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COMPARATIVE STUDIES ON THE ANTIOXIDANT, ANTI-INFLAMMATORY AND PHYTO-CHEMICAL CONSTITUENTS OF *PIPER BETEL* LEAVES FROM THE WESTERN GHATS AND NORTHERN REGION OF KARNATAKA

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ABSTRACT: Our present study aims at analyzing and comparing the antioxidant, anti-inflammatory and phytochemical property of betel leaves from two different domains of Karnataka that includes the Western Ghats and the Northern region. Initially, the ethanol extracts of all four samples (two from each domain) were prepared and checked for their anti-inflammatory and antioxidant activity. As a result, the betel leaf extract from the western region showed good antioxidant activity and a significant anti-inflammatory activity when compared with the standard Gallic acid. In connection to this, the phytochemical content such as Alkaloids, Tannins, Flavonoids, and Phenols showed positive in all four extracts, but the quantification studies showed that the extracts from the Western Ghats had a higher quantity of alkaloids and phenols when compared to the samples from northern regions. These results imply that the presence of functionally important groups depends on the composition of the soil in which they grow in. Hence having good biologically important activity in them can prove beneficial for their role towards formulating a cost-effective lead drug from the extracts with no side effects.

INTRODUCTION: Research on new natural antioxidants and anti-inflammatory agents has increased interestingly over the past few years. The production of reactive oxygen species (ROS), oxidative stress, and inflammation have a strong connection with various chronic diseases like neurodegenerative diseases, cardiovascular disease, osteoporosis and cancer, *etc.* [1, 2]. Hence, the external supply of antioxidants and anti-inflammatory agents can prevent and protect the body from the damaging effect of free radicals and in turn prevent the occurrence of many diseases [3].

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Naturally occurring plant materials are being utilized for a long time in home remedies, drug products, pharmaceutical industry, and cosmetic industries across the globe [4]. Despite this, our present lifestyle has confined us from natural life. Earlier mankind was most dependent upon the natural resources for the very need, but the present lifestyle was ignoring the rich natural heritage; this ignorance brings various health issues in modern lifestyles such as many old age problems, digestive disorders, and most neurodegenerative diseases [4].

However, nowadays, more studies on the detrimental effect of synthetic medicine, food additives, and preservatives used in the form of coloring agents on the human body have become popular. Simultaneously, researchers are now focusing on the beneficial effect of using natural resources in treating many ailments by isolation, characterization, and utilization of plant molecules

as potential disease-preventing agents [5] and have started to explore the benefits of natural herbs for their nutritive, antioxidant, antimicrobial and anti-inflammatory potential. One such natural herb that is being studied in research is betel leaf [6]. *Piper betel* also called as 'Pan' belongs to the family *Piperaceae* [7], which is an evergreen and perennial creeper basically emerged from Malaysia and cultivated across the world [8]. Due to the ethnomedicinal properties of the plant it is widely used in south-east Asian countries. The socio-economic importance of piper betel has been elucidated with every sphere of human life including medicinal, cultural, social, and religious aspect. Since ancient times piper betel is one of the most favorite herbs used for chewing. The fresh leaves are wrapped with the areca nut, catechu, and mineral slaked lime and consumed [9, 10] According to Ayurveda, betel leaf is called by its Vedic name Saptasira which is used as complementary medicines for purifying blood, appetizer, laxative, and voice. The key fundamental component of the piper betel leaf is a volatile oil, also called betel oil [11]. It contains a variety of biologically active components like Eugenol, Piperbetol, Chavibitol, Hydroxychavicol, Piperol A, and Methyl *Piper betol*. Hence *Piper betel* leaves have been used for various medicinal purposes, including nutritive, insecticidal, anti-oxidant, antimicrobial, antitumor, neuroprotective, anti-diabetic, and anthelmintic activity [12, 13, 14].

FIG. 1: LEAVES COLLECTED FROM DIFFERENT DOMAINS OF KARNATAKA

Western Ghats of India 'Sahyadri' (benevolent mountains), which is also known as a biodiversity hotspot, it covers (1.80.000 km^2) almost 6% of land of India [15]. The region has more than 30% of plant species found in India. The average annual rainfall is 3,456 mm. Due to which is considered as one of the highest rainfall regions in the country.

The temperature around the summer season is 20º-24 ºC. Northern Karnataka is also known as 'karunadu covers semi-arid plateau ranges from 300 to 730 meters (980 to 2,400 ft.), major fields are covered by red sandy loams and black soil. Annual rainfall ranges from 453-717 mm; the mean daily temperature in the summer season is around 31º-32 ºC. The region is drained by the river Krishna, Ghataprabha, Malaprabha, Tungabhadra, and Bhima.

In the present study, our aim is to assess and collate the biological activities that include preliminary phytochemicals analysis, antioxidant and anti-inflammatory potential of *Betel Leaf* extract from Western Ghats of Karnataka (Mangalore and Kodagu) and northern region of Karnataka (Hubbali and Chitradurga). Our study is also aimed at promoting the utilization of *Betel Leaf* in pharmaceutical and nutritive development.

MATERIALS AND METHODOLOGY: Plant Sample: Fresh, healthy betel leaves were collected from different regions of Karnataka (Western Ghats of Karnataka Mangalore and Kodagu and Northern regions of Karnataka - Chitradurga, and Hubbali). The leaves were then properly washed in distilled water to remove dirt and dust. They were then shade dried at 35-40 ºC and finely powdered and stored in the refrigerator until further use. The herbarium was deposited in the Department of Pharmacognosy, JSS College of Pharmacy, Mysuru, with the authentication number JSSCPM-PCOG- 1141.

