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Production of fermented tea petal decoction with insights into in vitro biochemical tests, antioxidant assay and GC-MS analysis

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Abstract

This research work was designed to attempt and propose the first report on production and biochemical characterization of fermented tea flower petal decoction or simply tea petal wine. The tea petal decoction and brewer's yeast or *Saccharomyces cerevisiae* were co-cultured for fermentation. Antioxidant activity and chromatographic separation of potential candidates were assessed. Primary investigations for qualitative characters on this fermented broth revealed the presence of steroids, tannin, flavonoids, phenol, cardiac glycosides, coumarin, caffeine etc. Our manufactured fermented broth showed high free radical scavenging activity after 2 months of aging. High DPPH scavenging activities were also observed in solvent fractions of acetone, ethanol and methanol. The antioxidant activity, alcohol percentage and other qualities were seen to be gradually increased during aging. Gas chromatography-mass spectrometry analysis revealed the presence of 44 compounds including many potential antioxidant molecules and other bioactive agents. Hopefully, presence of alcohol with medicinally active compounds and antioxidant activity will make it as acceptable as a good wine and tea flower as economically functional.

Keywords: Tea petal, Antioxidant, GC-MS, Fermentation, brewer's yeast

Introduction

Fermented beverages are associated with several health benefits as these are rich sources of substances like alcohols, fatty acids, terpenoids, flavonoids, anthocyanins, flavonols, catechins, other polyphenols and several other secondary metabolites of fermenting yeasts (German & Walzem 2000; Natella et al. 2001; Villano et al. 2006) which exhibit wide range of biological activities, mainly antioxidant activity. Antioxidants (e.g. catechin and their oligomers and proanthocyanidins, quercetin, resveratrol and other polyphenols) are the reason behind health protective properties of fermented beverages or wines which have been reported to have bioactivities like cardioprotective, anti-carcinogenic, anti-diabetic, anti-atherogenic, anti-

inflammatory, antiviral and antibacterial activities (Banc et al. 2014). Wine brewing is a traditional practice among various people and tribal communities where fruits, leaves, flowers etc. from various plants are used as raw materials.

Regarding metabolomics, tea plant has been studied by various researchers for a long period. However, most research on metabolomics of tea has been done mainly on leaves of the plant as leaf is the only organ for which tea plant is cultivated and praised for magnificent bioactive properties. Therefore, occurrence of signature tea leaf components, i.e., catechins, flavonol, caffeine, and other bioactive compounds like amino acids, saponins, terpenes etc. in tea flowers is of great interest. Recently, Chen et al. (2018) reviewed the metabolite profiling of tea flower. Their report summarized the presence of tea catechins (catechin, epicatechin, gallic catechin, epigallocatechin, gallic catechin gallate, epigallocatechin gallate etc.), flavonols,

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amino acids (theanine, L-phenylalanine), caffeine, saponins and polysaccharides of different cultivars from different regions. However, branches of tea bushes containing flowers or flower buds are subjected to complete pruning by planters to regulate proper vegetative growth and to achieve their economic goals. It is also reported that planters often use some chemicals (ethephon and α -naphthalene acetic acid) to suppress tea plant blossoming (Chen et al. 2018) to promote the yield and quality of tea leaves or, simply, in advance to upregulate the vegetative growth of plants to boost the economy. Moreover, due to successful and conventional application of asexual propagation, tea flowers which compete with tea leaves for water and nutrients, are generally considered as a waste resource of the plantation (Chen et al. 2018). Furthermore, flowers of any plant are commonly known to attract varieties of insect pests by releasing semiochemicals and other properties, which is another major issue that extends the demerits of blooming tea flowers in a tea garden. Overall, flowers of tea plant have zero economic value in plantations around the tea growing regions of the world.

However, tea flowers are reported as edible (<https://www.ediblewildfood.com/tea-plant.aspx>). Some tribal communities from tea growing regions and tea garden workers of north and northeast India are seen to consume the cooked (fried in oil) tea flower often, but there is an absence of ethnobiological survey on this. Not only metabolomics, but also biological activities exhibited by flowers of tea such as antihyperlipidemic, antihyperglycemic, antiobesity, and gastroprotective effects are also reported (Matsuda et al. 2016). Therefore, tea flowers could be better used in different applications. However, value-added utilization of tea flowers has not been achieved due to lack of sufficient research. Thus, ecofriendly approaches may provide better lead to valorization of this important resource.

The aim of this research was to explore a novel and possible way to valorize the considered waste of tea plantation, i.e., tea flowers, by using fermentation technology. Fermented tea petal decoction is the result of fermentation process where several metabolic pathways of yeast and reactions of its metabolites, compounds of the petals and sucrose are involved. The main objective was to brew fermented tea petal decoction and detect its antioxidant potential using in vitro biochemical experiments. Special attention was given on antioxidants, GC-MS analysis and separation of bioactive fractions through chromatography to study in depth.

