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# Effect of fermentation on the bioactivities of the aqueous extract of Viburnum coriaceum Blume – positive as well as negative inferences on experimental basis

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# Abstract

Products of natural fermentation have been considered as appealing targets for the study of drug discovery as they were diverse and complex in structure and biological activities. Significance of fermentation in drug preparation, its physicochemical parameters and clinical evaluation were extensively studied. Present study was focusing on fermentative modifications in the biological activities of the aqueous extract of *Viburnum coriaceum* Blume. Fermented and unfermented extracts were compared for their bioactivities. Free radical scavenging assays were performed for antioxidant activity. *In vitro* trypsin and lipoxygenase assays for anti-inflammatory activity and inflammation induced cell lines were studied for anti-neuroinflammatory activity. Pilocarpine induced rat models were studied for antiepileptic activity. Phytochemical screening of the extracts that befits the investigation was performed before starting the experiments. When the antioxidant, anti-inflammatory and anti-neuroinflammatory activities were found to be enhanced by fermentation, antiepileptic activity on animal models was found diminished. Qualitative analysis revealed the increased concentration of some classes of phyto-constituents and disappearance of some others after fermentation. Analysis of results uncovered the fact that fermentation has both positive and negative implications on the extract bioactivity. Disappearance of phytoconstituents and their derivatization may be the reason behind the negative effect.


# Introduction

Indian traditional medicinal system of Ayurveda avails the technology of fermentation extensively for drug preparation. Unani, Siddha, Chinese traditional systems etc. are also using this technology for the development of drugs. However, the scientific basis for this adoption and the theoretical basis explaining where and when the technology is to be applied is not explained anywhere in the Ayurvedic literature. Significance of fermentation in the conversion of compounds in the ayurvedic preparations and enhancement of bioactivity were explained by many in the preceding decades (Naveen et al. 2012). The traditional method of fermentation has more extensive biotransformation ability than commercially available yeasts (Naveen et al. 2015). *Woodfordia fruticosa* flowers are the fermenting agent in ayurvedic formulations. The significance of changes made by them, its physicochemical parameters, and clinical evaluations were extensively studied (Sabu and Haridas 2015). Derivatization of berberine (Chandra et al. 2017) and piperine (Sharanya et al. 2019) increases its propensity to react with suitable protein targets. Fermentative production of benzoic acid, an industrially significant compound, in the herbal wine increased its lipoxygenase inhibitory activity (Tomy et al. 2016). Products of natural fermentation have been considered appealing targets for the study of drug discovery as they were diverse and complex in structure and biological activities. The global health sector has contributed a number of revolutionary drugs from these products.

Aqueous extracts of *Mercurialis perennis* L. were fermented with lacto-bacterial strains for the derivatization of secondary metabolites. A complete conversion of metabolites hermidine, quinone and chrysohermidin were observed after the fermentation period (Lorenz et al. 2019). This derivatization increased the bioactivities possessed by the plant extracts. Fermentation with *Grifola frondosa* stimulated the lipoxygenase (LOX) inhibitory, antioxidant, and collagen biosynthetic activities of the aqueous extract of *Fructus arctii* (Kim et al. 2010). Sugar, protein, peptides, amino acids, and isoflavonoid content of the fresh soybean were found to increase with fermentation. This will increase its nutritional and bioactivity level (Vieira et al. 2009). Growth stimulating effect, hyaluronic acid production enhancement of the extract of *Astragalus membranaceus* var. *mongholicus* after fermentation with *Bacillus subtilis* was studied to analyze their *cosmetic* effect (Hsu and Chiang 2009). A number of researchers are working on the cosmeceutical effects of fermentation on plant extracts like anti-aging, whitening, anti-wrinkling, etc. (Sivamaruthi et al. 2018).

*Viburnum coriaceum* is a member of the Adoxaceae family, which includes more than 200 species (Prabhu et al. 2011). *Viburnum opulus*, which is phylogenetically related to *Viburnum coriaceum*, is a plant with very high medicinal properties (Clement et al. 2014). Though *Viburnum coriaceum* was least studied in the medicinal aspect, its formulations were reported to have anticonvulsive activity suggesting the neurological effects of the plant (Prabhu and Ponnudurai 2011). Our initial study revealed the bioactivities of the plant (Vijaytha et al. 2020) and present study was focusing on the modifications in the biological activities of plant extract by fermentation with *Saccharomyces cerevisiae*.

## Materials And Methods

# Plant material preparation and fermentation

Plant materials were collected, dried under shade and powdered. Root and aerial parts were powdered separately. The material was identified and authenticated by Dr. Prabhukumar K. M., Scientist and Head, Plant Systematics and Genetics Division, Centre for Medicinal Plants Research, Kottakkal, India. 5 g plant powder (2.5 g aerial parts and 2.5 g roots) was mixed with 50 mL of distilled water and boiled for 5 minutes and filtered. The filtrate was then centrifuged to get the clear aqueous extract. A fast-growing strain *Saccharomyces cerevisiea* MTCC 170 (Joginder et al. 2013) collected from Microbial Type Culture Collection (MTCC), Chandigarh was used for fermentation. Inoculums was prepared by mixing one loop of the culture in 100 mL of potato dextrose broth and incubated under shaking condition at room temperature for 18 h. 100 mL of the extract mixed with fermentation media components – potassium dihydrogen phosphate (1g/L), sodium chloride (1g/L), ammonium sulfate (2g/L), and sucrose (40g/L) was inoculated with 10% v/v of the inoculums and kept 30 days in a shaking incubator at room temperature for fermentation. After fermentation, extract was centrifuged and filtered to get a clear extract.