Preparation of Leaf Extracts: The betel leaf powder was weighed and suspended in distilled water, which was kept overnight for stirring by using a magnetic stirrer. The solution was then filtered, poured into petriplate, and then dried at room temperature to obtain the dry extract. The extract obtained was collected in an Eppendorf and stored until further usage.

Estimation of *in-vitro* Antioxidant Activity:

Diphenylpicrylhydrazyl (DPPH) Assay: DPPH method is used for free radical scavenging activity. To 0.05 ml of extract, 1 ml of 0.5 mM methanol solution is added thoroughly and kept in darkness at ambient temperature for one hour. The absorption of the mixture was measured at 515 nm. Ascorbic acid is used as a standard, and radical scavenging activity was evaluated by percentage inhibition formula [16].

% Inhibition = Absorbance of Control - Absorbance of Sample × 100 / Absorbance of Control

Inhibition of Lipid Peroxidation: Lipid peroxidation was measured according to Ruberto method. According to the protocol, 100 µl of the extract is added to 1 ml of egg homogenate, 1 ml of distilled water, 1 ml of $FeSO_4$ solution, and incubated at room temperature for 30 min. Soon after 2 ml of 20% acetic acid, 2 ml of TBA in 1.1% SDS, 1 ml of 20% TCA was added. The resultant solution was mixed thoroughly and allowed for incubation at 96 ºC for 2 h. 5 ml of Butanol was added to the cooled mixture and centrifuged at 3000 rpm for 10 min. topmost organic layer was collected and absorbance was measured at 530 nm. The percentage inhibition formula is used to calculate the inhibition of lipid peroxidation [17].

% Inhibition = Absorbance of Control - Absorbance of Sample × 100 / Absorbance of Control

Ferric Reducing Antioxidant Power Assay (FRAP Assay): This method is used to measure the reduction capacity of the plant extract. To 1 ml of extract, standard stock solutions of ascorbic acid were added with 3 ml of phosphate buffer (0.2M, pH 6.6), 3 ml of potassium ferric cyanide (1% W/V), and 3 ml of trichloroacetic acid (TCA) (10% W/V). The resulting solution was centrifuged at 3000rpm. The supernatant was collected and mixed with 3ml of distilled water and 0.5 ml of ferric chloride solution. The absorbance of the solution was estimated at 700 nm at a reaction time of 30min; the reduction influence of the extract was represented as the ascorbic acid equivalent (mg /g extract) [18].

Estimation of *in-vitro* Anti-Inflammatory Activity:

Human RBC Membrane (HRBC) Stabilization Method:

RBC Suspension Preparation: Human blood was collected and centrifuged at 3000 rpm. Then washed with the same volume of normal saline 3 times and reconstituted with normal saline as 10% v/v suspension.

Heat-Induced Hemolysis Assay: To 1 ml of plant extract, 1 ml of 10% RBC solution was added and heated at 56 ºC for 10 min, which was then centrifuged at 2500 rpm for 30 min and the supernatant was assembled and read at the absorption of 520 nm [19].

% Inhibition = Absorbance of Control -Absorbance of Sample × 100 / Absorbance of Control

Hypotonicity- Induced Hemolysis Assay: To1 ml of plant extract, 2 ml of hypo saline, 1 ml of phosphate buffer, and 0.5 ml of HRBC suspension was added and incubated at 37 ºC for 30 min and centrifuged at 3000 rpm for 10 min. The supernatant was discarded, and hemoglobin content was estimated at 560 nm. The hemolysis percentage was calculated by the following formula [20].

% Inhibition = Absorbance of Control -Absorbance of Sample × 100 / Absorbance of Control

Protease Inhibition Assay: To 2 ml of sample, 1 ml of trypsin is added, which is then incubated at

37 ºC for 30 min, then 2 ml of casein is added, and again the solution is allowed to incubate at 37 ºC for 3 h. After incubation, 3ml of TCA was added and centrifuged for 10 min at 9 ºC at 10,000 rpm. The supernatant was estimated at 420 nm against buffer solution as a blank and Gallic acid as standard. The following formula was used to calculate the percent of protease inhibition [21].

% Inhibition = Absorbance of Control -Absorbance of Sample × 100 / Absorbance of Control

Protein Denaturation Assay: Protein denaturation inhibition was assessed using Mizushima and Sakat *et al*. methods. 500 µl of 1% bovine serum albumin was added to100µl of the plant extract. This mixture was kept for 10 min at room temperature, followed by 20 minutes of heating at 51 °C; the absorbance of the solution is measured at 660nm once it has attained room temperature. Diclofenac sodium is used as standard, and the protein denaturation inhibition was calculated by using below formula [22].

% Inhibition = Absorbance of Control -Absorbance of Sample × 100 / Absorbance of Control

Preliminary Phytochemical Screening: The extract collected from the western and northern regions of Karnataka were further subjected to various standard qualitative test methods to determine the presence of various phytochemical components, including Alkaloids, Flavonoids, Terpenoids, Tannins, and Saponins [23].

Alkaloids:

Hager's Test: 1 ml of the extract was mixed with 1ml of Hager's reagent, and the presence of alkaloids is indicated by yellow-colored precipitate.

Wagner's Reagent Test: 2 ml of sample was mixed with 2ml of Wagner's reagent, and the presence of alkaloids is indicated by reddish-brown precipitate.

Flavonoids:

Alkaline Reagent Test: 1 ml of extract was taken, and to that 2 ml of 10% Sodium Hydroxide solution was added. The presence of flavonoids was confirmed by the appearance of yellow fluorescence.

