ISSN 2581-6217

World Journal of Pharmaceutical Science & Technology

Journal homepage: www.wjpst.com

Review Article

CLINICAL DATA MANAGEMENT SYSTEMS, SOFTWARE USE, ARTIFICIAL INTELLIGENCE(AI) AND RECENT UPDATES

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Received: 15-7-2022, Revised: 29-8-2022, Accepted: 10-9-2022

ABSTRACT:

Piper betel leaves were evaluated for its antiasthmatic activity, commonly known as pan. It is having long history to use in India as it has multiple therapeutic activities like antibacterial, lymphangitis, asthma, treating eczema and rheumatism. So selected, the plant *P. betel* is effective in antiasthmatic activity and antioxidant activity, but antihistaminic activity of *P. betel* is still not scientifically investigated. In the present study, the pharmacological evaluation of ethanolic extract and essential oil extract of leaves of *P. betel* has been done for their antiasthmatic and antioxidant activity on goat trachea.

Moreover, extracts of *P. betel* disturbed histamine aerosol induce bronchoconstriction in whole guinea pig, where essential oil was more effective comparatively to ethanolic extract. Thus, from the results obtained in the present investigation, it can be concluded that ethanolic extract and essential oil of *P. betel* possess antiasthmatic and antioxidant activity.

INTRODUCTION:

Piper betel Linn. was an edible plant with leaves that have been traditionally used in India, China and Thailand for prevention of oral malodour, since it has anti-bacterial activity against obligate oral anaerobes responsible for halitosis. Aqueous extracts of *Piper betel* have also been shown to reduce the adherence of

early dental plaque bacteria[1].*Piper betel* is a member of the Piperaceae family and its leaves have a strong pungent and aromatic flavour [2]. As well as use as a mouth freshener, the leaves are used for wound healing[3] and digestive and pancreatic lipase stimulant activities in traditional medicine[4](Prabhu et al., 1995). Antioxidant, anti-bacterial and anti-fungal[5].anti-inflammatory, anti-diabetic and radioprotective[6].activities of *Piper betel* have also been reported. In a clinical trial, *Piper betel* ointment cured and improved ringworm skin lesions at rates of 40 and 26%, respectively. *Piper betel* gel inhibited growth of dermatophytes that cause ringworm and growth of Candida species. More effectively than tolnaftate and with a similar inhibitory effect to that of clotrimazole[7]

Histamine was one of the important mediators of bronchoconstriction and inflammation[8].In the living organism, histamine [4-(2-aminoethyl) imidazole] is synthesized from the naturally occurring-amino acid, histidine, by the loss of a carboxyl group through bacterial or enzymatic decarboxylation[9].Histamine was released from degranulated mast cells. Targeting histamine, either prevention of its release from mast cells or use of histaminergic receptors antagonists becomes part of antihistaminic therapy[10]. In human body histamine was present in various biological fluids, in platelets, leucocytes, basophiles, and mast cells [11]. Major portion of histamine was stored in mast cells and circulatory basophiles[12]. Histamine also acts as a neurotransmitter participating in many cell physiological processes such as allergic reaction, inflammation, gastric acid secretion, central and peripheral neurotransmission[13].

Piper betel Linn, colloquially known as betel vine, is one of the most important plants in Southeast Asia. It is regarded as a propitious plant by the Indians, Nepali, Singhalese, Thai, and Vietnamese people and is used during auspicious functions, festivals, ceremonies, and sacred rituals[14]. The leaves, which are the most commonly used plant part, are pungent and possess aromatic flavour. They are widely consumed as a mouth freshener, either alone or in combination with the betel nut (areca nut *Areca catechu*), cloves, fennel, and so on. Betel leaf is also used along with tobacco leaves (*Nicotiana tabacum*) and habitual chewing of this betel quid has been conclusively proved to cause and enhance the risk of oral cancer[14]. However, unlike the prevailing belief, betel leaf by itself has no adverse health effects and, on the contrary, possesses myriad medicinal benefits.