# Phytochemical analysis

The extract before fermentation (EBF) and after fermentation (EAF) were analyzed qualitatively and quantitatively for studying the implication of fermentation on the plant's phytoconstituents. Presence of alkaloids (Morsy 2014; Santhi and Sengottuvel 2016), flavonoids (Morsy 2014; Gul et al. 2017), tannins (Morsy 2014), triterpenoids (Santhi and Sengottuvel 2016), glycosides (Morsy 2014; Santhi and Sengottuvel 2016; Gul et al. 2017), saponins (Santhi and Sengottuvel 2016; Gul et al. 2017) and anthocyanins (Rajesh et al. 2014) were analyzed under qualitative study. Alkaloids, flavonoids, tannins, and phenols were estimated as they contribute much in biological activities. Estimation of alkaloids was performed according to Harborne methodology (Morsy 2014; Harborne, A.J. 1998). Total flavonoid content was estimated by aluminium chloride calorimetric method (Chang et al. 2002; Sari and Elya 2017), total tannin content by modified Folin-ciocalteu method (Prabhavathi et al. 2016; Ci and Indira 2016), and total phenol content also by Folin-ciocalteu method (Ci and Indira 2016; Anesini et al. 2008).

# Bioactivity analysis

# Antioxidant activity

# DPPH radical scavenging assay

1, 1- Diphenyl-2-picrylhydrazyl (DPPH) is a free radical with the highest absorbance at 517 nm. The deep purple color of DPPH solution gets faded to golden yellow color while accepting hydrogen ions from donors. This can be proportional to the free radical scavenging activity and concentration of antioxidant compounds present in the plant extracts. DPPH solution (100 µM) and different concentrations (10, 20, 30, 40, 50 mg/mL) of plant extracts were

prepared in methanol. 2.8 mL of DPPH was mixed with 0.2 mL of plant extract and incubated at room temperature for 30 minutes. Absorbance was measured at 517 nm after shaken well. Ascorbic acid was the standard used (Pavithra and Vadivukkarasi 2015).

(%) DPPH scavenging activity = $\left(\frac{Absorbance of control - Absorbance of test}{Absorbance of control}\right) \times 100$

# ABTS radical scavenging assay

2, 2’ – azinobis (3 - ethylbenzothiazoline) – 6 – sulfonic acid (ABTS) salt generates free radicals when it reacts with strong oxidizing agents. Reduction in the blue-green color of the ABTS radical solution occurs when it absorbs hydrogen ions from the antioxidants and this can be measured at a wavelength of 734 nm. The working solution was prepared by mixing equal quantities of ABTS (7.4 mM) solution and potassium persulfate (2.6 mM) solution, incubated at room temperature for 12 h in dark. The absorbance of 1 mL of the working solution in 60 mL of methanol was measured and the value was adjusted to 1.17. Then the absorbance of the samples was measured. Different concentrations (2, 4, 6, 8, and 10 mg/mL) of the extracts were used for sample preparation. Samples were prepared by mixing 150 µL of the extract with 2850 µL of the working solution and incubated at 2 h in dark. Ascorbic acid was the standard used (Shalaby and Shanab 2013).

Percentage of ABTS scavenging activity = $\left(\frac{Absorbance of control - Absorbance of test}{Absorbance of control}\right) \times 100$

# Hydroxyl radical scavenging assay

Hydroxyl radicals are one of the highly reactive oxygen species developed inside the cells. In this assay competence of anti-oxidant compounds in the extracts with salicylic acid for scavenging the hydroxyl radicals was measured. Here 1 mL $FeSO_4$ (1.5 mM), 0.7 mL hydrogen peroxide (6 mM), 0.3 mL sodium salicylate (20 mM) and 1 mL of different concentrations (2, 4, 6, 8 and 10 mg/mL) of plant extracts were mixed and incubated at 37°C for 1 h. The absorbance was measured at 562 nm to detect the hydroxylated salicylate complex. Ascorbic acid was the standard used (Lipinski 2011).

$Percentage of scavenging = 1 - \left(\frac{A1 - A2}{A0}\right) x100$

$A_0$ - Absorbance of control

$A_1$- Absorbance of the reactants in the presence of extract

$A_2$ - Absorbance of reactants (except sodium salicylate) in the presence of extracts.

# Anti-inflammatory activity

# Trypsin inhibitory assay

Assay was performed using a spectrophotometric method developed by Sigma Aldrich (Enzymatic Assay of Trypsin Inhibitor. Sigma-Aldrich) with modifications. The substrate $N_\alpha$- benzoyl- L- arginine ethyl ester (BAEE) (0.25 mM) was prepared in phosphate buffer at pH 7.6 and its single unit will show an absorbance of 0.001 per minute at the same pH. Enzyme (0.05 mM) was prepared in ice cold HCl (0.001 M). 0.2 mL enzyme and 0.2 mL inhibitor were mixed and incubated for 10 minutes, and then 3 mL substrate was added to stop the reaction. Readings were taken at 253 nm by time scanning for 10 minutes. Phenyl methyl sulfonyl fluoride (PMSF) (0.1 mM) was the standard inhibitor used.

Percentage of inhibition = $\left(\frac{\mathrm{N-I}}{\mathrm{N}}\right) \mathrm{x}100$

N - Activity of the native enzyme

I - Activity of the enzyme in the presence of inhibitor

# Lipoxygenase inhibitory assay

Assay protocol developed by Anthon and Barrett (Surapneni and Chandrasada Gopan 2008) with modifications were used for the analysis. LOX (EC 1.13.11.12) type I-B (soybean) enzyme solution (0.93 µM) and linoleic acid substrate solution (0.32 mM) was prepared in borate buffer (0.2 mM) at pH 8.6. 2 mL of reaction mixture contained 50 µL enzyme, 360 µL substrate and 1.59 mL borate buffer. In the presence of an inhibitor (50 µL) buffer volume was reduced to 1.54 mL to maintain the total volume of the reaction mixture at 2 mL. Formation of hydroperoxy–octadecadienoic in the reaction mixture was measured in a spectrophotometer at 234 nm. Vanillin (1mM) was the standard inhibitor used.

