# PRODUCTION AND VALUE-ADDITION OF VINEGAR AND CHARACTERIZATION FOR ITS NUTRACEUTICAL PROPERTIES

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***Abstract***
*Vinegar is an acid condiment liquid produced from fermentation process of ethanol that yields acetic acid (ethanolic acid). Acetic acid concentration ranges from 4-8%in the natural vinegar. Present study focused on the vinegar production from the orange and pear along with the value addition of the medicinal plants like Coleus amboinicus (Mexican mint) and Tinospora cordifolia (Amirthaballi). Concentration of the vinegar was monitored from the day of inoculation, maximum concentration was recorded in the seventh day that is 0.12 N. Phytochemical constituents like cardiac glycoside, reducing sugar and terpenodies are present in the vinegar without value addition and cardiac glycoside, reducing sugar, flavonoids, terpenodies in aqueous extract of the medicinal plants. Cardiac glycoside, reducing sugar, flavonoids, glycosides and terpenodies shows positive results in the vinegar with value addition. The free radical scavenging activity of vinegar without value addition was 1.15 whereas vinegar with value addition was 0.40 was reported. Functional groups like alcohol, amine, aldehyde, imines, carbonyl, amide, carbon chloride were present in vinegar without value addition whereas vinegar with value addition consist primary and secondary amine, aldehyde, alkyl, amine and amide which was determined by FTIR analysis technique.*

**Key words:** Vinegar, Tinospora cordifolia, Coleus amboinicus, Nutraceutical.

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## 1. INTRODUCTION

The word Vinegar derived from the French words vine and aiger. It is a condiment made from different sugary and starchy materials by alcoholic and acetic fermentation (Cruess, 1958). Cider, rice, fruits musts, wine (red, white), juices (like pineapple, sugarcane, etc.), malted barley or pure alcohol are commonly used as substrates. Since from the ancient times for food preservation, cleaning, cooking and medicine vinegar has been used. Natural vinegar is high ranking food additive over synthesis vinegar as it carries essential amino acids from the substrates and which contains medicinal properties, it is able to treat the aches and gastric troubles (Er.Jay Hemke, 2020).

Pear is one of the highly nutritious fruits belong to Rosaceae family rich in vit-C, vit-A, copper, proteins, potassium. It is also containing provitamin A, folate and niacin. Folate and niacin are essential for cellular function and energy production. In the same way provitamin A supports skin health and wound healing; it is an excellent source of polyphenol antioxidant. Whereas its peal contains polyphenol of six folds more than pulp. Orange belongs to citrus fruit family. Citrus fruits are very good source of bioactive compounds like ascorbic acid, polyphenol, carotenoids and its antioxidant properties boost the benefits of vinegar (C.V. Davies, 2007). Amirthaballi (*Tinospora cordifolia)* is one of the traditional medicinal plants responsible for its various beneficial characters. Amirthabali (*Tinospora cordifolia*) leaves shows effective anti-microbial activity against *E. coli* (Kantesh M, 2017). Even it exhibit antidiabetic, anticancer, antioxidants, antitoxic properties (Reddy N.M, 2015). And rich in alkaloids like bereberine, palmatine, tembetarine, isocolumbin, syringing, and sitosterol. Among ancient medicine plants *Coleus amboinicus* plays a vital role,it is used to treat sour throat, skin problems and even for few respiratory disease. *Coleus amboinicus* is reported that it is enough able to boost immune system.

Due to their antioxidants, antitoxic, anticancer, antimicrobial, antidiabetics and immunity boosting properties it is able to produce a novel

product. The present study aimed to produce vinegar from pear and orange along the value addition by medicinal plants like *Coleus amboinicus*and *Tinospora cordifolia* (Ambrithaballi).

## 2. MATERIALS AND METHODS

The moderately ripened good quality pears and oranges were purchased in the city of Moodbidiri in the month of April 2021.
Fruits were washed well with tap water to remove the dust and dirt on the surface. Peeled orange and unpeeled pears were used as substrate. Seeds were eliminated from both the fruits, later juice was extracted. And 100 ml ofYEPD (yeast extract, peptone and dextrose) was prepared for the starter culture .Then 0.5 g of commercial yeast (*Saccharomyces cerevisiae*) was added to the flask and incubated at 28˚C in rotary shaker for 24 hours.