Materials and methods

Collection of tea petals and starter

Fresh flowers of tea (Fig. 1) were harvested from lower branches of mature (25 years old bush) *Camellia sinensis* (TV-26 clone) grown in the Experimental Tea Plantation at University of North Bengal, Darjeeling, India. Diseased flowers, pest affected petals, sun scorched or dry petals

were discarded soon after collection. Petals from healthy flowers were taken off using sterile forceps on a laminar airflow cabinet to avoid laboratory contamination. Dry brewer's yeast or *Saccharomyces cerevisiae* was bought from the local market to use as a starter because this is the most commonly used starter for brewing beer and wines at home by local brewers, moreover it is easily available in any local markets.

Decoction of flower petals, inoculation of starter and incubation

Tea flower petals and white sugar (sucrose as additional nutrient or carbon source for yeast) were mixed in a ratio of 1:1 (30 g each) with 500 ml sterile double distilled water and boiled at $100 \pm 5^\circ\text{C}$ for 20 min to prepare the decoction (Fig. 1). The decoction was filtered through sterile muslin cloth and final volume was adjusted to one liter by adding double distilled water. The jar was then autoclaved properly. Two grams of dried brewer's yeast or *Saccharomyces cerevisiae* were added in the decoction. Sterile polythene cover with pores was used to facilitate releasing of evolved CO_2 (Fig. 1) during fermentation. Muslin cloth, glass goods, polythene cover etc. were autoclaved and sterilized with 70% ethanol. The jar was incubated under a dark condition at $25 \pm 1^\circ\text{C}$ to produce fermented tea petal decoction (FTPD) which was collected at regular intervals for further studies. Following the same procedure, three jars were prepared as replicas and results of all further experiments were reported as means of those three replications.

Qualitative biochemical tests

Qualitative tests to determine presence of steroids, tannin, flavonoids, phenol, proteins, cardiac glycosides, coumarin, reducing sugar, starch and caffeine were conducted following the protocol of Majumder, Ghosh, Chakraborty, and Bhattacharya (2020); Majumder, Acharyya, et al. (2021). Tests were performed with samples collected on different stages of fermentation. Before adding starter and sucrose, same tests were performed on Tea petal decoction (TPD) or unfermented sample also in aim for comparison.

Time dependent alteration in physicochemical properties

Acidity (pH), optical density (OD), percentage transmittance (%T) and specific gravity (SG) of the broth were monitored on regular intervals during fermentation to study time dependent alteration in physicochemical characteristics. Acidity was determined by measuring pH by using a pre-calibrated pH meter to investigate the changes in acidity of the broth. OD and %T are determinants of sensory quality of any beverage, specially fermented beverages which were measured by a colorimeter at 420 nm. Specific gravity was determined as described by Pearson