Percentage of inhibition = $\left(\frac{\mathrm{N-I}}{\mathrm{N}}\right) \mathrm{x}10$

N - Activity of the native enzyme

I - Activity of enzyme in the presence of inhibitor

**Anti-neuroinflammatory activity**

# Cell collection, culturing, and inflammation induction

IMR 32 cell lines from National Centre for Cell Sciences (NCCS), Pune, India was used. Sub-culturing was done in Dulbecco's modified eagle's medium (DMEM) in a tissue culture flask (25 $cm^2$). DMEM was supplemented with L-glutamine, 10% FBS, sodium bicarbonate, and an antibiotic solution containing penicillin (100 U/mL), streptomycin (100 µg/mL), and amphotericin B (2.5µg/mL). Sub cultured cells were maintained in a humidified 5% $CO_2$ incubator (NBS Eppendorf, Germany) at 37ºC. Inflammation was induced by treating 60% confluent cells with 1 µL of 1µg/mL lipopolysaccharide (LPS). These induced cells were then treated with different concentrations of the extracts (5, 10, 20 mg/mL) and incubated for 24 h. Cell lysate was prepared after incubation for further analysis.

# Lipoxygenase assay

Axelrod method was employed for determining LOX activity (Axelrod 1981; Deepa et al. 2017). 50 µL of the cell lysate, 200 µL sodium linoleate in Tris HCl buffer at pH 7.4 to make 2 mL reaction mixture. Absorbance was measured at 234 nm to detect the presence of 5-hydroxyeicosatetraenoic acid.

Percentage of enzyme inhibition = $\left(\frac{\mathrm{N-I}}{\mathrm{N}}\right) \mathrm{x}100$

# Cyclooxygenase assay

The Cyclooxygenase (COX) activity was determined by Walker and Gierse method (Walker and Gierse 2010; Bindu et al. 2016). 100 µL of cell lysate was mixed with Tris-HCl buffer (pH 8) glutathione (5 mM) and hemoglobin (5 mM) and then incubated for 1 minute at 25°C. Arachidonic acid (200 mM) was added to initiate the reaction. After 20 minutes of incubation at 37°C 200 µL of 10% trichloroacetic acid in 1 N HCl was added to terminate the reaction. This was then

centrifuged and added 200 µL of 1% thiobarbiturate, boiled for 20 minutes and cooled, and again centrifuged for three minutes. COX activity was determined by reading absorbance at 632 nm.

Percentage of enzyme inhibition = $\left(\frac{\mathrm{N}-\mathrm{I}}{\mathrm{N}}\right) \mathrm{x}100$

# Antiepileptic activity

The antiepileptic activity of the extracts was studied on pilocarpine (PILO) induced animal models of temporal lobe epilepsy (TLE). Effects of peripheral inflammatory factors in the development of seizures were analyzed here. Blood and brain tissue parameters on account of this were measured.

# Toxicity study

Toxic effects of the extracts were analyzed according to the guidelines of OECD. Doses selected for the study were 250 mg/Kg, 500 mg/Kg and 1000 mg/Kg of the extracts (Kifayatullah et al. 2015).

# Pilocarpine induced convulsions

The seizure was induced by intraperitoneal (i.p) injection of PILO in Sprague Dawley rats (200–250 g). Animals of both sexes were randomly collected from Kerala Veterinary and Animal Sciences University, Mannuthy, Kerala, India and maintained in the Animal House facility (KULS/IAEC/2017/19) of the Department of Biotechnology and Microbiology, Kannur University, Kerala, India. Experiments were followed only after a period of one week of acclimatization and according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). 7 groups of 6 animals each were taken for the study. The first three groups were normal rats, negative control (vehicle-treated) and positive control (standard drug treated) respectively. The next four groups were treated with two doses (100 mg/Kg and 200 mg/Kg) of EBF and EAF respectively.

Animals were treated with the extracts and standard drug (10 mg/Kg diazepam) 30–45 minutes before induction (350 mg/Kg PILO). Peripheral effects of the inducer were reduced by i.p. injection of methyl scopolamine 30 minutes before induction. After PILO injection animals were kept 2 h undisturbed for observing latency and onset of seizures (Mante et al. 2017; Rapacz et al. 2016). Behavioral stages during the development of seizures were rated according to the Racine score (Racine 1972). First three stages (0, 1, 2) were non-convulsive stages and the next three stages (3, 4, 5) were convulsive stages (Phelan et al. 2015; Sharma et al. 2018). Blood samples were analyzed at the time of SE and 24 h after SE to measure the level of peripheral inflammatory parameters. Blood samples were collected by cardiac puncturing and prepared for the analysis (Marchi et al. 2007; Ali et al. 2012) of total and differential WBC count (impedance method- Horiba Pentra ES 60), quantification of IL-1β (ELISA - KB31165 IL-1β rat analyte kit, Krishgen biosystems, India) and CRP (ELISA – 88–7501 Rat CRP kit, Thermo Fisher Scientific). Animals were subjected to decapitation after 24 h of SE for the isolation of the brain. Brain tissues were collected and prepared (Craigie et al. 1963) for isolating the hippocampus (Löscher et al. 1984) and choroid plexus (Bowyer et al. 2012). Hippocampus being the centre of origin of seizures was isolated for measuring GABA and glutamate levels using thin layer chromatographic method (Sancheti et al. 2013). Serum albumin content (ELISA - E111-125, Bethyl laboratories) in the choroid plexus was measured as indicator of blood-brain barrier permeability (Westergren and Johansson 1990).