### 2.1. Fermentation

Before sowing for fermentation sugar level as been determined separately of each fruit juice by DNS method. And 200 ml of both orange and pear juice was transfer to the sterile 500 ml conical flask plugged with cotton once after it was inoculated by 3% of starter culture. The content were stirred properly and kept in static condition in room temperature.
The alcoholic fermentation process followed by acetic acid production via oxidization process. At regular interval of 3$^{rd}$, 7$^{th}$, 11$^{th}$ and 14$^{th}$ day the acidic concentration has been determined by titration method. After 15 days the content from the conical flask was filtrated and stored in a clean and sterile container at 4˚C for further use.

### 2.2. Value addition process

Each three gram of *Tinospora cordifolia* and*Coleus amboinicus* powder was mixed with 100ml of distilled water andkept in waterbath for 30 min at 70˚C. Obtained aqueous extract mixedwith vinegar in equal ratio and stored at 4˚ C for further use.

### 2.3. Characterizations of vinegar

#### 2.3.1 Sensory evaluation

The sensory parameters of vinegar like taste, color, appearance and odor were evaluated manually.

#### 2.3.2 Determination of acidity

Total acidity was determined using the standard method described by Amoa-Awua *et al.*, 1996. Total acidity was determined by titrating 10ml of samples against 0.1N NaOH and 1% of phenolphthalein as indicator.

#### 2.3.3 Phytochemical analysis

The following phytochemicals was examined in both vinegar without and with value addition, also in medicinal plants used for the value addition process. They are caridac glycosides, flavonaoids, saponins, tannins, terpenoids, glycosides, phlobatannins and reducing sugar.

#### 2.3.4 Antioxidant activity by DPPH method

(Brand Williams *et al.*, 1995)
The antioxidant property can be evaluated by DPPH method (2, 2-diphenly-1-picrylhydrazyl). It is based on the DPPH radical absorbance reduction at 517 nm caused by antioxidants action turns colorless or pale yellow when neutralized by antioxidants components.

## 3. RESULTS AND DISCUSSION

### 3.1 Sensory evaluation

Appearanceof both vinegar without and with value addition was clear. Color of vinegar without value addition was saffron whereas with value addition is brownish green. Taste of both the vinegar was pungent and odour was pleasant.

### 3.2 Determination of acidity

Acidity of vinegar was gradually measured in the interval of 3$^{rd}$, 7$^{th}$, 11$^{th}$ and 14$^{th}$ day. Acidity was maximum in day 7 (Figure: 01) and concentration after the value addition (final product concentration) 0.013N was reported.