Glycosides: To 1ml of sample extract, 1ml of glacial acetic acid, few drops of 5% $FeCl_3$, and few drops of concentrated H_2SO_4 are added. The reddish brown color that was formed at the junction of two layers confirms the presence of Glycosides.

Phenols: To 1ml of sample extract, 1 ml of alcohol, and then a few drops of FeCl3 solution was added, the appearance of greenish-yellow visualize the presence of Phenols.

Saponins: Add few drops of distilled water to 1ml of sample extract and shake vigorously; the appearance of honeycomb froth for a few minutes confirms the presence of Saponins.

Tannins: 2 ml of sample extract was added to few drops of 5% $FeCl_3$. The presence of tannins was confirmed by green color.

Steroids: Sample extract of 1ml is mixed with 1ml acetic anhydride and 2 ml of H_2SO_4; the presence of steroids is confirmed by the presence of an orange color.

Terpenoids: To 1ml of sample extract, 2ml of chloroform, and few drops of Conc.H2SO4is added, presence of terpenoids was confirmed by appearance of reddish-brown color.

Quantitative Estimation of Phytochemicals:

Total Phenolic Content: Folin-Ciocalteu method was followed to measure the total Phenolic content. Accordingly, 0.2 mL of plant extract, 4 ml of distilled water, and 0.8 ml of Folin-Ciocalteu reagent is added. After 1 min, 2 ml of 20% sodium carbonate and 7 ml of distilled water were added. The absorbance of the mixture is measured at 760 nm in triplicate for 30 min. Gallic acid as a standard and total Phenolic Content is expressed as an equivalent of Gallic acid per gram of extract [24].

Total Flavonoid Content: To1ml of sample extract, 0.5 ml of sodium nitrate solution is added and left for 5 min soon after 0.5 ml of 10% $AlCl_3$ solution and 1M of 0.5 ml of NaOH solution was added. After some time, mixture was diluted with distilled water, and the absorbance was measure at 510 nm. The standard used here is Quercetin, and finally, the flavonoid content is expressed as mg of Quercetin equivalent / g extract [25].

Statistical Analysis: Data obtained quantitatively were analyzed statistically by One Way ANOVA.

A difference was considered significant when p value was below 0.05 and results are expressed as the Mean ± Standard deviation (n = 3) at concentrations of 25%, 50% and 100%.B1 - Mangalore, B2-Madkeri, B3- Hubbali and B4-Chitradurga.

RESULTS AND DISCUSSION: *Piper betel*, a perennial plant is considered as a traditional herb and widely used in almost all countries of south Asia. Especially, the leaf of the betel vine has high prominence in treating many different types of ailments such as cancer, allergy, inflammation, gastritis, gingivitis and plaques, fertility problems, dermatitis, and wound healing activity *etc.* It can be consumed in the form of fresh leaves or in the form of concoction. Hence, betel leaf is widely studied for its medicinal properties such as anti-tumor, anti-mutagenic, anti-oxidant; anti-inflammatory, etc [26]. It is also used in the form of extracts or single isolated molecules. Among different solvent extracts, ethanol extract has shown good bioactivity and it may be due to the presence of important biomolecules in the betel leaves such as eugenol, hydroxyl chavicol and 4-chormanol, according to the study done by [27,28]. Also, the polarity of the solvent has a major effect on the extracting of major phytochemical groups such as phenolics. Hence, based on these pre-existing studies, we chose to use ethanol as a solvent for extraction as it has a good polarity index [29]. Plant extracts, as such, in their natural form possess a wide range of phytochemicals, unlike the single purified molecule. Hence the whole extract is preferred as it can provide a lead to current pharmaceutical industries in developing medicines, and also it can help in increasing the scope of the usage of herbal medicines in underdeveloped places which are unable to afford for modern medicines [30]. The antioxidant activity was checked by three methods and they are: DPPH (di-phenyl picryl hydrazine), inhibition of lipid peroxidation and by FRAP assay.

DPPH Scavenging Activity: The scavenging activity of all the four samples was tested against ascorbic acid as standard with the highest dose of 100 µg. The sample was used in three different doses starting from the lowest, *i.e.*, 25 µg, 50 µg, and 100 µg. DPPH is a widely approved method for screening of antioxidant activity of plant extract. In this assay, the violet-colored DPPH free radical that is stable at room temperature is reduced to a yellow-colored product known as diphenyl picryl hydrazine due to the interaction with the antioxidant analytes present in the extract [31].

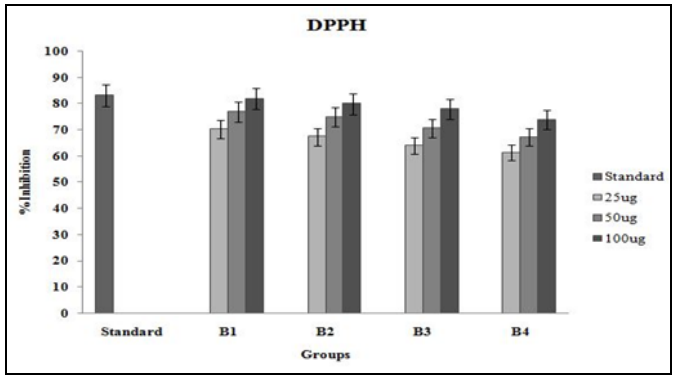

FIG. 2: DPPH ACTIVITY OF BETEL LEAF EXTRACT FROM DIFFERENT REGIONS

TABLE 1: DPPH ACTIVITY OF BETEL LEAF EXTRACT FROM DIFFERENT REGIONS

	DPPH			
Extract	**25%**	**50%**	**100%**	**P value**
Standard			83.31 ± 0.10	
B1	70.37 ± 2.57	77.03 ± 2.05	82.03 ± 0.05	.001
B2	67.43 ± 3.02	75.07 ± 1.95	80.10 ± 0.1	.001
B3	64.07 ± 4.0	70.77 ± 2.95	78.10 ± 0.1	.003
B4	61.4 ± 2.46	67.37 ± 3.00	73.87 ± 0.32	.002

The results obtained indicate that B1 and B2 at a higher concentration of 100 µg show good scavenging activity of 82.033% and 80.1 % when analyzed with the standard, which shows 83.1% of scavenging activity.