# Statistical analysis

Statistical analyses were performed using SPSS software and the final data is presented as mean ± SEM. All the data were evaluated by one way analysis of variance (ANOVA) followed by post hoc test and a value of $P < 0.05$ was taken as significance.

# Results

## Phytochemical analysis

**Table 1: Qualitative phytochemical analysis of aqueous extract of *Viburnum coriaceum* before fermentation and after fermentation**

| Secondary Metabolites | Aqueous extract before fermentation | Aqueous extract after fermentation |
|---|---|---|
| Alkaloids | + | - |
| Flavonoids | + | + |
| Tannins | + | + |
| Triterpenoids | + | + |
| Glycosides | + | - |
| Saponins | - | - |
| Anthocyanins | - | - |

**Table 2: Quantitative phytochemical analysis of aqueous extract of *Viburnum coriaceum* before fermentation and after fermentation**

| Secondary Metabolites | Aqueous extract before fermentation | Aqueous extract after fermentation |
|---|---|---|
| Alkaloids | 5.67±1.01% | - |
| Flavonoids | 297±4.87 (µg/mL) | 405±7.08(µg/mL) |
| Tannins | 582±10.45(µg/mL) | 674±15.3 (µg/mL) |
| Phenols | 256±7.9(µg/mL) | 435±6.03 (µg/mL) |

## Bioactivity analysis

Elevated activity was observed in EAF in all the *in vitro* assays performed for studying the antioxidant activity when compared to EBF (Fig. 1, 2 & 3). EAF also showed a better inhibition for trypsin and LOX in *in vitro* anti-inflammatory study (Fig. 4) and COX and LOX inhibition in the cell lines studied for anti-neuroinflammatory activity (Fig. 5 & 6). Whereas in the antiepileptic activity studied on animal model, EBF showed a better activity.

## Anticonvulsant activity

## Toxicity studies

100% mortality was observed in animals treated with the highest concentration of EBF whereas the mortality rate was found to be reduced to 66.66% in EAF but a sedative effect was observed in all the animals. At 500 mg/Kg dosage

sedative effect was observed in both EBF and EAF treated animals. 250 mg/Kg dosage also showed a slight sedative effect in the animals on both extracts.

### Observational studies after PILO injection

Rats were observed for a period of 2 hour after the PILO injection to get details about the latency, onset, and behavioral stages of SE. Animals treated with the second dose of EBF showed behaviors only up to the second stage of the Racine score, which was similar to that of the standard drug. No types of clonuses were observed in them and animals returned to normal condition within 4 – 5 hours of induction. In the first dose of EBF rats showed expressions up to 3$^{rd}$ behavioral stage. In the animals treated with EAF, behavioral stages were on the 4$^{th}$ and 5$^{th}$ stage of Racine score and 33.33 % mortality was observed in the group treated with the first dose of EAF due to SE (Table 3).

In the negative control group, the latent period for the onset of SE was 24 ± 7 minutes and this was found to be increased in the test groups. Groups treated with first and second dose of EAF had 35 ± 6 minutes and 45 ± 5 minutes respectively and in the groups with first dose of EBF latent period was 50 ± 3 minutes. No SE was observed in the groups treated with standard drug and second dose of EBF (Table 3).

**Table 3: Observational studies after pilocarpine injection during SE**

| Parameters | Group 1 (Normal) | Group 2 negative control (MS, PILO) | Group 3 positive control (MS, DZ, PILO) | Group 4 EBF - 1$^{st}$ dose | Group 5 EBF - 2$^{nd}$ dose | Group 6 EAF - 1$^{st}$ dose | Group 7 EAF - 2$^{nd}$ dose |
|---|---|---|---|---|---|---|---|
| Latent period (min) | Nil | 27 ± 7 | No SE | 50 ± 3 | No SE | 35 ± 6 | 45 ± 5 |
| Racine score | Nil | Stage 5 50% mortality | Stage 2 | Stage 3 | Stage 2 | Stage 5 33.33% mortality | Stage 4 |

### Blood and brain parameters

The parameters studied from blood and brain tissues also showed that the EBF is more active in reducing SE than EAF. The best result was shown by the second dose of EBF. The results (Table 4) revealed that there was an upregulation in the peripheral inflammatory markers upon injecting PILO. Animals treated with the standard drug (positive control) showed no significant difference ($p<0.05$) in the blood parameters from that of the untreated (negative control) animals. Diazepam, the standard drug used acts mainly in the brain cells to inhibit the development of seizures. That may be the reason for elevated blood parameters in the positive control groups. Blood samples collected 24 hours after SE showed normal values. The presence of serum albumin in the brain tissues indicated an increased permeability of the blood brain barrier (BBB). An elevated level of the excitatory neurotransmitter glutamate in the brain tissues was also found to be reduced upon treatment with the highest dose of EBF (Table 5).

**Table 4: Changes in the blood parameters during SE and 24 h after SE**

| Groups<br>Factors | 1<br>(Normal) | 2<br>(Negative control) | 3<br>(Positive control) | 4<br>(EBF- 1st dose) | 5<br>(EBF- 2nd dose) | 6<br>(EAF- 1st dose) | 7<br>(EAF- 2nd dose) |
|---|---|---|---|---|---|---|---|
| 1 hour after PILO injection | | | | | | | |
| WBC/ $mM^3$ | 8048±1000 | 2593±652 | 3664±505 | 5695±748 | 7473±560* | 3459±643 | 4450±697 |
| Lymphocyte (%) | 81.5±5.4 | 19.2±2.8 | 22.5±3.0 | 56.2±4.3 | 74.3±5.7* | 30.0±3.6 | 44.8±3.5 |
| IL - 1β (pg/mL) | 49.9±3.2 | 203.0±6.7 | 182.3±11.0 | 78.1±4.7 | 53.5±4.0* | 144.3±6.5 | 112.5±6.3 |
| CRP (pg/mL) | <2000 | <2000 | <2000 | <2000 | <2000 | <2000 | <2000 |
| 24 hours after PILO injection | | | | | | | |
| WBC/ $mM^3$ | >7300 | >7300 | >7300 | >7300 | >7300 | >7300 | >7300 |
| Lymphocyte (%) | >72 | >72 | >72 | >72 | >72 | >72 | >72 |
| IL - 1β (pg/mL) | <62 | <62 | <62 | <62 | <62 | <62 | <62 |
| CRP (pg/mL) | <2000 | <2000 | <2000 | <2000 | <2000 | <2000 | <2000 |