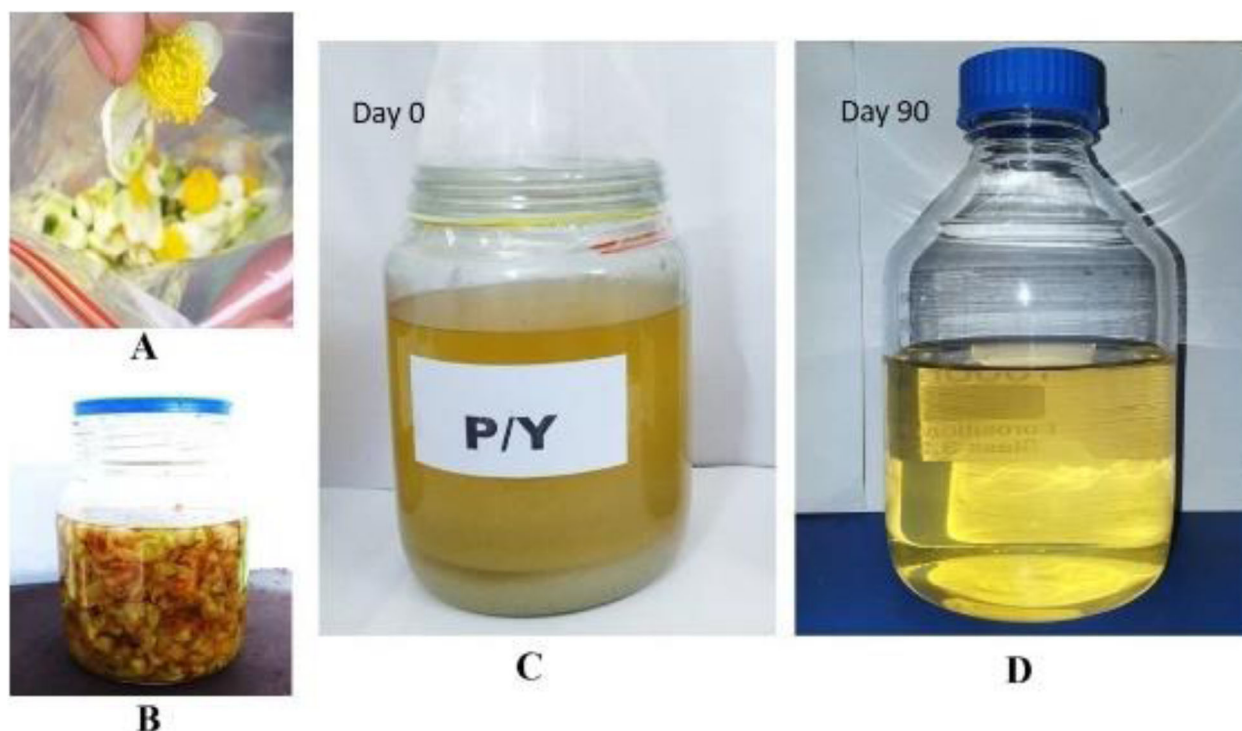


Fig. 1 Collection of tea petals, preparation of petal decoction and incubation. **A** Collection of tea petals, **B** Decoction of petals, **C** Fermented tea petal decoction broth at 0th day and **D** FTPD on 90th day of fermentation after bottling

(1976) from where amount of alcohol (%ABV) was estimated.

Gas chromatography-mass spectrometry analysis

The FTPD sample (5 ml) was drawn by sterile pipette on the 15th day of fermentation, air dried and extracted with the same amount of ethanol. Ethanol was chosen as GC-MS sample extraction solvent in this research to extract compounds of FTPD, because it has the polarity which is very close to alcoholic beverages. Sample was subjected to GC-MS analysis using the model instrument, GC-MS-QP2010 Plus (Shimadzu Co. Japan) attached with Rxi-5 fused-silica capillary column (0.25 μ m film thickness, 0.25 mm internal diameter and 30 m of length) following the protocol of Majumder, Ghosh, & Bhattacharya 2020 and Chakraborty et al. 2021. Analysis was performed by injecting 1 μ l each sample with a split ratio of 20:1. Injection temperature was 260 $^{\circ}$ C and interface temperature was set to 270 $^{\circ}$ C. Ion Source temperature was adjusted to 230 $^{\circ}$ C. Helium gas (99.9%) was used as carrier gas. Total flow rate was 16.3 ml/min and column flow rate was 1.21 ml/min. Mass spectra were recorded at 5 scan/sec with a scanning range of 40–650 m/z. The compounds were identified after comparing the spectral configurations obtained with that of the available mass spectral database, the NIST (National Institute of Standards and Technology) library. The

quantitative analysis in a total ion current mode (TIC) was performed as a traditional GC analysis based on the total peak area. The chromatogram (TIC) is based on the intensity of fragments produced by the ionization. Concentration of the amount of each compound was expressed as area percentage calculated from peak areas. The data obtained from GC-MS analysis were further analysed by studying available literatures.

Fractionation of FTPD and antioxidant activity (DPPH assay)

Following the protocol of Bhattacharya et al. (2009) fractionation or separation of FTPD components through column chromatography was done in order to assess free radical scavenging potential FTPD fractions. Dried FTPD was loaded on silica gel (Merck, 200–400 mesh size) and a series of nonpolar to polar solvents (hexane, benzene, chloroform, ethyl acetate, acetone, ethanol, methanol and water) were passed the column to obtain their respective fractions. Each fraction was vacuum evaporated at low temperature, dried and dissolved in 5 ml methanol (as methanol is used to prepare DPPH solution and for zeroing of spectrophotometer) for further antioxidant assay to find out probable potential fraction behind those activity and probable components responsible for this activity (based on their solubility in solvent fractions).

The free radical scavenging activity through DPPH assay was performed following the method described by Bhattacharya et al. (2009) and Majumder, Ghosh, et al. (2021) with minor modifications where antiradical activity was measured by a decrease in absorbance at 517 nm of methanolic solution of coloured 2,2-Diphenyl-1-picrylhydrazyl or DPPH (D9132 from Sigma-Aldrich) brought about by the sample. To 2800 µL of DPPH (100 µM) solution in methanol, 200 µL of samples (each FTPD fractions) were added and incubated for 30 min in dark at room temperature. Decreases in the absorbance in presence of the sample were noted at 517 nm by UV-Vis spectrophotometer (Cary 60 UV-Vis Spectrometer by Agilent). Results were expressed as the percentage of DPPH inhibition (%) occurring due to exposure of samples.

In vitro antioxidant assay on aging of broth

DPPH assay was performed on regular intervals after taking crude broth of FTPD as sample. The protocol of Majumder, Ghosh, et al. 2021, as done for the FTPD fractions, was followed in this experiment. DPPH assay was performed on a control sample or TPD also.