Commercial Application of Piper Betel Leaves:

There are some available commercial products containing betel leaves such as dietary supplements, mouthwash, medicinal products, and cosmetic and personal care goods including shampoo, soap, face cream, antiseptic lotions, toothpaste, and perfumes [13]. Current antimicrobial studies of betel leaves were focusing on oral pathogens, MDR Gram-negative and Gram-positive bacteria, and dermatophytes[16]. Thus, future development of medicinal products from betel leaves could be useful for preventing oral diseases, curing dermatophyte infections, and for the treatment and management of other infectious diseases. Additionally, a study has developed a simple, safe, cost-effective, and eco-friendly preparation of silver nanoparticles with polyaniline coating using water extract of betel leaves. The nanoparticles showed

potential antibacterial properties and could be further studied in various applications such as medical devices and pharmaceutical and biomedical industries[17].

In the food industry, essential oil is a promising food additive to protect and enhance the shelf life of products during processing and storage. BLEO is an ideal food preservative agent due to its antifungal and antioxidant properties[18]. Many experiments have investigated the antimicrobial properties of BLEO against foodborne pathogens[18]. Moreover, BLEO is not only beneficial to prevent spoilage of food products but also guarantees their safety for consumer health especially due to the ability of BLEO to suppress aflatoxin production. Aflatoxin, a mycotoxin from *A. flavus*, is an example of fungal contamination in food products. The toxin is known to be hepatocarcinogenic, teratogenic, mutagenic, and immunosuppressive. An investigation revealed that BLEO in apple juice could deactivate spores or inhibit spore germination which is required to limit fungal infection and mycotoxin production[19]. Further research on the overall acceptability of sensory aspects of the essential oil-treated foodstuffs is necessary to avoid market failure of the product [20].

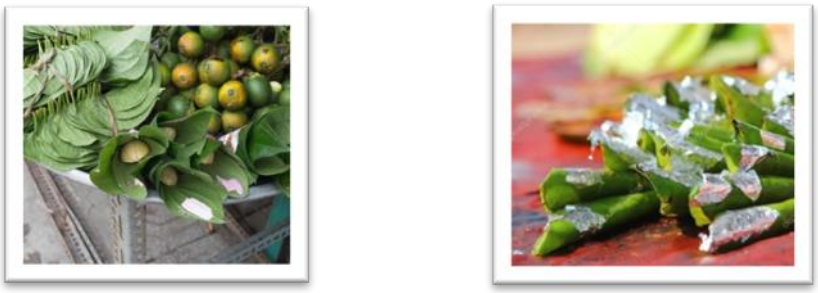

Fig 1. Piper Betel Leaves

Need and rationale

- To evaluate the antiasthmatic activity of Piper betel leaf
- To evaluate the anti-oxidant activity of Piper betel leaf
- To know the activities, present in the Piper betel leaf

Aim

- To evaluate the Antiasthmatic and Antioxidant properties present in the piper betel leaf

Objectives

- Evaluation of the properties present in the Piper betel leaf
- Knowing the effects of Piper betel leaf

Plan of work:

1. Literature survey
2. Selection of plant, methods and other materials
3. Procurement of the plant and other materials
4. Phytochemical studies
5. Study of activities
6. Methodology

MATERIALS AND METHODS:

Procurement of plant materials the leaves of *Piper betel* Linn. were collected from local habitat Pimple Gurav, Dist.-Pune, (Maharashtra) India.

DRYING AND SIZE REDUCTION:

After identification and authentication, leaves of *Piper betel* Linn. were subjected to drying under shade and then subjected for size reduction to coarse powder by pulverization. The powdered drug was stored in a tightly packed polythene bag.

Fig 2. Drying

Fig No 3.Grinding

EXPERIMENTAL WORK:

METHODOLOGY:

Extraction Of Drug:

1. Powdered leaves were charged into Soxhlet apparatus and exhaustive extraction was carried out using ethanol as solvent. The powdered drug was extracted with solvent until complete extraction was affected.
2. Extract was concentrated by distilling the excess of solvent to obtain the crude extractive. *Piper betel* leaves were cut into small pieces.
3. The pieces of leaves were placed in a round bottom flask with distilled water and hydro distillation was carried out for 4 h.

4. We added the leaves to this solution and kept it for about 7 days to get the extract. Then we filtered the solution with the help of filter paper and we obtained our extract.

Fig No.4 Extraction Process

Acute toxicity studies according to O.E.C.D. guideline:

As dose was increased further up to 400 mg/kg, total mortality was found. Hence 200 mg/kg dose was considered as effective dose. From these doses are selected as 100 mg and 200 mg in histamine induced bronchoconstriction.