***No significant difference with the normal rats (p<0.05) other values showed a significant difference with the normal**

**Table 5: Quantification of serum albumin, glutamate and GABA in brain tissue homogenate**

| Factors | 1<br>(Normal) | 2<br>(Negative control) | 3<br>(Positive control) | 4<br>(EBF- 1st dose) | 5<br>(EBF- 2nd dose) | 6<br>(EAF- 1st dose) | 7<br>(EAF- 2nd dose) |
|---|---|---|---|---|---|---|---|
| Albumin (ng/mL) | Nil | >1000 | 933.3±6.8 | 896.9±6.9 | Nil | 956.7±15.3 | 936.1±6.7 |
| GABA (ng/mg) | <270 | <270 | <270 | <270 | <270 | <270 | <270 |
| Glutamate (ng/mg) | 325.7±23.9 | 503.7±16.5 | 330.3±16.6* | 398.2±14.8 | 314.3±11.7* | 443.6±13 | 414.9±6.5 |

***No significant difference with the normal rats (p<0.05) other values in glutamate showed a significant difference with the normal**

# Discussion

Preceding decades increased the use of plants as the major source of bioactive compounds. Various technologies have developed to increase the access of bioactive compounds from the crude plant extracts. Fermentation is one among them, bound phenolic compounds got released to the extract as a result of cell wall degradation by microbial enzymes during fermentation which were otherwise been not available (Adebo and Gabriela Medina-Meza 2020). In traditional medicine quality of the products will be improved by fermentation. Changes will reflect in the bioactivities, stability and toxic effects of the plant compounds, which may either increase or decrease, got influenced by the fermentative conversion of the secondary metabolites. The bioavailability of metabolites also got increased by fermentation either by conversion of inactive components to active or by absorbing unwanted chemicals and making the rest easily available. Microorganisms in log phase produce different enzymes like protease, amidase, esterase, cellulase, amylase etc. which play key roles in the process of fermentation. Fermentation of *Mercurialis perennis* with Lactobacteria showed the appearance of two novel compounds, methyl hermidin and methyl hermidin quinone (Lorenz et al. 2019). Current study also assessed the influence of fermentation on different parameters of *Viburnum coriaceum* extracts. Phytochemical, toxicity and bioactivity analyses were performed on EBF and EAF to assess these influences.

Qualitative and quantitative phytochemical analysis let out the impact of yeast fermentation on the phytoconstituents of plants. Alkaloids and glycosides were found to be disappeared by the action of microorganism. Fermentation of plant extracts by yeasts were found to reduce alkaloids, sugars, phytates, etc. by degradation (Kasprowicz-Potocka et al. 2018; Antai, S and Nkwelang G 1999; Erukainure et al. 2010). Flavonoids, tannins, and triterpenoids were present in both EBF and EAF (Table 1) and the total amount was found to increase after fermentation (Table 2). This is by releasing bound phenolics from the cells or by enriching already present compounds (Adebo and Gabriela Medina-Meza 2020; Erukainure et al. 2010). Supporting the present analysis an enhancement in triterpenoid content was also observed in *Antrodia cinnamomea* when fermented with *Saccharomyces cerevisiea* (Wu 2015).

Antioxidant property of plant extract, have a key role in our body, is by scavenging the free radicals, chelating metal ions and by inhibiting some of the key oxidases. Both the extracts showed antioxidant activity with an enhancement in the EAF. The antioxidant activity was found to be increased by fermentation with *Saccharomyces cerevisiae.* In all the three experiments chosen for analyzing antioxidant activity this increasing pattern was observed (Figs. 1, 2 & 3). Increasing phenol contents in the extract can be a reason for this elevated antioxidant activity (Adebo and Gabriela Medina-Meza 2020; Chaiyasut et al 2017). Being a feasible strategy for enhancing bioactivities, fermentation can do much in increasing the anti-inflammatory potential of plant extracts also. Lipopolysaccharide insult on the intestinal epithelium can be cured by the fermented extracts of Rhizoma *Atractylodis macrocephalae* than the unfermented one (Bose and Kim 2013). Anti-inflammatory studies by extracts before and after fermentation of various plants have proved this (Hussain et al. 2016). *In vitro* assays with LOX and trypsin (Fig. 4) and anti-neuroinflammatory assay on IMR 32 cell lines with COX and LOX in the current study also add to this (Figs. 5 & 6).

Anti-convulsant activity studied *in vivo* also followed the same pattern with EBF showing higher activity than EAF (Tables 3, 4 & 5). Contradictory to current findings fermentation of *Viburnum coriaceum* by traditional methods showed a significant reduction in convulsions induced by the maximum electroshock method (Prabhu and Ponnudurai 2011) but they were not comparing the activity of fermented extract with the unfermented one. In the present study, conventional laboratory method was used for fermentation and it showed a decrease inhibition of convulsions induced by PILO. While fermenting whole extracts, total effect of the extract is the sum of the effects of compounds present in them. Fermentation may increase the effect of one compound and reduces the other. This may reduce the total effect of the extract in a particular treatment. Studies on decreasing bioactivity by fermentation were also reported (Gan et al. 2017). This may be due to the degradation of some bioactive compounds. Some reports also suggested that activity may decrease when it reached its maximum threshold (Verni et al. 2019). Though the activity

was negatively influenced by fermentation, beneficial part of the *in vivo* study was that toxicity of the extract was found to be reduced by fermentation.