Results

Qualitative biochemical tests

Qualitative tests revealed presence of steroids, tannins, flavonoids, caffeine, phenol, proteins, cardiac glycosides, coumarin, reducing sugar in FTPD sample which was collected on different stages during fermentation (from 15th day to 90th day). Nothing significant was observed which can differ these qualities between different aged samples. However, the most aged sample of our research (collected on 90th day) resulted better compared to less aged samples in these qualitative tests as its responses towards colour changes or other indicators were just quicker and sharper except reducing sugar, starch and protein which is a clear indicator of progress in fermentation process. But, on TPD, nothing significant was

observed to determine the presence of these qualities except starch, reducing sugar, protein, steroids and caffeine which was found to be present. The results of qualitative tests are exhibited by a heat-map in Fig. 2.

Time dependent alteration in physicochemical properties

A sharp decrease in OD and increasing in %T were observed in the broth upto 15th day (Fig. 3). It has also been observed that pH of FTPD decreases sharply up to the 15th day of fermentation then stabilizes gradually with passage of time (Fig. 3). Specific gravity was found to be 1.339 ± 0.177 on the 0th day that gradually decreased. Therefore, alcohol by volume or alcohol percentage (%ABV) showed a potential increase in aging ($37.54 \pm 1.245\%$ on 90th day). Both SG and %ABV, represented graphically in Fig. 3.

Gas chromatography-mass spectrometry analysis

GC-MS chromatogram of FTPD has revealed organic molecules, their derivatives and intermediates arisen from sole and interactive pathways of organic compounds present in tea petal decoction and fermenting agent during the process of fermentation. GC-MS detected the presence of 44 compounds including a number of known bioactive agents. The chromatogram of GC-MS detected compounds is represented in Fig. 4 and the list of those compounds is given in Table 1. The compounds detected by GC-MS were matched to databases like PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) and the good scents company or TGSC (<http://www.thegoodscentscompany.com/>) to study their shares over flavour and taste imparting characters in FTPD.

Fractionation of FTPD and antioxidant activity (DPPH assay)

We extended our study with in vitro antioxidant activity of the components present in FTPD by fractionating

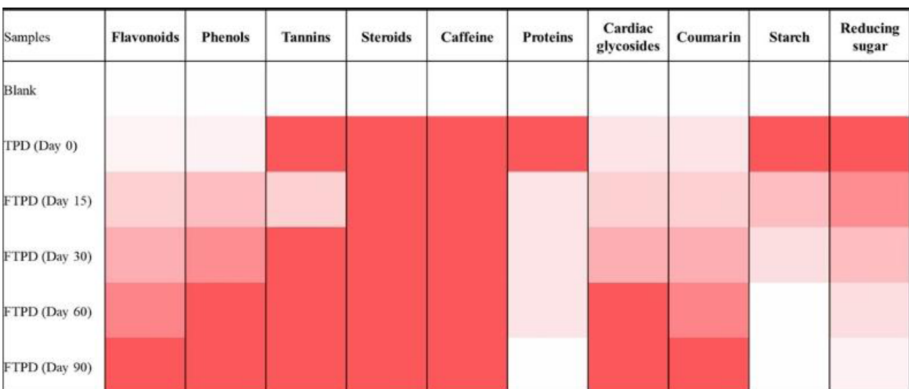
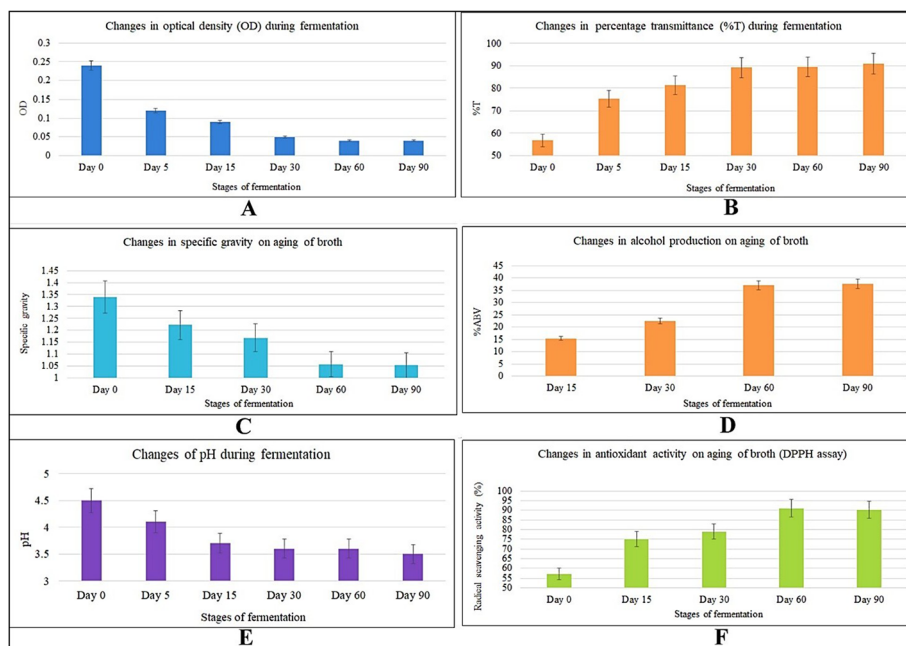