Calcium channel antagonist activity of the constituents of *Piper betel* has been reported, from this the dose for isolated goat tracheal chain preparation and isolated goat ileum preparation was selected as 100 mg.

MATERIALS AND METHODS:

Evaluation of anti-asthmatic activity using isolated Guinea pig ileum preparation:

The Guinea pigs (overnight fasted) were sacrificed and the ileum was mounted in an organ bath containing Tyrode solution which was continuously aerated at 3740.5°C. Bioassay of histamine 10 ug mL in plain Tyrode solution and in Tyrode solution containing 100 ug mL Piper longum Limm. extracts were performed. Percentage maximum contractile response was plotted to generate dose response curve of histamine, in the absence and presence of the plant extract (Pandit et al. 2008).

RESULTS

Evaluation of anti-asthmatic activity using isolated Guinea pig ileum preparation:

In the present study pet.ether, ale, and decoction extracts of fruits of Piper longum (100 µg mL) significantly (p-0.01) inhibited the histamine induced contraction of isolated Guinea-pig ileum preparation indicating its H, receptor antagonistic activity and supports the anti-asthmatic properties of the plant. There was decrease in % response in the presence PF, AF and DF at dose of 100 ug mL of Piper longum when compared to histamine (10 µg ml.) alone. The observations are given in Table 1.

Table 1: Effect of various extracts of *Piper longum* Linn. on isolated Guinea pig ileum preparation

Groups	Drug dose	Response (%)					
		0.1	0.2	0.4	0.8	1.6	3.2
		(mL)					
Control	Histamine (10 µg mL^{-1})	22.50±0.058	40.0±0.088	85.00±0.116	90.00±0.089	97.5±0.058	100.00±0.08
Standard	Histamine+CPM (10+10 µg mL^{-1})	1.00±0.003**	0.90±0.007**	0.83±0.009**	0.83±0.009**	4.7±0.011**	7.25±0.007**
PF	Histamine+PF (10+100 µg mL^{-1})	1.00±0.007**	1.00±0.006**	7.30±0.019**	9.75±0.009**	12.5±0.058**	22.50±0.058**
AF	Histamine+AF (10+100 µg mL^{-1})	9.25±0.027**	2.25±0.007**	5.00±0.012**	11.25±0.029**	30.0±0.029**	40±00.044**
DF	Histamine+DF (10+100 µg mL^{-1})	4.75±0.009**	16.30±0.005**	22.50±0.058**	30.75±0.089**	33.0±0.041**	40.70±0.033**

Values are in Mean±SEM, One-way ANOVA followed by Dunnett's t-test, Where* p<0.05 and **p<0.01 as compared to control, n = 5

Table No.1 : Evaluation of anti-asthmatic activity using isolated Guinea pig ileum preparation

PHYTOCHEMICAL ANALYSIS :

1) Test for Alkaloids :

To the extract added 1% HCl and 6 drops of Mayer's reagent and Dragendroff's reagent. An organic precipitate indicated the presence of alkaloids in the sample 24.

2) **Test for Flavonoids** :

5 ml of dilute ammonia solution were added to a portion of aqueous filtrate of each plant extract followed by addition of conc. H2SO4. A yellow coloration is observed which confirms the presence of flavonoids and it disappears on standing 25.

3) **Test of glycosides** :

Dissolve small amount of an alcoholic extract of the fresh or dried material in one ml of water.

Add a few drops of aqueous NaOH solution. Yellow color indicates the presence of glycoside 25.

4) Test for Steroids :

2 ml of acetic anhydride was added to 0.5gm of ethanolic extract of each sample with 2 ml of H_2SO_4. The color change from violet to blue or green indicated the presence of steroids 26.

5) Test for Tannins :

5 ml of extract was added to few drops of 1% lead acetate. A yellow precipitate indicated the presence of tannins 26.

6) Test for Terpenoids :

5 ml of each extract was added to 2 ml of chloroform and 3 ml of conc. H_2SO_4 to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the terpenoids 25.

7**) Test for Saponins** :

The extract with 20 ml of distilled water was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam indicated the presence of saponins 24.