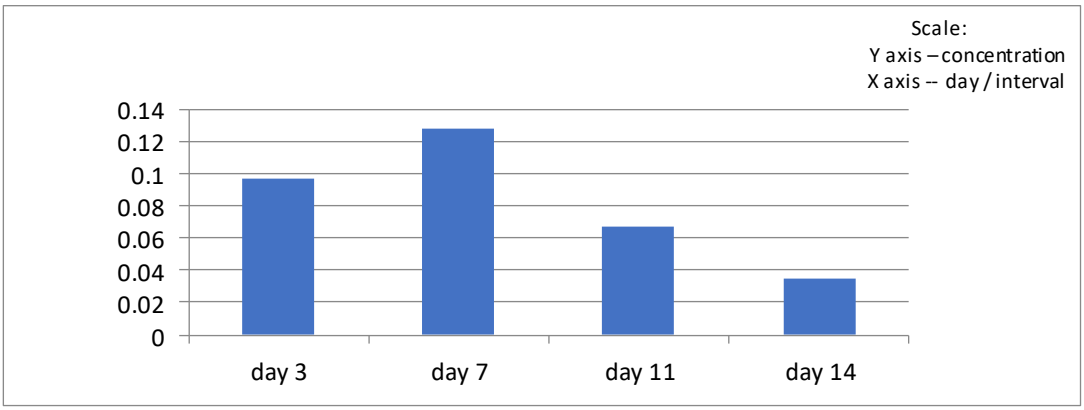


**Figure 1. Total acidity level of vinegar**

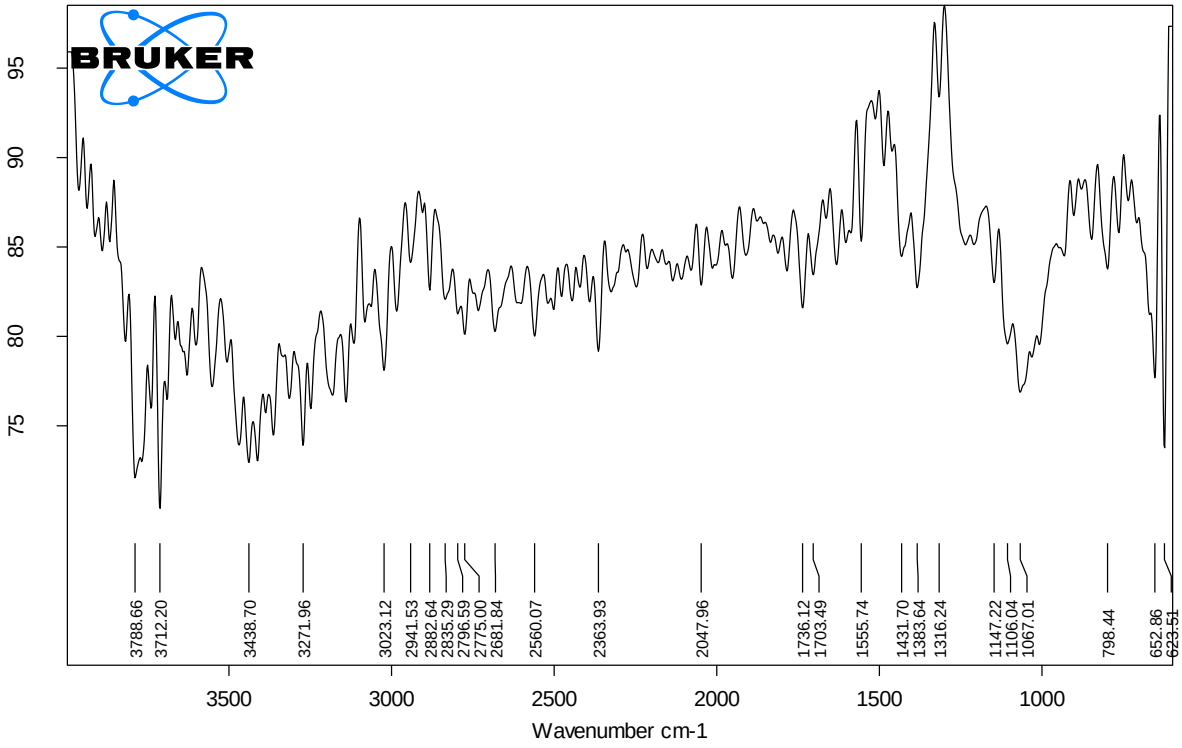


**Figure 2. Fourier Transforms Infrared Spectroscopy of vinegar without value-addition**

### 3.3 Qualitative Phyotochemical analysis

The obtained vinegar was subjected for phytochemical analysis in which the vinegar without value addition shows positive results for cardiac glycoside, reducing sugar and terpenodies. And the vinegar with value addition contains cardiac glycoside, reducing sugar, flavonoids, glycosides and terpenodies. Aqueous extract of medicinal plants used for the value addition consist cardiac glycoside, reducing sugar, flavonoids and terpenodies.

### 3.4 Antioxidant activity by DPPH method

The free radical scavenging activity of the vinegar with value addition was higherthan with vinegar without value addition. The scavenging activity of vinegar without value addition was 1.15 (32%) in 30 µg/ml of solid extract,whereas scavenging activity of vinegar with value addition was 0.40 (69 %) in 30 µg/ml.

### 3.5 Fourier Transforms Infrared Spectroscopy of vinegar without value-addition

Fourier Transforms Infrared Spectroscopyanalysisusedto identify the possible functional groups present the molecules. The obtained spectrum was ranges between 1000-4000 $cm^{-1}$. The peaks observed in 3788.66- 3712.20 $cm^{-1}$ reflects the presence of alcohol moiety, 3439.70 $cm^{-1}$ amine, 3023.12 $cm^{-1}$ assigned alkane group, 2835.29 $cm^{-1}$ assigned aldehyde moiety, 2363.93 – 2047.96 $cm^{-1}$ assigned alkyl group, 1703.12 $cm^{-1}$ assigned with carbonyl group, 1555.74 $cm^{-1}$ assigned with amide group, 1147.22 $cm^{-1}$ assigned with esters and 798.44 $cm^{-1}$ assigned with carbon chloride.

### 3.6 Fourier Transforms Infrared Spectroscopy of vinegar withvalue-addition

The obtained spectrum was ranges between 1000-4000 $cm^{-1}$. The peak 3365.59 $cm^{-1}$ assigned with primary or secondary amine, 2881.58 $cm^{-1}$ aldehyde moiety, 2359.60 $cm^{-1}$ assigned with alkyl group, 1316.55-1401.92 $cm^{-1}$assigned with amine group and 1036.71 $cm^{-1}$ assigned with amide moiety.