Fig. 2 Heatmap showing levels of different qualities, tested on TPD and FTPD samples (taken at different stages of fermentation)



it through a silica column using a series of non-polar to polar solvents with an intention to separate them on the basis of their preference for solvent. In DPPH assay, antioxidant molecules act as a proton donor where the free radical is scavenged and absorbance is decreased thereby rendering a change in colour (Manivasagan et al. 2015). Series of FTPD fractions showed a tendency of increasing DPPH scavenging activity from nonpolar to polar end. Satisfactory percentages of inhibition were observed in solvent fractions of acetone ($95.36 \pm 1.114\%$), ethanol ($88.25 \pm 1.54\%$) and methanol ($92.29 \pm 0.52\%$), whereas others exhibited moderate activity (Fig. 5). Control sample or TPD resulted in nothing significant as antioxidant property was also very

much low ($22.8 \pm 2.333\%$ in DPPH assay) compared to FTPD.

Changes in antioxidant activity on aging of broth

Determination of hydrogen donating ability or DPPH assay was performed with a crude sample of FTPD to determine changes in antioxidant potential of fermentation broth with time. Sample, collected on regular intervals during aging, showed significant increase in DPPH scavenging potential justifying improvement of free radical scavenging activity (Fig. 3-F). Free radical quenching activity was comparatively low ($57.12 \pm 2.1\%$ on 0th day) initially after 15 days it was increased to $75.07 \pm 1.45\%$. However, the rate of increase was highest between 30th

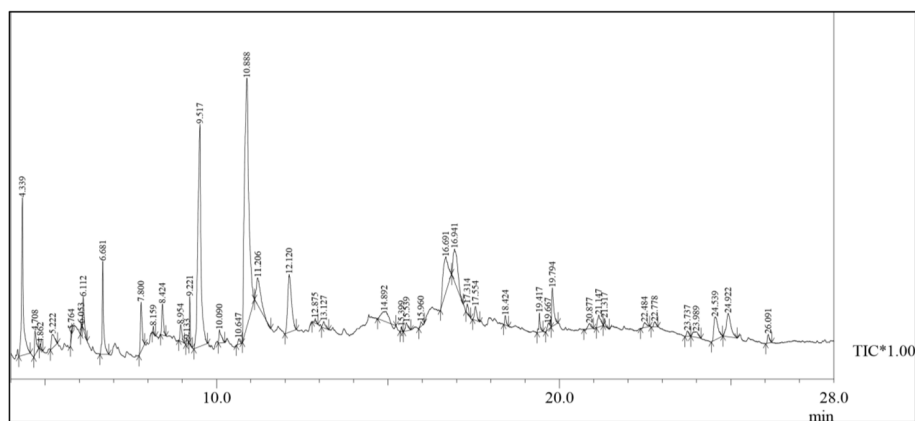
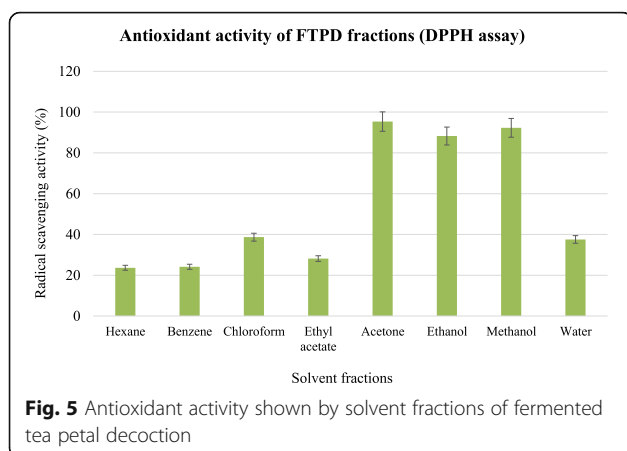


Table 1 GC-MS peak report for the sample fermented tea petal decoction (FTPD) with names of compounds as detected