Sr. No.	Phytochemical		Results				
			Aqueous extract	Ethanolic extract	Methanolic extract	Butanolic extract	Acetone extract
1	Steroids		+++	+	++	+++	+
2	Diterpenes: Copper acetate test		++	+	+	+++	+
3	Phlobatannins		-	-	-	-	-
4	Tannin:	Lead acetate test	+++	+	++	-	++
		$FeCl_3$	-	+	++	+	++
5	Cardial Glycosides: Keller-Killani test		+	-	-	-	-
6	Flavonoid:	Alkaline Reagent Test	++	+	-	+	++
		NH_4OH	++	+	-	+	-
		Mg turning test	-	-	-	-	-
		Zn dust test	+	-	-	-	-
7	Anthocyanin		-	-	-	-	-
8	Phytosterol: Salkowski's test		-	-	-	-	-
9	Alkaloids	Wagner's reagent	+	-	-	++	-
		Hager's reagent	++	-	-	+++	-
10	Phenols: $FeCl_3$ test		+	-	-	-	-
11	Emodins		-	-	-	+	-
12	Coumarin		+++	+	-	-	++
13	Leucoanthocyanin		-	-	-	-	-
14	Saponin: Foam test		+++	+	+	-	+

Key: (+) Positive test, (-) Negative test, '+' low; '++' moderate; '+++' high

Table No.2 : Phytochemical Tests

Test Results

Result Of Alkaloids

Result Of Flavonoids

Result Of Glycosides

Result of Steroids

Fig No 5. Tests for present constituents

ANTIOXIDANT STUDY

1. Introduction

Antioxidants is any substance that directly scavenges Reactive Oxygen Species (ROS) or indirectly acts to up-regulate antioxidant defences or inhibit ROS production[17].It protects human being from deadly diseases such as cardiovascular disease, cancerous disease[18],neurodegenerative disease, Parkinsons and Alzimers disease [19]. Antioxidant supplements or antioxidant-rich food can help in reducing the oxidative damage from free radicals and active oxygen species[20]. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and trolox are widely used as antioxidants in the pharmaceutical and food industry. However, they have been showing toxic or mutagenic effects[21]. Due to the toxicity of synthetic antioxidant, emphasis is given on development and isolation of natural antioxidants (polyphenols, tannins and saponins) from plant species[22]. Out of these, phenolics are the major contributor of antioxidant activity in plant extracts due to their higher value in total content[23], synergistic effectiveness as hydrogen donating capacity, reducing agent and free radical scavengers. Wong et al., 2006, have optimized the extraction conditions for the maximum recovery of total phenolics which is an important aspect in the field of natural antioxidant for preservation of food. Piper betel L. is the perennial dioecious vine which belongs to Piperaceae family. It is originated from Malaysia and cultivated in tropical and subtropical parts of India. Due to ethno-medicinal properties, the plant is widely used in south-east Asian countries.

Piper betel is the most favourite herb used for chewing purpose according to native people. Traditionally piper betel has socio-economic importance and gained valuable attention at ceremonial events. In Ayurveda (Indian medicinal system) betel leaf is known by its Vedic name *Saptasira* and used as adjuvant with different medicines for voice, purifying blood, laxative and appetizer. The essential oil contains high safrol along with eugenol, allyldiacetoxybenzene and chavibitol acetate as major constituents in Sri Lankan betel variety leaves[24]. Antioxidant action of betel leaf is very high, due to the presence of phenolic compound hydroxy-chavicol (4allyl pyrocatechol) which have been proved to be preservative for vegetable oils up to the concentration of 0.03% without imparting their taste and odour [25]. Use of betel leaf extract as an antioxidant in butter cake retarded its oxidation and extends its shelf life. It was found to be a better source of antioxidant as compared to BHT and BHA [26]. Hydroxy-chavicol shows anti-carcinogenic activity[27].The biological activity of major bioactive constituents such as allylpyrocatechol, hydroxychavicol, chavibetol of betel leaf were described[28]. In the present study six variety of betel leaves were collected from different regions of India and extracted with five different solvents. Selection of solvents for extraction was made on the basis of good extraction efficacy for plant phenolics[29].