The scavenging percentage of B3 (78.1) and B4 (73.8) are comparatively lower to that of the extracts of western regions and the standard drug.

Also, the activity of all four extracts decreases as the concentration decreases, and thus, a dose-dependent scavenging activity can be observed among the extracts.

Inhibition of Lipid Peroxidation: One of the major cause of uncontrolled oxidative stress is the imbalance between the antioxidants and pro-oxidant levels in the cells tissues, and organs

already damaged by oxidative stress or free radical stimuli such as radiation, UV rays, environmental toxins, and exposure to viral infections, insecticides, etc. These free radicals or reactive oxygen species are the major cause of lipid deterioration and are easily prone to lethal peroxidative modification. Hence based on the peroxidation process, a molecule of Malan oldehyde (MDA) interacts with two molecules of Thiobarbituric acid (TBA) to form malanoldehyde TBA complex hence changing to red color, which can be absorbed in 500 nm [32].

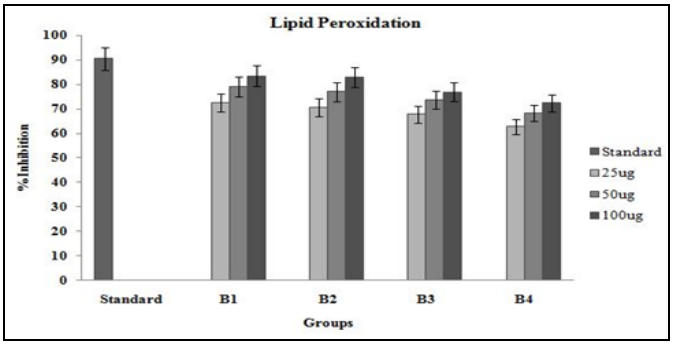

FIG. 3: LIPID PEROXIDATION ACTIVITY OF BETEL LEAF EXTRACT FROM DIFFERENT REGIONS

TABLE 2: LIPID PEROXIDATION ACTIVITY OF BETEL LEAF EXTRACT FROM DIFFERENT REGIONS

Lipid Peroxidation				
Extract	**25%**	**50%**	**100%**	**P value**
Standard			93.17 ± 2.23	
B1	72.7 ± 3.55	79.07±2.00	83.40 ± 0.1	.004
B2	70.70 ± 3.11	77.10±3.05	83.17± 0.15	.003
B3	67.77 ± 3.57	73.77±3.55	77.53 ± 0.47	.018
B4	62.77 ± 3.47	68.40±4.41	72.20 ± 0.2	.032

The percentage of inhibition of lipid peroxidation by the standard compound at 100 µg is 90.6% and whereas both B1 and B2 show the inhibition at 83 % at highest concentration compared to the rest two lower concentration. But the extracts B2 and B3 collected from the northern part do not show much significant inhibition of lipid peroxidation when taken in all the three concentrations which thus cannot be correlated to the standard. This effect can be due to the presence of high levels of phenols and flavonoids in the extract.

Ferric Reducing Antioxidant Power (FRAP) Assay: The ferric reducing antioxidant activity of the plant extracts is measured by the reduction of ferric tripyridyltriazine complex to produce a colored ferrous tripyridyltriazine [33]. The anti-oxidant activity generally depends on the phytochemical group to break the free radical chain by donating a hydrogen atom Eugene.

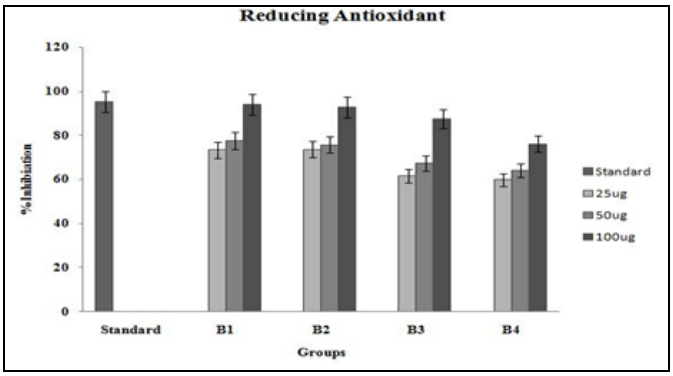

FIG. 4: REDUCING ANTIOXIDANT ACTIVITY OF BETEL LEAF EXTRACT FROM DIFFERENT REGIONS

TABLE 3: REDUCING ANTIOXIDANT ACTIVITY OF BETEL LEAF EXTRACT FROM DIFFERENT REGIONS

Reducing Antioxidant				
Extract	**25%**	**50%**	**100%**	**P value**
Standard			95.37 ± 0.55	
B1	73.47 ± 4.33	77.66 ± 2.51	94.03 ± 0.05	.004
B2	73.70 ± 3.01	75.66 ± 3.56	92.80 ± 0.34	.001
B3	61.46 ± 3.45	67.33 ± 4.03	87.37 ± 2.12	.003
B4	59.80 ± 2.57	64.00 ± 3.00	76.10 ± 5.56	.027

Here in our study, we could find good ferric reducing activity by different extracts at 100 µg with the percentages being 94.0%, 92.8%, 87.3%, and 76.1% from both the domains, which could be correlated to that of the standard used at the highest concentration. At a concentration lower than that of 100 µg all the four samples showed less activity compared to the standard hence showing the dose-dependent reducing power of the extracts.