Peak index	Retention time	Area	Name of compounds	Area%
1	4.339	7,493,570	Furfural	7.36
2	4.708	1,113,308	Furfuryl alcohol	1.09
3	4.862	148,272	α -Angelica lactone	0.15
4	5.222	1,157,282	Butanoic acid, 2-ethyl-, methyl ester	1.14
5	5.764	160,116	2,2-Diethoxyethanol	0.16
6	6.053	132,320	alpha-Methylene-gamma-butyrolactone	0.13
7	6.112	1,283,202	α -Ketoglutaric acid	1.26
8	6.681	3,458,352	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanone	3.39
9	7.800	2,273,303	Levulinic acid	2.23
10	8.159	154,701	2-Thiopheneacetic acid, decyl ester	0.15
11	8.424	1,382,285	Methyl 2-furoate	1.36
12	8.954	815,679	Glycerol 1-ethyl ether	0.80
13	9.133	136,634	1,4-Diethoxy-2-butene	0.13
14	9.221	927,102	1,6-Benzodioxocin-7,8,9,10-d4, 2,3,4,5-tetrahydro-	0.91
15	9.517	17,637,462	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	17.31
16	10.090	795,178	Succinic acid diethyl ester	0.78
17	10.647	233,996	5-Acetoxyethyl-2-furaldehyde	0.23
18	10.888	27,832,952	5-(Hydroxymethyl)-2-furaldehyde	27.32
19	11.206	3,214,226	Methyl 3-hydroxy-4-methylpentanoate	3.15
20	12.120	5,760,559	cis-Dimethyl morpholine	5.65
21	12.875	181,856	Stearyl alcohol	0.18
22	13.127	419,317	Ethyl 2-formyl-1-cyclopropanecarboxylate, trans	0.41
23	14.892	1,571,892	Levogluconan	1.54
24	15.399	104,973	1-Hexadecene	0.10
25	15.539	548,296	4,6-Dimethyl-5-(nitromethyl)-3-heptanone	0.54
26	15.960	184,347	Serricornin	0.18
27	16.691	4,932,676	(E)-Dodec-5-en-4-olide	4.84
28	16.941	3,972,945	Hydrazinecarboxamide, 2-(2-methylcyclohexylidene)-	3.90
29	17.314	443,107	13-Hexyloxacyclotridecan-2-one	0.43
30	17.554	853,252	3-Butyl-4-nitro-pent-4-enoic acid methyl ester	0.84
31	18.424	399,936	Caffeine	0.39
32	19.417	659,214	Palmitic acid	0.65
33	19.667	482,627	3',5'-Diacylthymidine	0.47
34	19.794	2,040,684	5,5'-Oxybis(5-methylene-2-furaldehyde)	2.00
35	20.877	365,591	Isoshyobunone	0.36
36	21.147	965,532	Cyclohexanecarboxylic acid, 4-butyl-, 4-hydroxyphenyl ester	0.95
37	21.317	236,281	Stearic acid	0.23
38	22.484	376,154	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	0.37
39	22.778	273,329	Unknown compound	0.27
40	23.737	314,627	1-Methoxybicyclo[2,2,2]oct-5-en-2-yl methyl ketone	0.31
41	23.989	756,041	Pyrazole-4-carboxaldehyde, 1-methyl-	0.74
42	24.539	2,459,557	5,5'-Oxybis(5-methylene-2-furaldehyde)	2.41
43	24.922	2,806,373	Cyclohexanecarboxylic acid, 1-(1-bromocyclohexylcarbonyl	2.75
44	26.091	424,542	2-Cyclohexen-1-one, 3-bromo-2-methyl-	0.42



(78.88 \pm 1.26%) and 60th (90.91 \pm 2.2%) day of fermentation and, thereafter, no significant change in DPPH scavenging activity was seen. IC₅₀ value for DPPH scavenging activity was also measured on the 90th day (after bottled) which was found to be 57.9 \pm 1.56 μ l. Free radical quenching activity was found very low (22.8 \pm 2.333%) in TPD.

Discussion

Antioxidant activity and other qualities were seen improved in aged samples compared to the samples of the first 15 days. This indicated that fermentation can input more qualities. Not only in DPPH assay, TPD did not responded well in other biochemical tests too except starch, reducing sugar, protein, steroids and caffeine. In tea flowers, presence of caffeine, saccharides, amino acids etc. were previously reported (Chen et al. 2018). Thus, supporting the metabolomics, qualitative detection of group of molecules like reducing sugar, protein and caffeine in its decoction brings no confusion at all.

FTPD was analysed through GC-MS where metabolomics components were studied by reviewing established reports on them. Among the 44 compounds, three most abundant components, i.e., 5-(hydroxymethyl)-2-furaldehyde (27.32%); 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (17.31%) and furfural (7.36%) are reported as potential antioxidant molecules having lots of other biological activities (Hameed et al. 2015; Khan et al. 2012). The most abundant 5-(hydroxymethyl)-2-furaldehyde, was previously reported as a prime wine component which is mostly found in aged samples of different wines (Serra-Cayuela et al. 2014). So, this could probably be synthesized in FTPD during storage or fermentation saccharides (Chen et al. 2018) present in it. Tea flowers were previously reported to contain a huge amounts (20–30% of total dry weight) of saccharides (Chen et al. 2018; Weng 2004). So, presence of metabolites derived from saccharides (due to fermentation of caramelization) in its fermented decoction is completely valid. Another highly