Extraction efficacies of phenolics are mainly affected by time, temperature, solubility, sample to solvent ratio, physical state of the sample and polarity of solvents[30]. Extraction of betel leaf was done by keeping all the above parameters constant and changing polarity of solvents. To our knowledge enormous study has

been carried out on the antioxidant activity of different betel leaves variety[31]. However, there is no literature available on the photochemiluminescence study of betel leaf. The present research is focused on two objectives (1) To investigate the photochemiluminescence activity of betel leaf extract of each variety and its comparison with other antioxidant evaluation methodology namely (DPPH), (ABTS) and (FRAP). (2) To determine the effect of solvents in extraction of polyphenols and antioxidants as well as study the correlation between different antioxidant activity assays with total phenol content.

2. MATERIALS AND METHOD

2.1 Chemicals

Standard compounds for the determination of radical scavenging activity viz., DPPH (2, 2-diphenyl1-picrylhydrazyl), ABTS+ (2, 2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid), gallic acid and catechin were purchased from Sigma-Aldrich Fine Chemicals (St. Lous. MO). TPTZ (2,4,6-tripyridyl-striazine) was procured from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India), FolinCiocalteu reagent, methanol, ethanol, ethyl acetate, acetone, acetic acid, hydrochloric acid were obtained from Merck (Darmstadt, Germany). PHOTOCHEM antioxidant (ACW) kit used was obtained from Analytikjena (Konrad-Zuse-Strasse 1, Germany). Potassium per sulphate, acetate buffer, anhydrous sodium acetate, hydrochloric acid, ferric chloride, ferrous sulphate was purchased from Central Drug House (Pvt.) Ltd. (New Delhi, India).

2.2 Plant Materials and Extractions

Based on the shape, colour, taste and aroma, many verities of betel leaves are found in India. The leaves were stored at -40oC for 12 hours, lyophilized in freeze dryer (Labconco, Kansas City, USA) and stored in dark at 4oC before extraction. One gram freeze dried powder of each betel leaf variety was dissolved in 25 mL of solvent (80% methanol, 80% ethanol, 80% acetone, 80% ethyl acetate and distilled water) and extractions were carried out in shaking incubator (Lab Tech, LSI-2005RL, Hyderabad, India) for 2 hours. The extracts were filtered through muslin cloth and centrifuged at 4000 rpm for 15 min. The supernatant was filtered through 0.45µm Nylon-66 membrane syringe filters (mdi Membrane Technologies LLC, California, USA) and stored at 4oC for further analysis.

2.3 Determination of Total Phenolic Contents

Total phenol content (TPC) of different variety of betel leaves was determined according to the Folin-Ciocalteu method with slight modification (Singleton and Rossi, 1965). 200 µL sample of leaf extract was transferred into a test tube containing, 1 mL of freshly diluted (10 fold) Folin-Ciolateu reagent. The mixture was allowed to stand at room temperature. After 8 min, 3 mL of 7.5% (w/v) sodium carbonate was added to the mixture and shaken manually. Then the mixture was incubated at room temperature for 60 min. The absorbance was recorded at 765 nm using UV – visible spectrophotometer (Shimadzu, UV-2600 Kyoto,

Japan). Acidified methanol was used as blank. The calibration curve was plotted against gallic acid and expressed in terms of mg GA equivalents per g dry weight basis (mg GAE/g dw). The linearity of the range for TPC was measured as 0.05 – 0.5 mg GA/mL (R2=0.9929).

2.4 Antioxidant Activity

2.4.1 DPPH Radical Scavenging Activity

The free radical scavenging activity of the leaf extract was measured by using DPPH• radical with some modifications[32]. According to Payetet al.(2005), 100 µL extracts was reacted with 3.9 mL of 0.004% (80% methanol) DPPH solution. The reaction mixture was allowed to incubate in dark for 60 min. Then absorbance was measured against methanol at 515 nm using spectrophotometer (Shimadzu, UV2600 Kyoto, Japan). Catechin was also used as a reference in this assay. A standard curve was obtained using catechin standard solution at concentration 0-1 mg/mL. The absorbance of the extract was compared to that of catechin standard curve and all results were expressed as mg Catechin equivalent per g dry weight basis (mg CE/g dw).