# Conclusion

Drastic changes were observed in the field of herbal medicines in the past decades, fermentation methods are being used for the development of many medicinal formulations and drug candidates. It can either enhance the bioactivities or reduce the toxicities possessed by the constituents in medicine. Many microorganisms perform molecular degrading process in fermentation for synthesizing novel compounds, converting highly toxic to less toxic, less active to highly active or even highly active to less active; accounts for the reduction or enhancement of bioactivities. Anti-oxidant, anti-inflammatory, immune-modulatory, anti-infective, anti-allergic, anti-diabetic effects etc. were found to be greatly influenced by fermentation positively or negatively. Some fermented formulations were supposed to possess high toxic compounds due to contamination with pathogenic microorganisms hence validation and standardization of the fermentation conditions and microorganism to be used is more important to get a valuable result.

# Declarations

### Availability of Data and Materials

The data are available from the corresponding author upon request.


### Funding Statement

No fundings were received


### Competing Interests

The authors declare that they have no competing interests.

### Authors' Contributions

The authors declare that all the works stated in the article were done by themselves. V V performed all the experimental works and compiled the data. H M and E J V conceived the research program, and guided the work. H M gave final shape to the manuscript. All the authors have read and approved the final manuscript.

### Ethics approval statement

Al the experiments were carried out according to the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA), India and ARRIVE (Animal Research: Reporting of In Vivo Experiments) after getting approval from the Internal Animal Ethical Committee of Kannur University, Kerala, India (KULS/IAEC/2017/19).

### Convention on biodiversity

The authors procured permission from the Forest Department, Govt. of Kerala, India to visit and collect the plant sample from the forest areas for research purposes as per Order No. WL 10-47474/2014 dated 1/12/2015 and accordingly procured the sample for the study.

### Consent to Participate

Not Applicable

### Consent to Publish

Not Applicable

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# Figures

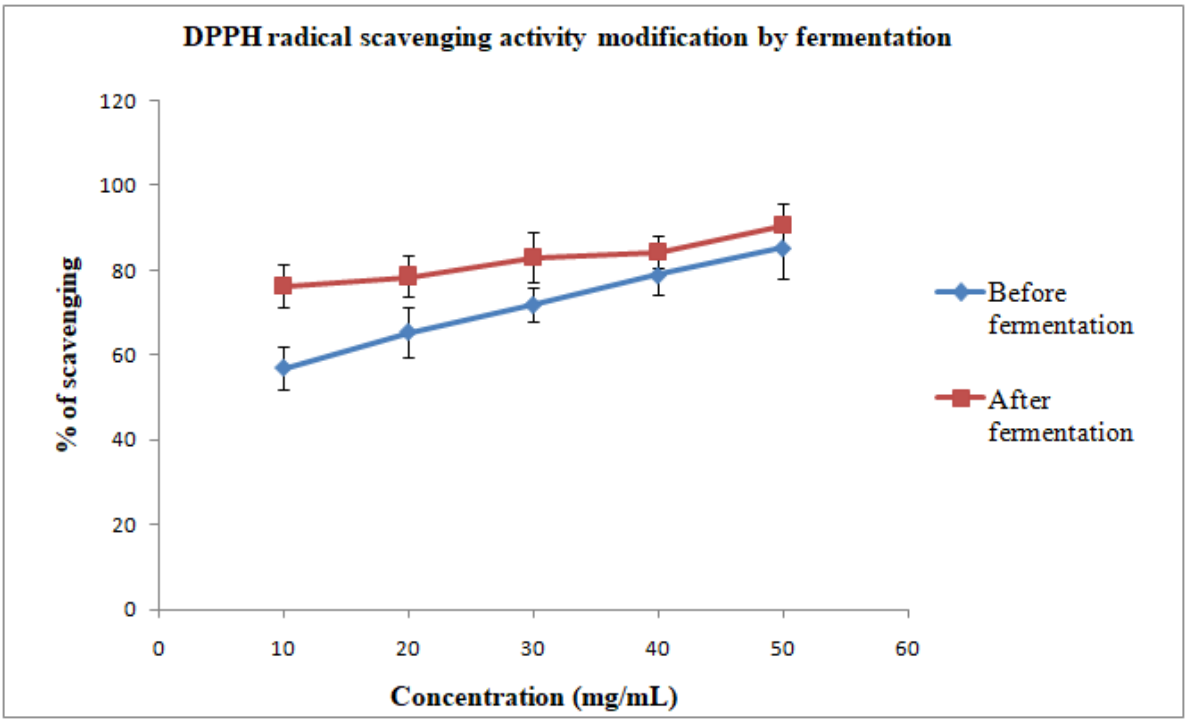



Figure 1

DPPH radical scavenging activity of the plant aqueous extract before and after fermentation