Anti-inflammatory Assay: Under anti-inflammatory assays, three main experiments were carried out, and they are: Human RBC Membrane stabilization Method (heat-induced hemolysis and hypotonicity induced hemolysis) Albumin protein denaturation inhibition Assay, and Protease denaturation assay.

Human RBC (HRBC) Membrane Stabilization Assay: Inflammation is one of the major causes of many existing and emerging disorders. It plays a role that is often linked with oxidative stress. Hence most of the research is done by focusing on ameliorating inflammation by using phyto-chemicals, and the commonly used preliminary *in-vitro* anti-inflammatory assay is the human RBC membrane stabilization assay. During inflammation, the lysosome releases the lysosomal enzymes into the cytosol, thereby triggering the level of inflammation in the surrounding tissues. Hence most anti-inflammatory drugs work by blocking the release of the lysosomal enzymes or by maintaining the stability of the lysosomal membrane.

But as human red blood cell mimics the lysosomal membrane, this particular study was carried out to check the ability of the phytochemicals present in the extract to maintain the stability of the membrane by two methods, *i.e.*, heat-induced and hypotonicity induced hemolysis assay [34].

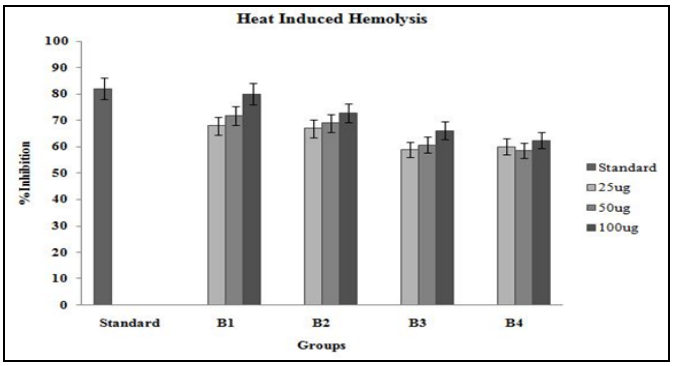

FIG. 5: HEAT INDCED HEMOLYSIS ACTIVITY OF BETEL LEAF EXTRACT FROM DIFFERENT REGIONS

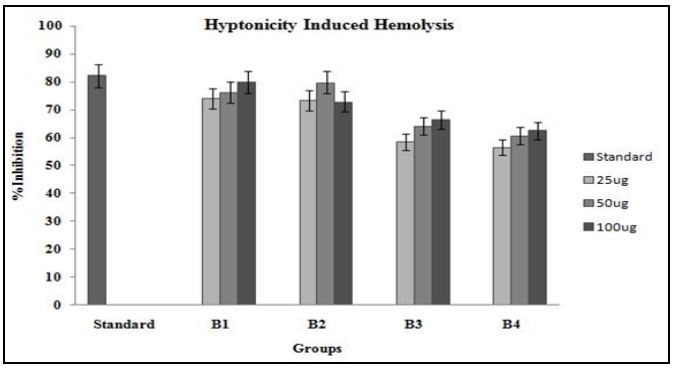

FIG. 6: HYPOTONICITY - INDUCED HEMOLYSIS ACTIVITY OF BETEL LEAF EXTRACT FROM DIFFERENT REGIONS

TABLE 4: HEAT-INDUCED HEMOLYSIS ACTIVITY OF BETEL LEAF EXTRACT FROM DIFFERENT REGIONS

Heat-Induced Haemolysis				
Extract	**25%**	**50%**	**100%**	**P value**
Standard			82.20 ± 0.20	.
B1	68.07 ± 3.10	71.77 ± 3.08	79.97 ± 0.45	.003
B2	67.17 ± 3.01	69.00 ± 2.40	72.83 ± 1.04	.054
B3	59.23 ± 2.04	60.67 ± 2.53	66.27 ± 0.46	.008
B4	56.70 ± 6.45	58.67 ± 2.61	62.47 ± 0.50	.206

TABLE 5: HYPOTONICITY - INDUCED HEMOLYSIS ACTIVITIES OF BETEL LEAF EXTRACT FROM DIFFERENT REGIONS

Hypotonicity - Induced Haemolysis				
Extract	**25%**	**50%**	**100%**	**P value**
Standard			82.20 ± 0.20	
B1	74.00 ± 7.9	76.07 ± 2.90	79.97 ± 0.45	.374
B2	73.2 ± 3.41	79.67 ± 3.50	72.83 ± 1.04	.024
B3	58.43 ± 2.41	64.17 ± 4.05	66.27 ± 0.46	.031
B4	56.43 ± 2.41	60.7 ± 3.045	62.47 ± 0.50	.042

As a result, we could find that at the highest concentration of 100 µg, the extract from the Western Ghats protects the stability of the membrane in both heats induced and hypotonicity induced hemolysis at a percentage of 79% and 72% when compared to the extracts from the northern domain of Karnataka which showed a lesser percentage of protection in all three doses (25 µg, 50 µg, 100 µg) in contrast to a standard which showed 82.2 % of protection of membrane stability in its highest concentration of 100 µg.

Albumin Protein Denaturation: Protein denaturation is a well-observed process in inflammation. Denaturation can happen due to several factors that include external stress such as heat, strong acid or solvent, and some organic salts. It is a process by which the protein loses its secondary and tertiary structure resulting in damage to surrounding tissues and thereby affecting the biological function.