2.4.2 ABTS Radical Scavenging Assay

For the ABTS˙+ assay the method previously described was followed (Re et al., 1999). The working stock solution for ABTS˙+ was prepared by mixing the equal quantities of 7.0 mM ABTS˙+ solution and 2.45 mMpottasiumpersulphate and allowing them to react for 16 hours in dark. At the time of measurement, prepared working solution was diluted with 80% ethanol to the absorbance level of 0.70±0.02 at 734 nm. A 100 µL of sample leaf was allowed to react with adjusted ABTS˙+ working solution for 6 min. Catechin was used as standard and ABTS radical scavenging activity of all the extracts were expressed as mg Catechin equivalent per g dry weight basis (mg CE/g dw).

2.4.3 Ferric Reducing Antioxidant Potential (FRAP) Assay

Ferric reducing antioxidant potential of extract was analysed using the method proposed by Firuzi et al.(2005). FRAP solution was prepared by adding 200 mL of 300 mM acetate buffer (which was adjusted to pH of 3.6 by the addition of acetic acid) to 20 ml of 20 mM ferric chloride hexahydrate (dissolved in distilled water) and 20 mL of 10 mM 2,4,6-tri-(2-pyridyl)-striozine (TPTZ) (dissolved in 40 mMHCl). Ferrous sulphate standard graph was prepared by taking different concentration of ferrous sulphate (0.1-1 mM). 120 µL distilled water/standard/sample was added to 4 mL of the FRAP solution and absorbance was taken at 593 nm after 4 min. Ferrous sulphate equivalent concentration in mM was calculated from the standard graph and expressed as mmol ferrous sulphate equivalent/g on dry weight basis (mmol Fe+2/g dw).

3. RESULTS AND DISCUSSION

3.1 Total Phenol Content

Phenolics (secondary metabolites) of plant origin are the symbol of antioxidant properties which protect the human health from several diseases and have played the vital role in reducing free radicals produced by oxidation reactions[33].It has been noted that chavicol, allylprotocatechol, chavibetol and eugenol are the major pungent component of betel leaf responsible for antioxidant activity (The Wealth of India, 1989). This findings are in relation with previous one where chavibetol, allylprotocatechol were fractionated through column chromatography (Rathee et al., 2006). In earlier study, lesser value of total phenol content in betel leaf was found due to the use of high polar solvent (Maisuthisakul, 2008). The effect of solvent polarity on the extraction yield of total phenolics was elucidated by some researchers (Zhao et al., 2006). In the present study we have tried to extract as much polyphenols from the betel leaves by using five different solvent systems. The results obtained for TPC have been depicted in Table 1. According to the polarity of extraction solvent, a wide variability was observed among the each betel leaf variety. The TPC for six variety betel leaf extract in five different solvents were found in the range of 0.29 to 2.62 mg GAE/g dw for PA, 0.08 to 2.59 mg GAE/g dw for PB, 0.09 to 2.38 mg GAE/g dw for PC, 0.07 to 2.87 mg GAE/g dw for PD, 0.16 to 2.07 mg GAE/g dw for PE and 0.04-1.16 mg GAE/g dw for PF. The observed descending order of contributed extraction solvent for TPC can be arranged as 80% methanol > 80% acetone > 80% ethanol >80% ethyl acetate > water. Highest TPC was observed in 80% methanol extract while lower TPC was observed in water extract. It is reported that, water: methanol (1:1) extract of P.betel, P.betleoides and P.wallichii was found to be the best solvent with significantly higher ($P<0.01$) phenolic content as compared to the other solvent (Rathee et al., 2006). On the other hand lower yield of TPC in aqueous betel leaf extract was also reported (Abrahim et al., 2012). Our finding matches with the reported results. PD variety from West Bengal showed highest TPC among the all varieties. Our findings are in agreement with the previous results where betel variety of West Bengal had more phenol content compared to the Sweet and Mysore variety [34].