### 3.7 Overview of the functional groups

Vinegar without value-addition consist alcohol, primary amine, aldehyde, alkyl group, amide, esters, carbon chloride and in vinegar with value-addition consist primary or secondary amine, aldehyde, alkyl group, amide, imines.

### Discussion

In present study *Saccharomyces cerevisisae was* used for the vinegar production by *Citrus sinensis* and*Pyrus pyrifolia*. Similar kindofwork done by Joshi *et al.*, 2002, Fleet et *al.*, 2003, Kocher *et al.*, 2006used *Saccharomycescerevisisae* .Trcek *et al.*, 2000 and2005 used *Acetobacter aceti*, *Acetobacter intermedius* for cider vinegar production respectively. Gonzalez *et al.*,2005 and Vegas *et al.*, 2010 used *Gluconobacter oxydans.* The study done by Yuan*et al.*, 2017, *Acetobacter aceti* CICC 21684 was used for cider vinegar production.Garcia-Garcia *et al.*,2009 used*Acetobacteriaceae* and *Gluconobacter. Saccharomyces cerevisiae* with *Lactobacillus plantarum* was used for the citrus vinegar production by Chen Y *et al.*, 2017.

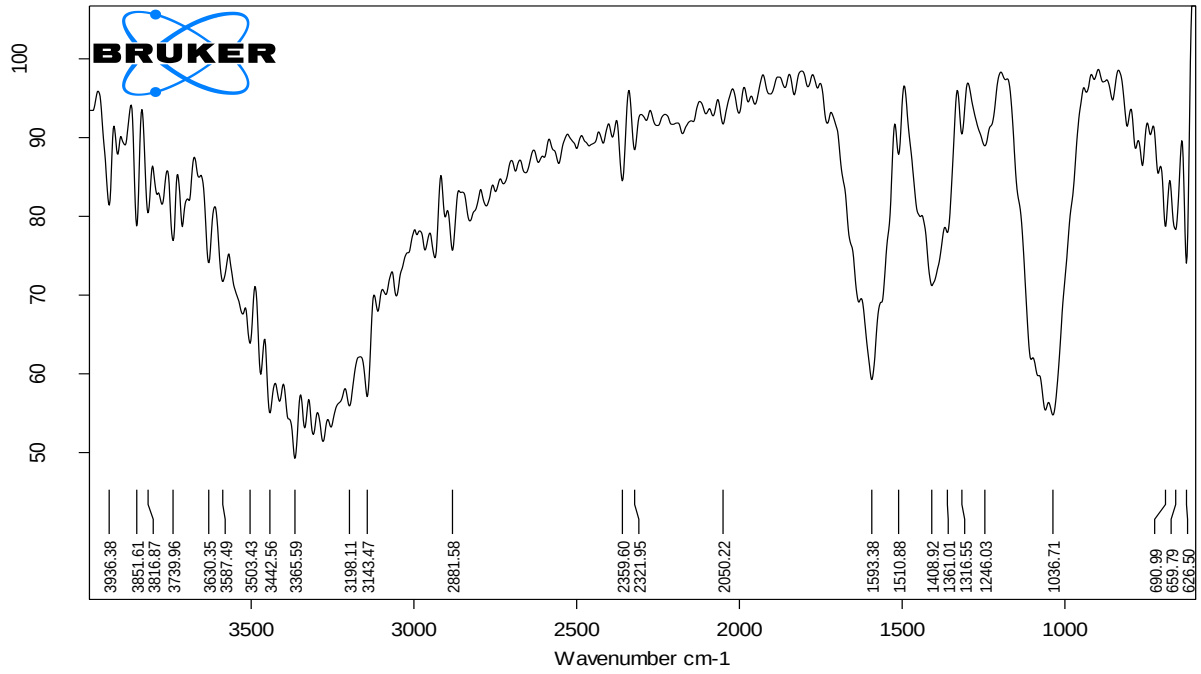


**Figure 3. Fourier Transforms Infrared Spectroscopy of vinegar with value-addition**

**Table 1. Overview of the functional groups present in the vinegar without and with value-addtion**

| Wave number ($cm^{-1}$) | Vinegar without value -addition | Vinegar with value addition |
|---|---|---|
| 3500-4000 | Alcohol | functional group absent |
| 3000- 3500 | Amine | Primary Or Secondary Amine |
| 2500-3000 | Aldehyde | Aldehyde |
| 2000-2500 | Alkyl Group | Alkyl (-CN Stretching) |
| 1500-2000 | Amide | Imines |
| 1000-1500 | Esters | Amides |
| 500-1000 | Carbon chloride | functional group absent |