valued honeycomb derived component; 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (Kanbur et al. 2009) also confirms fermentation process or breakdown of sucrose and petal components by yeast. Interestingly, this compound is mainly found in royal jelly, which is actually a result of natural fermentation of saccharides of flowers occurring in beehives. Furfural and its derivatives detected in FTPD i.e., furfural, furfuryl alcohol, 5,5'-oxybis(5-methylene-2-furaldehyde) etc. are reported as metabolites of fermenting microbes and common aroma components of wine and wine derived spirits like brandy, whiskey, rum etc. (Dumitriu et al. 2020; Okaru & Lachenmeier 2017). Furfuryl alcohol occurs mainly due to enzymatic or chemical reduction of furfural during aging of a wine. Butanoic acid, 2-ethyl-, methyl ester is also reported to exhibit wine aroma imparting property (Ishikawa et al. 2010). Moreover, FTPD compounds i.e., butanoic acid, furfural, 2,2-diethoxyethanol etc. are reported volatile aroma compounds of some famous Chinese liquors which are probably considered as aging markers of some wines (Xu et al. 2017). Gastroprotective anti-ulcer agent (Maria et al. 2000) alpha-methylene-gamma-butyrolactone, an antioxidant (Salat et al. 2012) was detected in FTPD which could be derived very common wine component i.e., gamma-butyrolactone (a major component of red wine reported by Vose et al. 2001). Antioxidant compound α -ketoglutaric acid (Long & Halliwell 2011) is a metabolite of wine yeast (as an intermediate in Krebs cycle) which was probably derived during fermentation. Furthermore, GC-MS of FTPD has revealed more other components which are well documented as aroma imparting components (potential fruity and wine like aroma in wines), these are, 2,4-dihydroxy-2,5-dimethyl-3(2H)-furanone (Chukwu et al. 2017); methyl 2-furoate (European Food Safety Authority 2004); Methyl 3-hydroxy-4-methylpentanoate (Lytra et al. 2015); 1-hexadecene (Vararu et al., 2016) etc. Butanoic acid and diethyl succinate are also reported as fermentation metabolites in ciders (Wilson et al. 2021) which strongly supports the metabolomics of FTPD. Antioxidant agent levulinic acid and its precursor succinic acid, both are reported as products of glucose fermentation and mainly found in aged wine and beer samples (Antonetti et al. 2020). 5-Acetoxy-methyl-2-furaldehyde, a sweet taste modulator of wine, has been detected in FTPD, interestingly, this compound is also found in traditional medicinal balsamic vinegar, another fermented product (Cámara et al. 2006; Hillmann et al., 2012). Alañón et al. (2010) reported that levoglucosan is a carbohydrate derived bioactive wine component which is mainly formed in alcoholic beverages during aging. Interestingly, this component was also detected in FTPD. Compounds like furfural; 2-furanmethanol; pentanoic acid, 4-oxo-; 2-furancarboxylic acid, methyl ester; 5-(hydroxymethyl)-2-furaldehyde; stearyl alcohol; palmitic acid;

stearic acid etc. were also found to contribute a sweet, caramel like, astringent and wine like flavour towards development of a wine like taste.

Being insect pheromone; major compound (E)-dodec-5-en-4-olide (Dickschat et al. 2005) and trace compound serricornin (Lozanova et al. 2005) are probably transferred either from pollinators or could be originated in petal as semiochemicals which are actually biosynthesized in plants aiming to attract insects for pollination.

Three abundant and major FTPD compounds- 5-(hydroxymethyl)-2-Furaldehyde; 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- and furfural are well-known potential antioxidant molecules (Yi & Kim 1982; Yu et al. 2013) and are soluble in acetone, ethanol and methanol. However, major compound 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- is a reported sugar derived product which is a possible derivative of a flavonoid biosynthesized during fermentation by yeast because it is reported as a compound with flavonoid fraction and as a fungal secondary metabolite having antifungal properties (Teoh & Mat Don 2014). Interestingly, column chromatographic fractions of methanol, acetone and ethanol solvents showed a high antioxidant potential where presence of these molecules could be a reason behind this. Antioxidant fatty acid derivatives like palmitic acid, stearic acid and stearyl alcohol were detected in FTPD which were previously reported as fatty acids of red wine (Yunoki et al. 2004). Yi & Kim 1982 reported antioxidant activity of wine compound levulinic acid, furfural, 5-hydroxymethyl furfural, and pyrazines and interestingly, all of these were detected in FTPD (Table 2).

Bioactivity of signature tea leaf compound caffeine as a central nervous system stimulant is well established but its presence in tea petal wine is definitely a good finding of this research. This may contribute a significance in metabolomics of tea plant as presence of caffeine in tea flower was also reported (Chen et al. 2018). Not only in GC-MS analysis, but also in results of qualitative biochemical tests, presence of caffeine and steroids was determined in both TPD and FTPD samples.

