Abstract—This is the first report from India on a beverage resulting from alcoholic fermentation of the juice of sea buckthorn (Hippophae rhamnoides L) using lab isolated yeast strain. The health promoting potential of the product was evaluated based on its total phenolic content. The most important finding was that under the present fermentation condition, the total phenolic content of the wine product was 689 mg GAE/L. Investigation of influence of bottle ageing on the sea buckthorn wine showed a slight decrease in the phenolic content (534 mg GAE/L). This study also includes the comparative analysis of the phenolic content of wines from other selected fruit juices like grape, apple and black currant.

Keywords—Alcoholic fermentation, Hippophae, Total phenolic content, Wine

I. INTRODUCTION

The rich nutritional and bioactive substances in Hippophae rhamnoides L. commonly known as sea buckthorn have attracted many people who are interested in the production or utilization of nutritional and medicinal sea buckthorn products for preventive or curative purposes. There is strong recent or re-emerging interest in such products in Europe, North America and Asia [1]. The berries of sea buckthorn are among the most nutritious and vitamin-rich fruit known. They contain large amounts of essential oils and vitamins C. Sea buckthorn is also rich in protein, especially globulins and albumins, carotene, fatty acids (saturated and unsaturated), free amino acids, flavonoids and vitamins E [2], [3], [4], [5].

Beneficial effects of the sea buckthorn berries and berries fractions on human health have been extensively investigated and substantiated by studies, suggesting a great potential of the berries for maintaining and promoting human health [4], [6], [7].

Oral administration of sea buckthorn berry juice increased the plasma HDL-cholesterol concentration and decreased the susceptibility of LDL to oxidation in human. Flavonoid extract from sea buckthorn berries showed in vivo antithrombotic properties in an animal model, probably due to inhibition of platelet aggregation. Moreover, in vitro assays evidenced distinct cytoprotective properties of sea buckthorn berry extracts, which could be attributed to their anti-oxidant activity [8].

Based on established reports of nutritional and medicinal properties of the berries, we thought it is worthwhile to develop a product which can serve as “functional foods” which are specialized foods capable of providing additional physiological benefits such as preventing or delaying onset of chronic diseases, as well as meeting basic nutritional requirement.

As the fermentation process has many advantages like increasing the shelf life of product, improving the aroma and texture of the product and in some cases enhancing the nutritional value of the final product, an attempt was made to produce sea buckthorn wine using alcoholic fermentation. To the best of our knowledge, this is the first report on production of sea buckthorn wine, using lab isolated yeast strain. So far only malolactic fermentation of sea buckthorn juice has been reported by [9].

In the present study we report a comparative analysis of total phenolic content of the sea buckthorn wine and other selected fruit wines from grape, apple and blackcurrant produced with same strain under similar conditions. As this is the first report on development of alcoholic fermented product from sea buckthorn juice, the influence of bottle aging was also investigated on the phenolic content of the sea buckthorn wine in order to investigate the effect of storage condition on the total phenolic content.

II. MATERIALS AND METHODS

A. Strains

The strains of S. cerevisiae, used in this study were of two type i.e. lab isolated yeast strain (S4) and two industrial strains from two breweries, viz, Vintage and Minchy’s Pvt Ltd.
B. Sources of juice samples
Commercially available fruit juice of apple, grape, black currant and sea buckthorn were purchased from Real and Leh Berry, respectively. To avoid variation between batches, we obtained a large consignment of the same batch from a single vendor.

C. Chemicals
Chemicals used in this study were Yeast Extract, Malt Extract, Peptone, Glucose (Dextrose), Diammonium ortho phosphate (DAP), Gallic acid, Folin & Ciocalteu’s Phenol Reagent (F-C), Sodium Carbonate. All reagents used for the study were of analytical grade. Gallic acid and F-C reagent were purchased from Sigma–Aldrich.

D. Wine production
Different fermentation was set up using different strains. The pasteurized juices (100ml) were inoculated with 0.2g (wet weight) of the yeast strains. To the juice 0.2g of DAP and 9g of dextrose was also added prior to fermentation. The wine fermentation was set at 30°C at stationary phase for 7 days.

E. Total phenolic content
TPC was determined using the Folin-Ciocalteu reaction [10], with gallic acid as the standard.

III. RESULTS AND DISCUSSION
As sea buckthorn juice has many therapeutically useful compounds, an attempt was made for the first time to develop a fermented product using a lab isolated strain. For the fermentation, 14 strains of yeast were collected from different locations of state of Himachal Pradesh (India). Of them, one strain S4, was found to have highest ethanol tolerance (13% v/v) and hence was used for the development of the final product. The wine was made using the juice of the berry and the S4 strain. Earlier studies performed in our lab showed that the final wine product was of semi-dry style with a pH of 3.02 [11].