In the present study, vinegar before value addition consist cardiac glycoside, reducing sugar, terpenoids. Aqueous extract of medicinal plants (*Coleus amboinicus* and *Tinospora cordifolia*) contain cardiac glycoside, reducing sugar, terpenoids, flavonoids. In the vinegar with value addition, the positive results for cardiac glycoside, reducing sugar, terpenoids, flavonoids and glycosides was reported. Ethanol, sugar, tannins, ester, volatile acids presence was noted from the study done by Joshi *et al.,* 2002. Acetic, citric, lactic, and succinic acids in malt vinegar was reported by Saiz-Abajo *et al.,* 2005. In plum vinegar acetic, tartaric and lactic acid is presencewas reported byLin *et al.,* 2009. Alcohol and sugar, flavonoid and phenolics, organic acid and phenolic compounds presence was reported in the Yamada *et al.,* 2008, Capanoglu E *et al.,* 2016, Hua-Bin Li *et al.,* 2019 respectively. Budak *et al.,* 2011 and Guzel Seydim et al., 2019 producedapple cider and grape vinegar phytochemical analysis reported the presence of gallic acid, catechin, epicatechin, chlorogenic acid, caffeic acid, and *p*-coumaric acid and gallic acid, catechin, epicatechin, chlorogenic acid, caffeic acid, syringic acid, and ferulic acid resepectively. Twelve phenolic acids andflavonoid, phenols presencewas reported by Hasim K *et al.,* 2017 and Driss Ousaaid *et al.,* 2021respectively.

In present study antioxidant activity in vinegar without value addition shown 0.40 (32%) and vinegar with value addition 1.15 (69%)by DPPH method. Study done by Yongyuth *et al.* 2016, they found the activity in 0.05-0.30 range. Activity value ranges from 7.72 – 17.96 with respect to ABTS assay GV5 and GV3 was reported by Hasim K *et al.,* 2017. Similar work done by Lucilla Domingues *et al.,* 2017 recorded the total antioxidant activity by FRAP method, $11.0 \pm 1.67$ mmol $L^{-1}$ for orange, $4.8 \pm 0.5$ mmol $L^{-1}$ for mango, $18.6 \pm 2.33$ mmol $L^{-1}$ for cherry and $3.7 \pm 0.3$ mmol $L^{-1}$ for banana vinegar. The study done by Hua-Bin Li *et al.,* 2019 was reported 0.570 and 0.760 of antioxidant activities by DPPH and FRAPS method respectively. Antioxidant activity -0.58876 was recorded by Driss Ousaaid *et al.,* 2021 via DPPH method. In the present study the functional groups like alcohol, amine, aldehyde, imines, carbonyl, amide and carbon chloride present in vinegar without value addition whereas vinegar with value addition consist primary and secondary amine, aldehyde, alkyl, amine and amide which was determined by FTIR analysis technique. Similar type of work as done by Kadiroglu P *et al.,* 2018, Reina R R,*et al.,* 2017,reported that commercial vinegar consist C-H group, C=H moiety, hydroxyl group, ethanol group and acetic acid, ester, alcohol groups respectively.The study carried by Calle J L*et al.,* 2021, reported the presence of C-O stretching of alcohol, hydroxyl, aromatic and acid group in the sherry wine vinegar.

## 4. CONCLUSIONS

In the present studyacidity of vinegar was gradually monitored in the interval of three days that is $3^{rd}$, $7^{th}$, $11^{th}$ and $14^{th}$ day. Acid concentration was maximum in theseventh day was reported. Examination of phytochemicals in the obtained vinegar of both without and

with value addition was performed.And also for the aqueous extract of medicinal plants used for value addition was subjected for the phytochemical analysis. Cardiac glycoside, reducing sugar, terpenoid, flavonoids are detected in the present study. The free radical scavenging activity was determined for both the vinegar without value addition and with value addition that is 1.15 (32%) and0.40 (69%) respectively. Functional groups like alcohol, amine, aldehyde, imines, carbonyl, amide, carbon chloride present in vinegar without value addition whereas vinegar with value addition consist primary and secondary amine, aldehyde, alkyl, amine and amide which was determined by FTIR analysis technique

Hence the vinegar with value addition of *Coleus amboinicus* (Mexican mint) and *Tinospora cordifolia* (Amirthaballi) can be used as nutraceuticals product which is good at antioxidant property and rich in phytochemicals. And also further study can be on the antidiabetic, anti fungal and anti microbial studies.

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