Thus, albumin denaturation is considered as one of the screening assays for the anti-inflammatory activity of phytochemical groups present in the extract [35]. Hence, based on the study, there is again a higher percentage of inhibition of protein denaturation as the concentration increases.

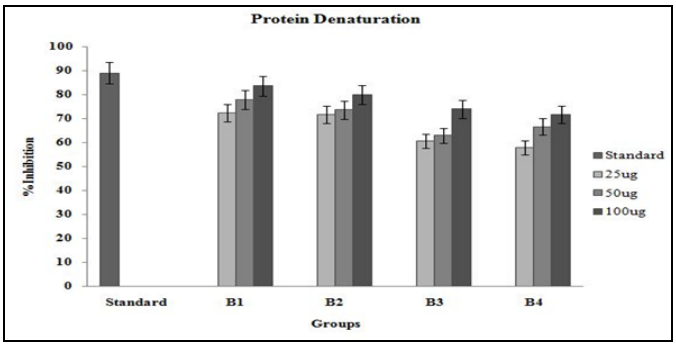

FIG. 7: PROTEIN DENATURATION ACTIVITY OF BETEL LEAF EXTRACT FROM DIFFERENT REGIONS

TABLE 6: PROTEIN DENATURATION ACTIVITY OF BETEL LEAF EXTRACT FROM DIFFERENT REGIONS

Protein Denaturation				
Extract	**25%**	**50%**	**100%**	**P value**
Standard			89.07 ±2.10	
B1	72.33 ± 3.78	78.07 ± .2.0	83.67 ± 2.51	.006
B2	71.67 ± 3.05	73.67 ± 2.51	80.03 ± 3.05	.028
B3	60.67 ± 3.05	63.17 ± 2.84	74.13 ± 3.20	.003
B4	58.03 ± 3.00	66.67 ± 2.51	71.67 ± 2.51	.002

Among the three different doses of extracts tested, the percentage of inhibition of albumin denaturation is found to be 83.6 % and 80% in a concentration of 100 µg in samples collected from different zones of Western Ghats. Whereas, the samples from the northern domain did not show significant inhibition of denaturation in all three doses when evaluated with the standard, which showed 89% of inhibition of albumin denaturation at a single highest concentration. Therefore, a dose-dependent activity can be observed from the above results

Proteinase Inhibitory Assay: Proteinase is an enzyme that is found abundantly in the neutrophils called serine proteases. The lysosomes of these neutrophils are said to release these enzymes into the cytosol, which then exaggerates the intensity of the inflammation by damaging the tissues [31, 36]. Hence, a good proteinase inhibitor can act as a potent anti-inflammatory lead molecule. In connection to this, our studies showed that the extracts from both the Western Ghats and northern region are actively showing antiproteinase activity provided the concentration is higher *i.e.*, 100 µg. The percentage of inhibition of all four extracts is 93.5%, 90.55%, 86.33% and 83.2% respectively. These values can be correlated to the value of the standard, which shows 96.33% of antiproteinase activity at 100 µg.

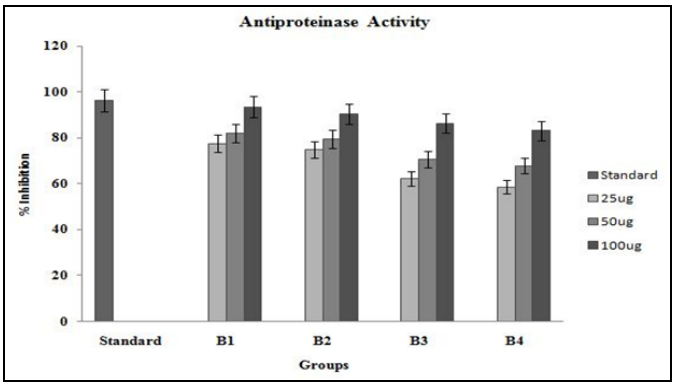

FIG. 8: ANTI-PROTEINASE ACTIVITY OF BETEL LEAF EXTRACT FROM DIFFERENT REGIONS

TABLE 7: ANTI-PROTEINASE ACTIVITY OF BETEL LEAF EXTRACT FROM DIFFERENT REGIONS

Antiproteinase Activity				
Extract	**25%**	**50%**	**100%**	**P value**
Standard			96.33 ± 0.41	.000
B1	77.67 ± 2.51	82.03 ± 3.01	93.5 ± 0.50	.000
B2	74.90 ± 2.66	79.43 ± 2.55	90.50 ± 0.50	.000
B3	62.30 ± 3.05	70.80 ± 3.07	86.33 ± 0.41	.000
B4	58.53 ± 2.40	67.90 ± .36	83.20 ± 0.26	.000

Phytochemical Analysis: As the extracts from different domains showed varying antioxidant and anti-inflammatory activity, we wanted to further screen the phytochemical group as we hypothesize that the phytochemical groups might be the reason for the biological activity and also proceed with the quantitative analysis of the groups. Hence, being on par with the analysis, the ethanol extract of all 4 domains showed the presence of major phytochemical groups such as flavonoids, alkaloids, phenols, and tannins [37]. Followed by qualitative analysis as mentioned above, the quantitative analysis was also done for flavonoids and phenols by considering that the anti-inflammatory and antioxidant activity might be due to the presence of major groups in the extracts [38].

