amount of polyphenol (41%) followed by protein (36.4%), ascorbic acid (28%) and low content of carbohydrate (Fig.-4).

From the comparative study of nutraceutical value among whisky, grape wine and Nutraceutical wine produced in this investigation and it was observed that Nutraceutical wine has low ethanol content and higher polyphenol in comparison to other two types and which indirectly determine the quality of wine (Fig-5).

Table-1

Name of Phytochemical	Qualitative analysis Method Adopted
Phenol	Take 1 ml sample 1 ml $FeCl_3$ (10%) and take the observation
Carbohydrate	Take 2ml Filtrate add Bradford Reagent then Heat the solution in water bath and take the observation
Flavonoid	Take 0.5ml Sample add 10 drops of HCl followed by Zinc dust and take the observation
Alkaloid	Take 1ml Sample add Hager's reagent and take the observation
Terpenoid	Take 1ml Sample add 2ml Chloroform followed by H_2SO_4 3ml and take the observation
Ethanol	Take the sample add $K_2Cr_2O_7$ followed by H_2SO_4 the Heat the solution and take the observation
Methanol	Take the sample add KI followed by NaOH and take the observation

Table-2

Incubation time Period	Observation	Result
2 days after fermentation	more yeast viable cell	yeast cell are present In large number
4 days after fermentation	more yeast viable cell	yeast cell are present In large number
6 days after fermentation	more yeast viable cell	yeast cell are present In large number
8 days after fermentation	less or death yeast cell or viable cell are present	yeast viable cell present in small number
Before the last day of primary fermentation	less viable cell are present	yeast viable cell present in small number
Secondary fermentation	less viable cell are present	yeast viable cell present in small number
2 days after secondry fermentation	less viable cell are present	less viable cell are present
5 days after secondry fermentation	less viable cell are present	less viable cell are present
Last days after secondry fermentation	no viable cell	yeast cell was dead
After fining or clarification	no viable cell	no yeast was present

Table-3

Type of chemical	Observations	Inference
Ethanol	Light Green solution	Present
Methanol	Clear (No precipitation)	Absent
Phenol	Green Colour	Present
Carbohydrate	Red precipitate	Present
Flavonoid	Dirty Pink Colour	Present
Alkaloid	Prominent Yellow Precipitate	Present
Terpenoid	Reddish Brown Colour	Present

Fig-1 A-C: showing plants of *Mahua longifolia* Tree (A); branches containing flower (B); Flower bunch (C).

Fig-2: A-E showing different steps of production of wine. Dry flower of Mahua (A); Yeast inoculant (B), Fermentation apparatus (C); Microscopic photo graph showing no traces of yeast in the sample (D) Filtration of the sample through filter paper (E).

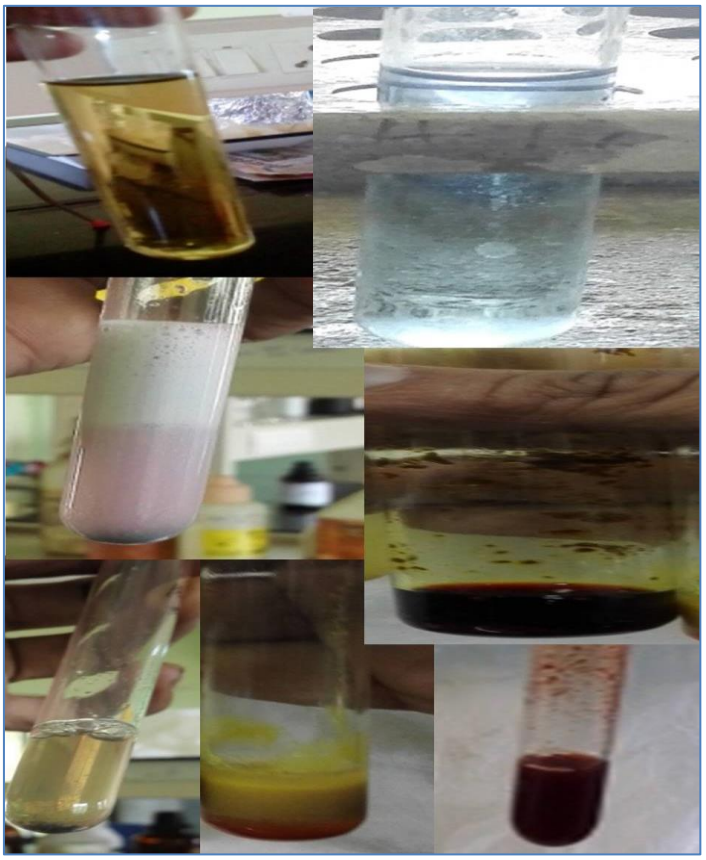

Fig-3 A-G: Qualitative analysis of wine produced from the Mahua flower fermentation. Ethanol (A); Methanol (B); Flavinoids (C); Terpenoid (D); Phenol (E); Alkaloids (F); Carbohydrate (G).