A. Total Phenolic Content
Phenolic acids have been associated with color, sensory qualities, and nutritional and antioxidant properties of foods [12]. Phenolics behave as antioxidants, due to the reactivity of the phenol moiety [13]. Current thought links the high antioxidant content of fruits and foods with the inhibition of oxidative damage diseases such as coronary heart disease, stroke and cancer [14]. With respect to wines, it is a well established fact that red wines gets its antioxidant and anti-inflammatory properties due to the phenolic constituents [15], [16], [17]. Wines are unique because they contain both antioxidant and alcohol. Its antioxidants are protected once they go through the body by the same process that our body uses to detoxify ingested ethanol. In the liver there are two NAD-dependent enzymes, alcohol dehydrogenase converts ethanol to acetaldehyde and aldehyde dehydrogenase converts acetaldehyde to acetate, these enzymes produce NADH in each step. This NADH is then capable of recycling used up antioxidants by reducing them and in the process regenerates NAD+, which in turn is required to detoxify more ethanol [18]. In this regard, studies based on phenolic content of berry wines have shown that they are more effective than red and white wines. By the study of [19], on the basis of specific phenolic content, summer cherry, blackberry and blue berry wines were 30-40% more efficient in superoxide radical scavenging than red grape wines. More recently [20] evaluated the antioxidant activities of fruit wines and arranged them in the following order bilberry > blackberry > black mulberry > sour cherry > strawberry > raspberry > apple > quince > apricot > melon. So far the most detailed study reported on fruit wine phenolics and antioxidant activity is by [21] the amount of total phenolics in the berry and fruit wines and liquors ranged from 91 to 1820 mg GAE/L. Therefore certain fruit wines can be classified as functional food, owing to their established healthful protective effects, as functional foods are dietary components that may provide a health benefit beyond basic nutrition. In this backdrop we found that sea buckthorn which is also another berry has not been explode for oenological studies. The result in our lab showed that total phenolic content of sea buckthorn juice was 933 mg GAE/L.

Based on these result, we used sea buckthorn juice to develop a wine product and investigate whether it could be consider as a functional beverage on the basis of phenolic content.

For the development of wine, the more essential factor was yeast strain and the fermentation time. Recently there has been report of reduction of catechin, rutin and quercetin levels by interaction with wine-related micro-organisms like Pedicoccus pentosaceus ATCC 25745, Saccharomyces cerevisiae ATCC 36026, and a probiotic strain, Bifidobacterium longum DJO10A [22]. This would have important implications for the overall bioavailability of phenolics in the sea buckthorn functional beverage. Therefore, in this study, three strains of S cerevisiae were examined for their ability to absorb and/or transform the phenolics.

Fig. 1 Study of effect of different strains in total phenolic content of sea buckthorn wine
According to Fig. 1, the highest amount of total phenolic content which was retained in the wine was obtained by fermentation with lab strain S4 (689 mg GAE/L) in comparison to strains from Vintage and Minchy’s Pvt. Ltd. (634 and 544 mg GAE/L), respectively. These observations point to the fact that the strain (S4) has lesser absorption potential for the phenolic as compared to the other strains and hence was used for the future experiments.

From the present work it can be inferred that the intake of phenolic with 100 ml of sea buckthorn wine is at least 68.6 mg GAE which falls in the range of intakes of phenolic compounds from some other well known beverages like black tea 57-357 mg, linden flower 0.27-24 mg, sage, 1-105 mg, grape molasses (1-42 mg), violet carrot juice (6-110 mg) and tarhana (5-149 mg) per day, respectively [24]. Hence, sea buckthorn wine can also serve as a good supply of phenolic compounds.

Apart from the source of the fruit and the fermentation conditions another important factor which affects the phenolic content of fruit wine is the reactions that take place during the process of aging. A comparison of phenolic content in young and bottle aged wine (4 months) showed a slight decrease in total phenolic from 68.9 to 53.4 mg GAE/serving (Fig. 3). Similar results showing a decrease in phenolics, have been also reported by [25] in red wines. Another study on the changes of phenolic content in blended and aged wine by [26], registered an increase in total phenolic concentration after 9 months of aging followed by a decrease at 23 months of aging in the bottle. These changes are possibly due to the transformation of phenolic compounds into condensed forms that possess slightly different chemical properties and reactivities towards the Folin-Ciocalteau reagent [27].

Based on the results indicated in the present study, the fact emerges that sea buckthorn wine can be a potential health promoting product. However the antioxidant activity of wine has to be evaluated before drawing any further conclusion. The phenolic content of wine further can be improved by...
blending. Application of technology for improvement of sensory qualities of wine need to be evaluated for acceptance in the wider section of the population.

IV. CONCLUSION

Considering the phenolic potential of this sea buckthorn wine, more effort should be devoted to increase its production and to optimize its phenolic availability by alternative processes like blending.

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