TABLE 8: PRELIMINARY QUALITATIVE PHYTOCHEMICAL ANALYSIS OF ETHANOL EXTRACT OF BETEL LEAF

Phytochemicals	B1	B2	B3	B4
Alkaloids	+	+	+	+
Phenols	+	+	+	+
Flavonoids	+	+	+	+
Glycosides	+	+	+	+
Terpenoids	+	+	+	+
Tannins	+	+	+	+

(+ = Present and - = absence of phytoconstituents)

TABLE 9: QUANTITATIVE ANALYSIS OF MAJOR PHYTOCHEMICAL GROUPS

Samples	Total Phenol (ug/mg)	Total Flavonol (ug/mg)
B1	512.00 ± 1.15	233.00 ± 1.52
B2	489.00 ± 2.08	252.60 ± 1.33
B3	442.33 ± 1.45	295.00 ± 2.88
B4	452.33 ± 1.85	273.00 ± 1.52

As mentioned in **Table 1** the phenolic content in the extracts from the western region is higher with a value of 512 µg/mg and 489 µg/ mg than the samples from the northern domain, which has a lower quantity of 442 µg/mg and 452 µg/mg of phenolic. But, the total flavonoid content is more in the sample obtained from the northern region with a quantity of 295 µg/mg and 273 µg/ml when compared to that of the western regions.

The high content of phenols can be correlated to the increased antioxidant activity as they can block the release of free oxygen radicals formed from the substrate by donating hydrogen atoms or electrons [39]. Both phenols and flavonoids have OH- groups that are directly linked to carbon atoms of benzene ring as a result of which they can be held responsible for two major bioactivities that is antioxidant and anti-inflammatory [40].

CONCLUSION: The antioxidant and anti-inflammatory activity of extracts collected from four different domains of Karnataka are highly dependent on the presence of different phyto-chemical groups. Also, their varying biological activity and the presence of phytochemical groups can be due to the environmental condition and soil composition, because of which there is a lower activity in the extracts collected from the dry areas of Karnataka *i.e.* Chitradurga and Hubbali. Overall from the above results, it can be concluded that betel leaves from the region that are rich with minerals and moisture can act as a good source of anti-inflammatory and antioxidant agents with abundant phytochemical groups that results in increased biological activity and thus being a prominent source for the synthesis of sustainable drugs.

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REFERENCES:

1. Matschke, Veronika, Carsten Theiss and Johann Matschke: "Oxidative stress: The lowest common denominator of multiple diseases. Neural Regeneration Research 2019; 14(2): 238.
2. Sharifi-Rad and Mehdi: Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases." Frontiers in Physiology 2020; 11: 694.
3. Mitra and Krishna A: Antioxidants: A Masterpiece of Mother Nature to Prevent Illness." Journal of Chemical Reviews 2020; 243-56.