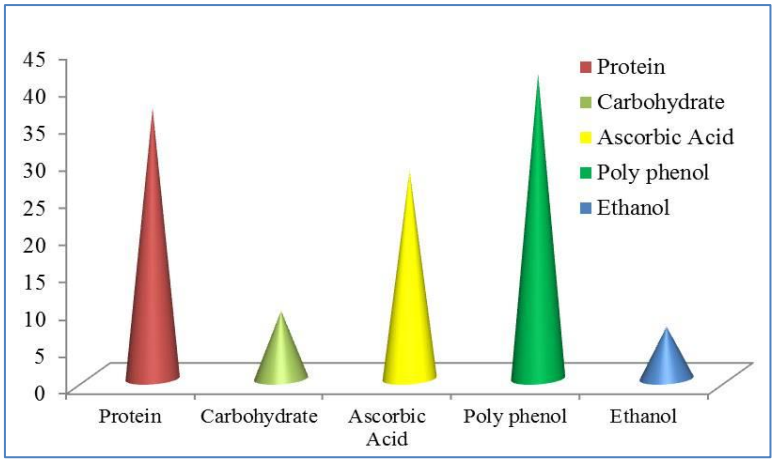

Fig-4: Quantitative analysis of Different molecules present in the wine produced from Mahua flower

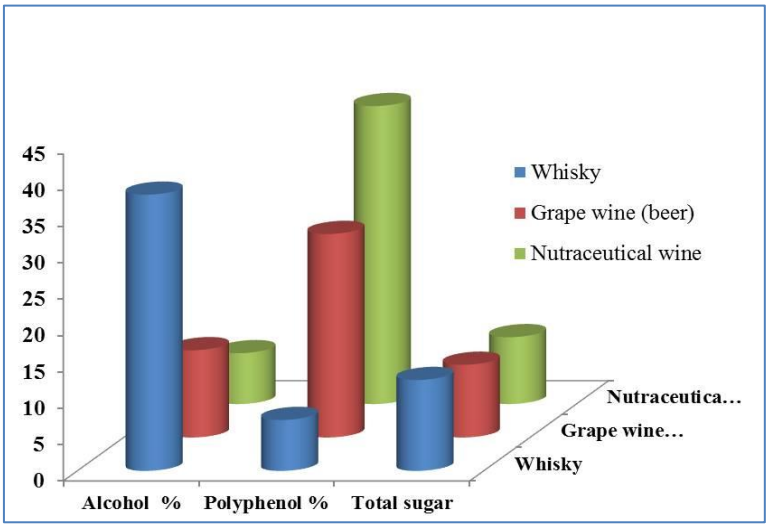

Fig-5: A comparative Quantitative analysis of Different molecules among three different types of wines

4. DISCUSSION

Moderate consumption of wine has so many positive effects but excessive consumption of wine is injurious to human health. One of the important constituent of wine is ethanol which concentration determines the quality of the wine as it act as the enzyme inducer and positively influences the effect of polyphenol on phase-I & II metabolism. In this investigation the wine produced from Mahua contains only 7% which is very low than the wines produced from other sources like grape, peaches, plum, apricot, banna etc. (Shrikanta *et al*., 2014; Mishra *et al*., 2016), which indicated that wine production from Mahua is a good alternative. Even if it is less than the wine produced from mixture of Mahua flower extract and pomegranate fruit juice and Mahua flower extract with Guava (Sony and Dey, 2013; Priyanka *et al*. 2019). Polyphenol are the most abundant antioxidant found mainly in fruits and beverages. The wine produced through the current protocol contained 41% polyphenol which is more than the report published earlier (Sony and Dey, 2013; Priyanka *et al*. 2019).presence of high content of polyphenol particularly resveratrol proved that the wine has potential beneficial effect on human health which is same to the report published earlier (Karlsen *etal*., 2010; Kalpana, 2011; Xie *et al*., 2011 and Weingerl, 2012). The content of Ascorbic acid is also quite more than the earlier report (Priyanka etal 2019). The results of comparative analysis among the whisky, grape wine and the wine produced through this protocol shown that ethanol and sugar content is low but polyphenol content, protein content were very high in the neutraceutical wine than that of wine and grape wine, which signifies the potential benefit of the production of wine from the Mahua flower.

5. CONCLUSION

The neutraceutical wines are far better than many beverages (alcoholic as well as non- alcoholic) with response to nutrition as well as price's point of view. The results of the current investigation shown that the Mahua is a better substrate for preparation of wine due to their availabity and easy processing method. The nutritional and chemical analysis revealed that the wine produced from Mahua is rich in ascorbic acid, polyphenol, free fatty acids, and proteins and has very low content of sugar and alcohol. From this it can be concluded that Mahua can be used for commercial

production of nutraceutical wine and may turn out to be a great product in the market.

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