3.2 Antioxidant Activity

The highest level of DPPH scavenging activity (Table 2) was observed for 80% ethanol extract of PF (133 mg CE/g dw) variety followed by PE (127.5 mg CE/g dw), while the lowest activity was observed for water extract of PA (2.48 mg CE/g dw) variety. Overall 80% ethanol was found to be the more suitable solvent over 80% methanol. The trend of antioxidant capacity of betel leaf extracts provided by DPPH assay after extraction with 80% ethanol was like PF>PE>PD>PC>PB>PA. However these results appeared to contradict a previous TPC report where 80% methanol exhibited more suitable solvent over 80% ethanol. There was significant difference ($P<0.05$) in the DPPH scavenging activity of different solvent extract for a particular variety except for PA and PC, where the result was not significantly different for solvent 80%

acetone and 80% ethyl acetate. In another study, concentration dependent scavenging of DPPH radical was observed by three varieties of betel (Bangla, Sweet and Mysore)[34].Highest DPPH scavenging activity was observed by the Bangla (IC50 52.43 µg/mL) variety followed by Sweet and Mysore variety. The DPPH study of three betel leaf varieties using seven different solvents shows that there is no significant difference in the activity using the solvent methanol and ethanol. The lowest IC50value obtained for methanol: water (1:1) extract was 36.5,45.5 and 50.4 µg/mL respectively for the P.betle, P.betleoidesand P.wallichiivariety respectively[35] (). ABTS.+ assay is used for both hydrophobic and lipophilic antioxidant system on the other hand DPPH is used in case of lipophilic antioxidant system[36]. Betel leaf extract values for ABTS.+ assay are shown in Table 3. Most of the values shows slightly more ABTS.+ scavenging capacity as compared to the DPPH method when catechin was used as standard in both the cases. Our findings are in agreement with the previous results reported (Khanam et al., 2012), which shows lower antioxidant capacities for most of leafy vegetables equivalent to trolox, quercetin and ascorbic acid after comparison of DPPH assay to the ABTS assay. Highest ABTS scavenging activity was obtained for 80% ethyl acetate extract of PA (79 mg CE/g dw) variety and the lowest value was for water extract of PF (6.45 mg CE/g dw) variety followed by PB (6.53 mg CE/g dw). 80% ethyl acetate was found to be the best extraction solvent for ABTS study of four (PA, PB, PC and PD) varieties out of six varieties. For PE (35.52 mg CE/g dw) and PF (26.82 mg CE/g dw) variety, 80% ethanol was found to be the best extraction solvent with significant difference. The highest and lowest FRAP value was reported for 80% ethyl acetate extract of PA (1.35 mmol Fe+2/g dw) variety and water extract of PF (0.04 mmol Fe+2/g dw) variety (Table 4). 80% ethyl acetate was found to be the best extraction solvent for FRAP study of PA, PB, PC and PD variety. 80% Methanol was found to be most effective for PE (0.80 mmol Fe+2/g dw) variety and 80% acetone was the best for FRAP study of PF (0.41 mmol Fe+2/g dw) variety. In a similar study, methanol: water (1:1) reported to be the best solvent for ABTS and FRAP study of different betel leaf variety (Tamuly et al., 2013). PCL assay was chosen for antioxidant study of betel leaf extract because the superoxide radical (O2 one of the dangerous reactive oxygen species) is directly linked with health issues and give antioxidant activities in nano molar range.

Table No.3 : Total phenol content of six variety betel leaf extracted with five different solvents

Sample TPC (mg GAE/g dw basis)

	80% Methanol	80% Ethanol	80% Acetone	80%Ethyl Acetate	Water
PA	2.62 ± 0.036^{c}	2.04 ± 0.87^{b}	2.73 ± 0.30^{c}	1.94 ± 0.27^{b}	0.29 ± 0.02^{a}
PB	2.59 ± 0.020^{c}	1.85 ± 0.066^{b}	1.89 ± 0.25^{b}	2.06 ± 0.22^{b}	0.08 ± 0.02^{a}
PC	2.38 ± 0.02^{c}	1.74 ± 0.10^{b}	2.37 ± 0.31^{c}	2.13 ± 0.13^{c}	0.09 ± 0.02^{a}
PD	2.87 ± 0.12^{c}	1.8 ± 0.03^{b}	1.72 ± 0.13^{b}	1.84 ± 0.05^{b}	0.07 ± 0.01^{a}
PE	2.07 ± 0.14^{c}	0.84 ± 0.06^{c}	0.93 ± 0.11^{c}	0.37 ± 0.01^{b}	0.16 ± 0.02^{a}
PF	1.16 ± 0.02^{c}	1.1 ± 0.03^{c}	1.45 ± 0.20^{d}	0.52 ± 0.02^{b}	0.04 ± 0.01^{a}

Values are mean ± standard deviation of triplicate analyses. Results of each solvent extraction were analyzed separately. Different letters in the same row are significantly different ($p<0.05$) as measured by Tukey's B test.