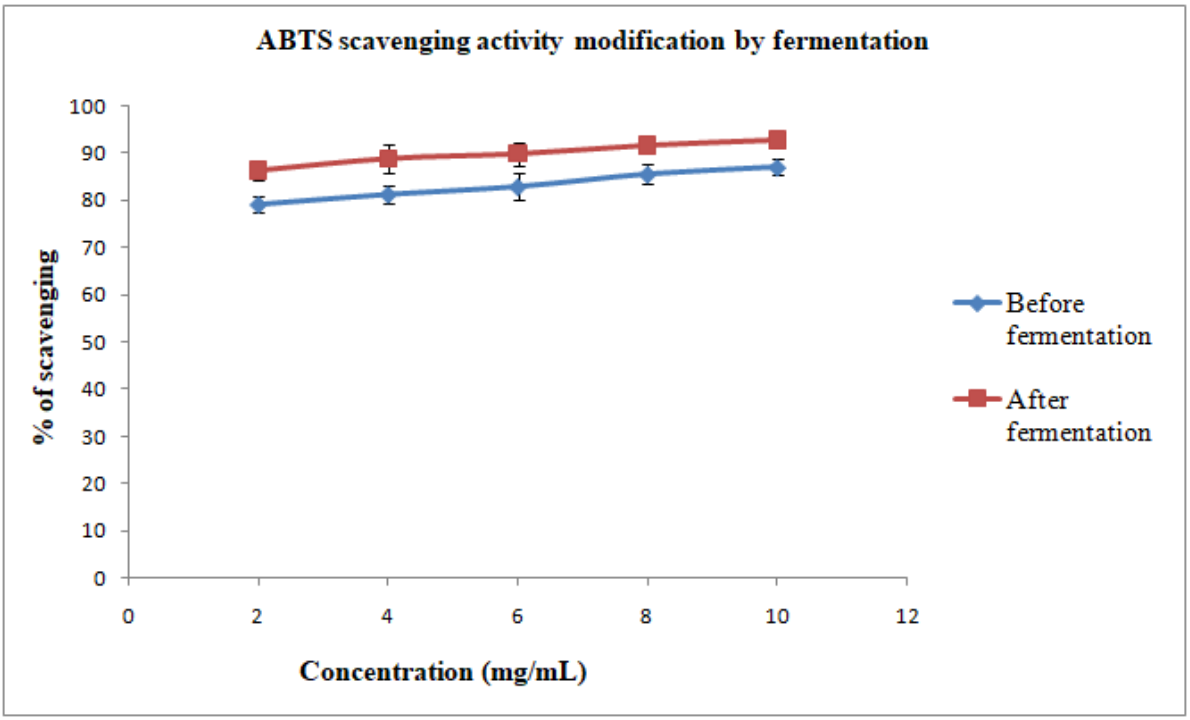



Figure 2

ABTS radical scavenging activity of the plant aqueous extract before and after fermentation

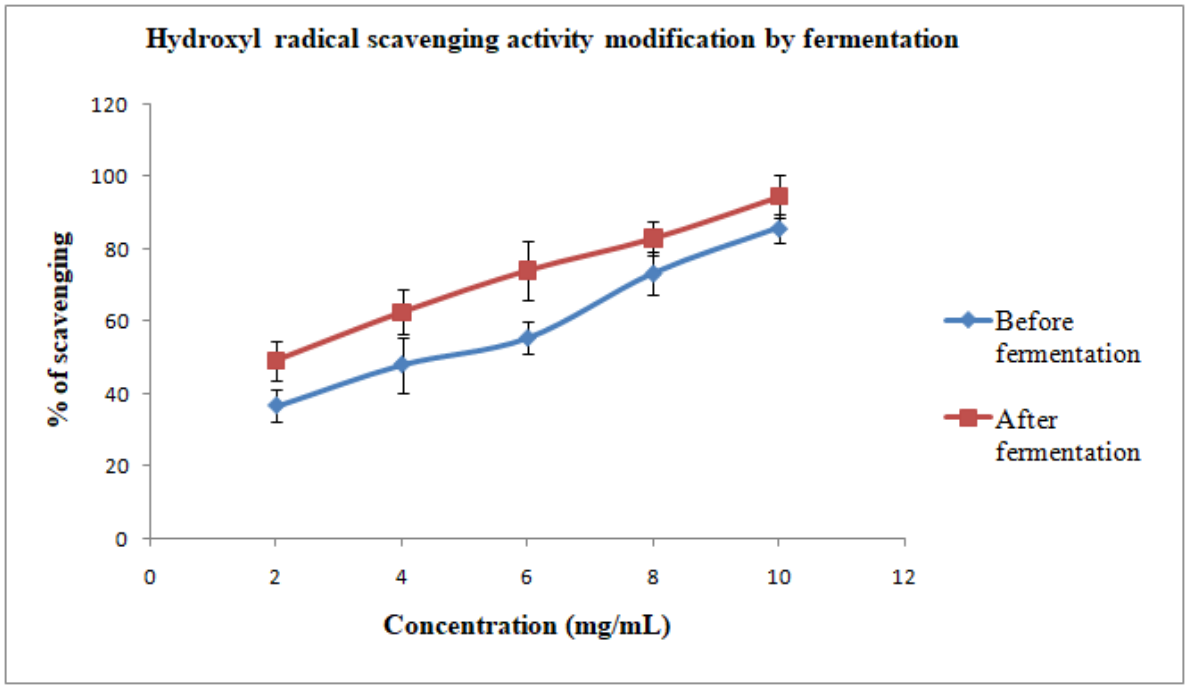



Figure 3

Hydroxyl radical scavenging activity of the plant aqueous extract before and after fermentation