Determination of alteration in physicochemical properties like pH, OD, %T and specific gravity were important to judge fermentation rate and acceptability of a beverage as wine. Typically, pH of wine ranges between 3 to 4 (Hale 2019) and in our FTPD it was found 3.5 ± 0.2 on 90th day (when bottled) which is very acceptable. Generally, during fermentation, yeast or other microbes cause acidification by their metabolic process. As a result, the broth is turned into acidic which further accelerates the fermentation rate as well as reflected by the results. Increasing acidity is a factor which indirectly assures the progress in the fermentation process. This result demonstrated that the rate of fermentation was at

Table 2 List of reported antioxidant components (references are given in round brackets) found in fermented tea petal decoction (FTPD) through GC-MS analysis

Serial no.	Name of compounds	Area%
1	Furfural (Yi & Kim, 1982)	7.36
2	Furfuryl alcohol (Yi & Kim, 1982)	1.09
3	alpha-Methylene-gamma-butyrolactone (Salat et al., 2012)	0.13
4	α-Ketoglutaric acid (Long & Halliwell, 2011)	1.26
5	Levulinic acid (Yi & Kim, 1982)	2.23
6	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (Yu et al., 2013)	17.31
7	5-(Hydroxymethyl)-2-furaldehyde (Yi & Kim, 1982)	27.32
8	Stearyl alcohol (Yunoki et al., 2004)	0.18
9	Palmitic acid (Yunoki et al., 2004)	0.65
10	5,5'-Oxybis(5-methylene-2-furaldehyde)	4.41
11	Stearic acid (Yunoki et al., 2004)	0.23
12	Pyrazole-4-carboxaldehyde, 1-methyl- (Yi & Kim, 1982)	0.74

its peak during the first 15 days of fermentation. Furthermore, optical density (OD) was measured to determine the concentration of particles in FTPD while results of percentage transmittance was proportional to the amount of light that passed through the sample. So, these were assessed to observe changes in OD and %T which actually indicates chemical changes during fermentation. Generally, during fermentation of wine or other alcoholic beverages, the colour of fermentation broth becomes gradually lighter and transparent except the surface area where yeast cells sometimes form a film (also called flor or kahm yeast) and the bottom portion where dead yeast bodies or debris usually remain as sediments. So, OD and %T were monitored on regular intervals upto 90th day of fermentation (doing minimum disturbance to the broth) to measure and determine the changes. The results indicated that the rate of chemical changes was high in FTPD during that period, which is also positively correlated with the result of pH. After 15th day till 90th day, during aging, the same changes on OD and %T occurred but very slowly as the broth became more clear and transparent in appearance consequently indicating a slowdown or end to the metabolic process or reactions inside the broth (Fig. 1). Specific gravity (SG), another important factor, was also assessed to determine the point of acceptability of a fermenting beverage like wine. Typically, like pH and OD, SG of a broth is also found to be decreased after fermentation. The results reflected by pH, OD, %T, specific gravity, %ABV and changes of free radical scavenging property on aging (Fig. 3) were clear indicatives of a fact that fermentation process controlling metabolic changes took

place till 60th day of fermentation and then stopped gradually. A probable reason for this result is either depletion of carbon source or occurrence of stationary or death phase attained by the yeast cells naturally to conclude the fermentation process. The results judged not only the fermentation rate but also acceptability of FTPD as a fermented drink or wine. However, further experiments by preparing broth using varied sugar sources with varied amounts and proper growth study are required to point out the key factors that are responsible to limit the fermentation process.

Moreover, no significant change in DPPH scavenging activity was seen between 60th day and 90th day, which also indicated that after a month of aging the metabolism (production or development of secondary metabolites) was gradually stopped. DPPH free radical scavenging activity exhibited by column fractions, as shown in the results, also justified the antioxidant property of FTPD. FTPD derived antioxidant compounds (61.91% peak area) discussed above (listed in Table 2), strongly supports the high free radical scavenging activity shown FTPD in DPPH assay.

Conclusion

Antioxidants play important roles in human life by destroying free radicals and protecting the human body against many major diseases and disorders. Moreover, a major part of our regular diet, mainly, green foods and beverages like tea and wines are major sources of antioxidants where application of tea petal decoction or its wine will be significant being rich in bioactive components. Moreover, our aim of this research was to study the possibilities wine production from tea flower petal to make it economically useful where qualitative analysis, antioxidant assay and GC-MS based metabolomics were considered for characterization. Hopefully, concept and outcomes from this research have shown the possible and biotechnological way to valorize tea flower which will help tea planters to make use of this unused part of tea plant commercially and tea flowers to get praised by scientific communities, oenologists from wine industries to pharmaceuticals.

Abbreviations

GC-MS: Gas chromatography-mass spectrometry; FTPD: Fermented tea petal decoction; TPD: Tea petal decoction; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; OD: Optical density; %T: Percentage transmission; SG: Specific gravity; %ABV: Alcohol by volume (%)

Acknowledgements

Not applicable.

Authors' contributions

SM and MB conceived the idea and designed the protocol. SM, SS, AG, SA, SS and SC performed all laboratorial work. SM and MB compiled data and wrote the draft manuscript. All the authors read and approved the manuscript.

Funding

Not applicable.

Availability of data and materials

All data analysed during this study are included in this article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 16 August 2021 Accepted: 19 September 2021

Published online: 02 November 2021

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