4. Calixto, João B: The role of natural products in modern drug discovery." Anais da Academia Brasileira de Ciências 2019; 91.
5. Di Sotto, Antonella, Vitalone A and Giacomo SD: "Plant-Derived Nutraceuticals and Immune System Modulation: An Evidence-Based Overview." *Vaccines* 2020; 8(3): 468.
6. Madhumita M, Guha P and Nag A: Bio-actives of betel leaf (*Piper betle* L.): A comprehensive review on extraction, isolation, characterization, and biological activity. Phytotherapy Research 2020; 34(10): 2609-27.
7. Bajpai V, Kumar N and Kumar B: Phytochemistry of Piper betle Landraces. CRC Press; 2020 Jun 8.
8. Guha, Proshanta and Nandi S: Essential oil of Betel leaf (*Piper betle* L.): A novel addition to the world food sector." Essential Oil Resear Springer Cham 2019; 149-96.
9. Vikash C, Shalini T, Verma NK, Singh D, Chaudhary SK and Asha R: *Piper betel* Phytochemistry, traditional use & pharmacological activity a review. International Journal of Pharmaceutical Research and Develop 2012; 4(4): 216-23.
10. Sengupta R and Banik JK: A review on betel leaf (pan). International Journal of Pharmaceutical Sciences and Research 2013; 4(12): 4519.
11. Shah SK, Garg G, Jhade D and Patel N: *Piper betle* phytochemical, pharmacological and nutritional value in health management. Int J Pharm Sci Rev Res 2016; 38: 181-89.
12. Chauhan ES, Aishwarya J, Singh A and Tiwari A: A review-Nutraceuticals properties of *Piper betel* (Paan). Am J Phytomed Clin Ther 2016; 4(2): 28-41.
13. Sarma C, Rasane P, Kaur S, Singh J, Singh J, Gat Y, and Dhawan K: Antioxidant and antimicrobial potential of selected varieties of *Piper betle* L.(Betel leaf). Anais da Academia Brasileira de Ciências 2018; 90(4): 3871-78.
14. Rekha VPB, Kollipara M, Gupta BRSS, Bharath Y, and Pulicherla KK: A review on *Piper betle* L: Nature's promising medicinal reservoir. American Journal of Ethnomedicine 2014; 1(5): 276-89.
15. Kumar KA, Chandrashekar KR, Bhagya N and Vivek MR: *Ex-situ* conservation of threatened plant species of the Western Ghats. Zoo's Print 2020; 35(1): 9-15.
16. Akar Z, Küçük M and Doğan H: A new colorimetric DPPH• scavenging activity method with no need for a spectrophotometer applied on synthetic and natural antioxidants and medicinal herbs. Journal of Enzyme Inhibition and Medicinal Chemistry 2020; 32(1): 640-47.
17. Ruberto G, Baratta MT, Deans SG and Dorman HD: Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum maritimum* essential oils. Plantamedica 2020; 66(08): 687-93.
18. Blanche EO, Valère KT, Judith MM and Rosalie NN: Antiradical Activity and Ferric Reducing Antioxidant Power of *Pleurotus pulmonarius*, *Pleurotus floridanus* and Pleurotussajor-caju Formulations Extracts *in-vitro*. Food and Nutrition Sciences 2020; 10(10): 1202.
19. Thirumagal K and Geetha RV: *In-vitro* anti-inflammatory activity of *Plumeria acuminata* using membrane stabilization assay. Drug Invention Today 2019; 11(8).
20. Puttaswamy N, Malojiao VH, Mohammed YHE, Sherapura A, Prabhakar BT and Khanum SA: Synthesis and amelioration of inflammatory paw edema by novel benzophenone appended oxadiazole derivatives by exhibiting cyclooxygenase-2 antagonist activity. Biomedicine & Pharmacotherapy 2018; 103: 1446-55.
21. Benedek B, Kopp B and Melzig MF: *Achilleamille folium* L. sl–Is the anti-inflammatory activity mediated by protease inhibition. Journal of Ethnopharmacology 2007; 113(2): 312-17.
22. Pandey AK, Kashyap PP and Kaur CD: Anti-inflammatory activity of novel Schiff bases by in vitro models. Bangladesh Journal of Pharmacology 2017; 12(1): 41-43.
23. Yadav RNS and Agarwala M: Phytochemical analysis of some medicinal plants. Journal of Phytology 2011; 3(12).
24. Chang MY, Lin YY, Chang YC, Huang WY, Lin WS, Chen CY and Lin YS: Effects of infusion and storage on antioxidant activity and total phenolic content of black tea. Applied Sciences 2020; 10(8): 2685.
25. Ahmad M, Mohammad N, Aziz MA, Alam MA, Hossain MS, Islam MR and Uddin MG: Comparison of antioxidant role of methanol, acetone and water extracts of *Andrographis paniculata* Nees. Journal of Medicinal Plants Research 2020; *14*(8): 428-37.
26. Paranjpe R, Gundala SR, Lakshminarayana N, Sagwal A, Asif G, Pandey A and Aneja R: Piper betel leaf extract: anticancer benefits and bio-guided fractionation to identify active principles for prostate cancer management. Carcinogenesis 2013; 34(7): 1558-66.
27. Sarma C, Kumari S, Rasane P, Singh J, Amin R, Choudhury N and Kaur S: Exploringnutraceutical potential of selected varieties of *Piper betle* L. Carpathian Journal of Food Science & Technology 2018; 10(1).
28. Muruganantham N, Solomon S and Senthamilselvi MM: Anti-oxidant and anti-inflammatory activity of *Cucumis sativas* (cucumber) flowers. Int J Pharm Sci Res 2016; 7(4): 1740-45.
29. Abu F, Taib M, Norma C, Moklas M, Aris M and Akhir MS: Antioxidant properties of crude extract, partition extract, and fermented medium of dendrobiumsabin flower. Evidence-Based Complementary and Alternative Medicine 2017.
30. Rasoanaivo P, Wright CW, Willcox ML and Gilbert B: Whole plant extracts versus single compounds for the treatment of malaria: synergy and positive interactions. Malaria Journal 2011; 10(1): 4.
31. Kambizi L, Bakare-Odunola MT, Oladiji AT, Kola-Mustapha AT, Amusa TO, Atolani O and Quadri AL: Proteinease inhibition, membrane stabilization, antioxidant and phytochemical evaluations of leaves, seeds and calyces of four selected edible medicinal plants. Cogent Chemistry 2017; 3(1): 1314064.
32. Murdifin M, Pakki E, Alam G, Manggau MA, Muslimin L, Rusdi M and Wahyudin E: Lipid Peroxidation Inhibitory Activity *In-vitro* of *Mezzetia parviflora* Becc. Wood Bark Polar extract. Pharmacognosy Journal 2017; 9(2).
33. Adebiyi OE, Olayemi FO, Ning-Hua T and Guang-Zhi Z: *In-vitro* antioxidant activity, total phenolic and flavonoid contents of ethanol extract of stem and leaf of *Grewiacar pinifolia*. Beni-Suef University Journal of Basic and Applied Sciences 2017; 6(1): 10-14.
34. Kardile MV, Mahajan UB, Shaikh HM, Goyal SN and Patil CR: Membrane stabilization assay for anti-inflammatory activity yields false positive results for samples containing traces of ethanol and methanol. World J of Pharmacy and Pharmaceutical Sci 2016; 5: 493-97.
35. Sangeetha G and Vidhya R: *In-vitro* anti-inflammatory activity of different parts of *Pedalium murex* (L.). inflammation 2016; 4: 5.
36. Silvestre-Roig C, Braster Q, Ortega-Gomez A and Soehnlein O: Neutrophils as regulators of cardiovascular inflammation. Nature Reviews Card 2020; 17(6): 327-40.
37. Sweatha R, Vinitha V, Thilagavathy D, Yuvasri L, Subhashini S and Sakthivel R: A study on phytochemical analysis and antibacterial activity of *Piper betel* Varieties (Kamar and Kumbakonam Vetrilai). Eureka 2019; 2582: 1571.

38. Uddin MF, Uddin SA, Hossain MD and Manchur MA: Antioxidant, cytotoxic and phytochemical properties of the ethanol extract of Piper betle leaf. International Journal of Pharmaceutical Sciences and Research 2015; 6(10): 4252.
39. Johri S, Khan N and Khan N: Phytochemical Screening, Biochemical Estimations and Spectroscopic analysis of Various Extracts of Piper betel Leaves. Int J Pharma Res Health Sci 2017; 5(6): 1939-44.
40. Kaurinovic B and Vastag D: Flavonoids and phenolic acids as potential natural antioxidants. InAntioxidants 2019 Feb 1 (pp. 1-20). London, UK: IntechOpen.

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