Table No.4 : DPPH radical scavenging activity of six variety betel leaf extracted with five different solvents Sample DPPH (mg CE/g dw basis)

	80% Methanol	80% Ethanol	80% Acetone	80%Ethyl Acetate	Water
PA	68.25±1.67^{c}	32.94±0.86^{b}	71.43±1.30^{d}	79±1.70^{e}	12.41±1.33^{a}
PB	46.93±1.49^{b}	68.38±1.08^{d}	55.95±1.28^{c}	78.32±2.17^{e}	6.53±1.30^{a}
PC	36.04±1.83^{b}	58.03±0.86^{c}	58.92±1.28^{c}	63.71±1.09^{d}	7.44±1.08^{a}
PD	70.30±2.60^{d}	49.90±2.30^{c}	42.83±0.71^{b}	78.10±1.68^{e}	8.81±0.73^{a}
PE	32.50±1.98^{b}	35.52±0.85^{b}	32.16±1.53^{b}	12.40±2.87^{a}	15.85±0.64^{a}
PF	26.2±0.63^{c}	26.82±2.58^{c}	24.11±0.85^{c}	22.63±0.64^{b}	6.45±1.06^{a}

Values are mean ± standard deviation of triplicate analyses. Results of each solvent extraction were analyzed separately. Different letters in the same row are significantly different ($p<0.05$) as measured by Tukey's B test.

Table No.5 : Antioxidant capacity of six variety betel leaf extracted with five different solvents measured by ABTS method Sample $ABTS^{\cdot+}$ (mg CE/g dw basis)

	80% Methanol	80% Ethanol	80% Acetone	80%Ethyl Acetate	Water
PA	63.56±0.89^{d}	54.35±2.15^{c}	21.36±0.06^{b}	23.25±0.02^{b}	2.48±0.005^{a}
PB	58.95±0.18^{e}	55.37±1.25^{d}	19.04±1.6^{b}	22.71±0.09^{c}	4.46±1.04^{a}
PC	38.90±1.8^{c}	74.96±1.8^{d}	20.94±1.47^{b}	23.35±0.07^{b}	4.92±0.57^{a}
PD	51.78±1.46^{d}	77.71±1.62^{e}	16.52±0.41^{b}	22.42±0.13^{c}	2.96±0.94^{a}
PE	25.40±1.10^{d}	127.5±0.25^{e}	9.32±0.80^{b}	18.22±1.10^{c}	3.56±0.36^{a}
PF	35.25±2.20^{e}	133.5±1.77^{d}	17.47±0.84^{c}	21.42±0.64^{b}	7.20±0.98^{a}

Values are mean ± standard deviation of triplicate analyses. Results of each solvent extraction were analyzed separately. Different letters in the same row are significantly different ($p<0.05$) as measured by Tukey's B test.

Table No.6 : Antioxidant capacity of six variety betel leaf extracted with five different solvents measured by FRAP method Sample FRAP (mmol Fe^{+2}Eq./g dw basis)

	80% Methanol	80% Ethanol	80% Acetone	80%Ethyl Acetate	Water
P A	0.65±0.03[b]	0.67±0.06[b]	0.90±0.04[c]	1.35±0.01[d]	0.09±0.005[a]
P B	0.74±0.05[b]	0.70±0.07[b]	0.68±0.04[b]	0.95±0.05[c]	0.05±0.005[a]
P C	0.64±0.02[b-c]	0.56±0.07[b]	0.69±0.04[c]	0.90±0.05[d]	0.04±0.005[a]
P D	0.52±0.04[c]	0.24±0.03[b]	0.39±0.02[c]	0.21±0.01[b]	0.07±0.005[a]
P E	0.80±0.03[c]	0.63±0.04[b]	0.56±0.06[b]	1.10±0.11[d]	0.07±0.005[a]
P F	0.21±0.01[b]	0.30±0.03[c]	0.41±0.005[d]	0.38±0.03[d]	0.04±0.005[a]

Values are mean ± standard deviation of triplicate analyses. Results of each solvent extraction were analyzed separately. Different letters in the same row are significantly different ($p<0.05$) as measured by Tukey's B test.

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