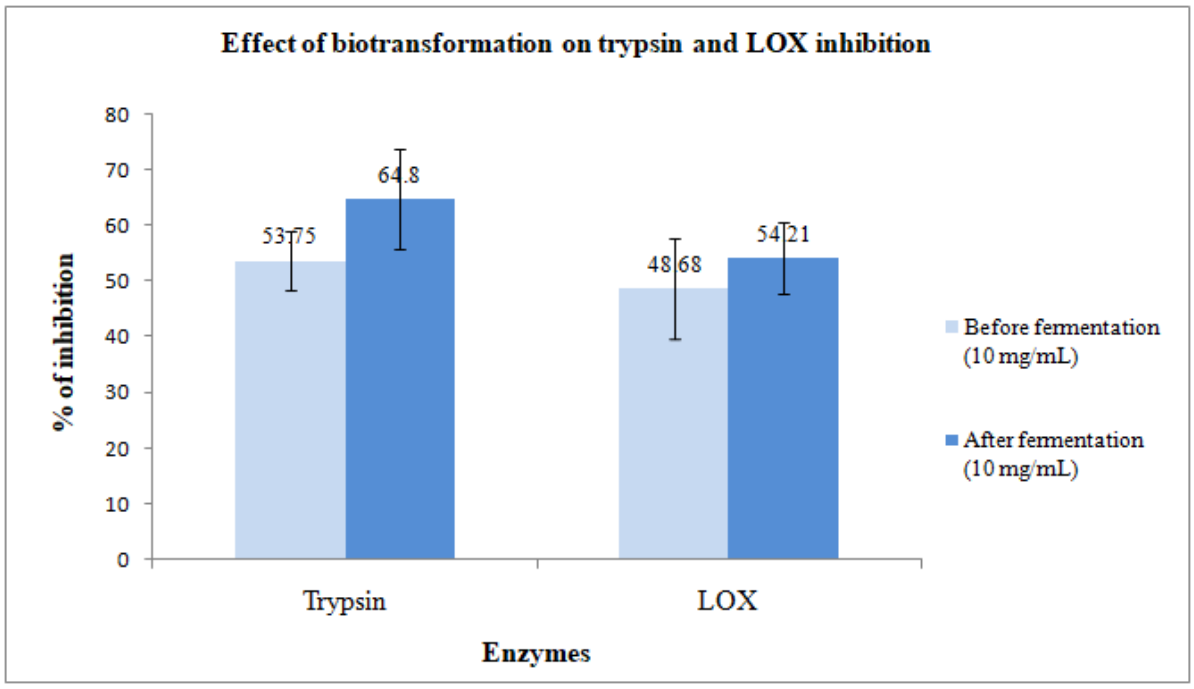



Figure 4

Anti-inflammatory activity of the plant aqueous extract before and after fermentation

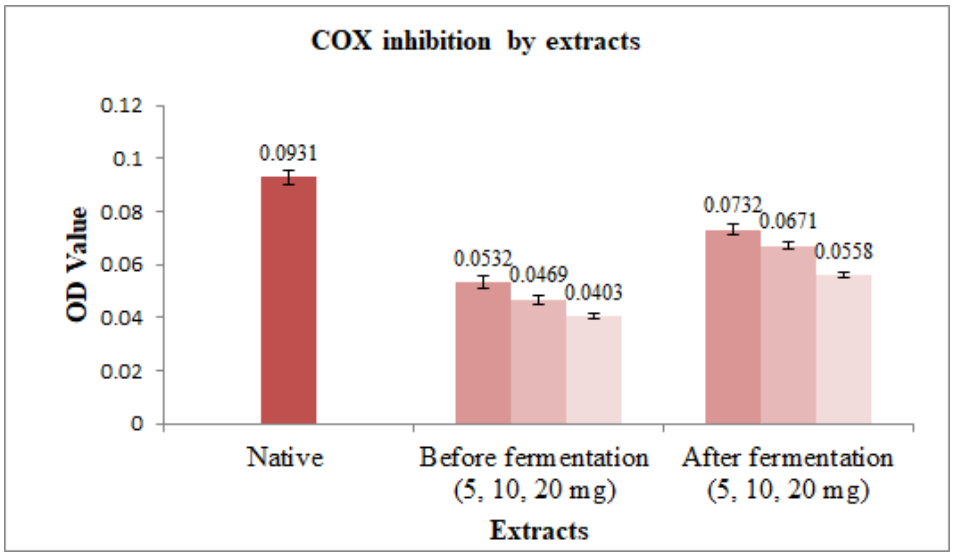



Figure 5

COX inhibitory activity of the extracts in inflammation induced cell lines

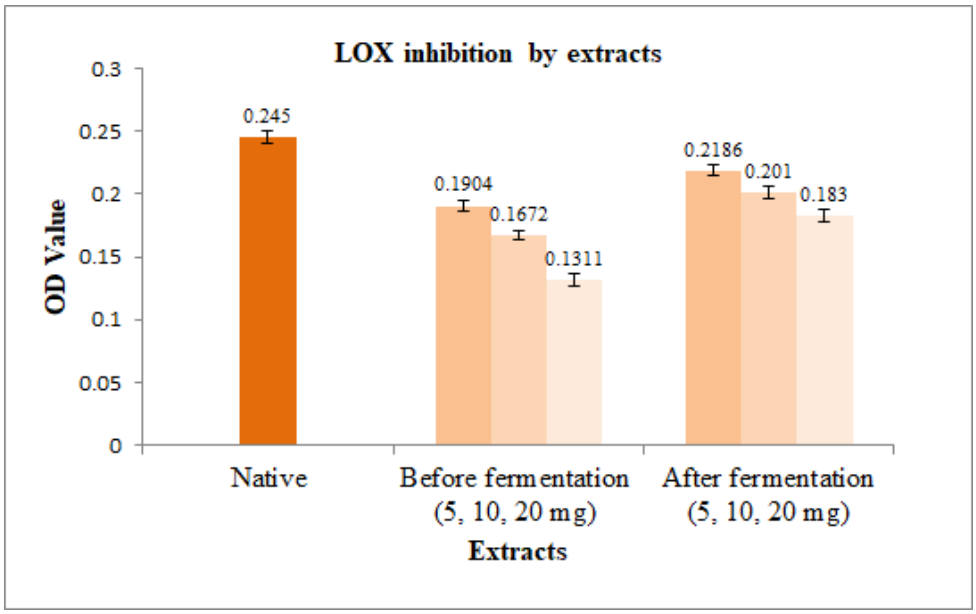



Figure 6

LOX inhibitory activity of the extracts in inflammation induced cell lines