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Research Article

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Antioxidant and Metal Chelating Activities From Various Solvent Extracts in *P. betel*, *C. papaya* Leaves and *T. aestivum* Grass

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ABSTRACT

In the present study, three plants Triticum aestivum, Piper betel and Carica papaya were used to evaluate the total phenolic and flavonoid content, metal chelating and antioxidant activity. Successive extractions were done by soxhelet apparatus using different solvent of different polarity petroleum ether, ethyl acetate, n-butanol. Aqueous extract was prepared by pulverization method. The results revealed that butanolic and aqueous extract showed higher total phenolic and flavonoid content in all three plants. N-butanol extract of Triticum aestivum and Piper betel showed high metal chelating and antioxidant activity as compared to Carica papaya. The results indicated that n-butanol extract of both plants have the potential to be a source of natural antioxidant and as a potent antihemolytic agent.

Keywords: *T. aestivum; P. betel; C. papaya*; Metal chelating activity; FRAP

INTRODUCTION

Plants are potential sources of natural antioxidants. It produces various antioxidative compounds to counteract reactive oxygen species (ROS) to survive. In recent years much, attention has been devoted to natural antioxidant and their association with health benefits [1]. Medicinal plants, rich in secondary metabolites, offer a pool of preventive and therapeutic options [2]. Plant derived antioxidants such as polyphenols, carotenoids, flavonoids, phenolics, ascorbic acid (vitamins C) and E have multiple biological effects, including antioxidant activity [3]. An added benefit of plant-derived bioactive compounds has minimal side effect, compared to those of synthetic drugs [4]. Antioxidants are substances that can prevent or retard oxidation of lipids, proteins and DNA: and to protect the compounds or tissues from damage caused by oxygen or free radicals [5].

Oxidative damage and haemolysis caused by reactive oxygen species (ROS) have a main role in the development of diseases such as thalassemia, glucose-6-phosphate dehydrogenase deficiency and sickle cell anaemia. RBCs are the primary targets of free radicals, due to their high membrane concentrations of polyunsaturated fatty acids and O_2 transport associated with redox active haemoglobin molecules, which are effective promoters of ROS [6,7]. Antioxidant and other supportive therapies protect RBCs against oxidative damage [8,9]. Enrichment with vitamin E and C is effective in preventing LDL oxidation in patients with thalassemia. Iron chelators mobilize tissue iron by forming soluble, stable complexes that are then excreted in the faeces and urine. Chelation therapy reduces iron-related complications and thereby improves quality of life and overall survival [10].

Wheat (*Triticum aestivum*), a cereal grass belongs to family Gramineae (Poaceae) [11]. Wheatgrass provides a concentrated amount of nutrients, including iron, calcium, magnesium, amino acid and vitamin A, C, E and chlorophyll in large amount [12]. Wheatgrass is also reported play an important role in treatment for cancer [13], joint pain and serve as an antioxidant [14]. The effectiveness of the plant extracts is mainly due to the presence of bioactive constituents like phenolics, flavonoids and others [15].

Piper betel belongs to the Piperaceae family Piper betel (Paan). It is a medicinal plant, traditionally used in catarrhal and pulmonary affections, as a digestive and carminative and as stimulant of pancreatic lipase [16-18]. Scientifically, studies have reported the biological benefits of *P. betel* to include inhibition of platelet aggregation [19] and immunomodulatory properties [20]. Some of these observed biological activities were attributed to high antioxidant activities of this plant [21,22].

Carica papaya (Papaya) belongs to family caricaceae. It is commonly called pawpaw is an important plant consumed as a fresh fruit or processed into dessert [23]. An infusion of the mature papaya leaves is also taken as an antidote for fever and malaria [24].

For thousands of years, mankind has known about the benefits of drugs from nature. Even today, plant materials remain an important resource for combating illness. The aim of this study is to compare the antioxidant and metal chelating activity of herbal extracts in three plants i.e. *Triticum aestivum* grass, Piper betel leaves, *Carica papaya* leaves.

MATERIALS AND METHODS

Sample Preparation

Fresh *P. betel* leaves were procured from village Sandalpure Antri of Gwalior, M.P. The grass of *T. aestivum* was cultivated and leaves of *C. papaya* were collected from ITM University, Gwalior, Turari Campus. The leaves were cut into small pieces and dried in shade at room temperature, followed by grinding into fine powder. Powdered leaves (50 g) was extracted with soxhelet apparatus using different solvent viz petroleum ether, ethyl acetate, n-butanol according to their polarity. For aqueous extraction, 20 g powder leaves were added into 100 ml distilled water was incubated in a orbital shaking incubator for 24 h at room temperature, supernatant was evaporated to 1/5th of original volume and stored at 4°C in air tight bottles.

Total Phenolic Content

The total phenolics in the extract were determined using Folin-Ciocalteu method [25]. To each sample solution (1.0 ml) and the standard (gallic acid) was added 5 ml of folin-ciocalteu (sigma-aldrich) and 4 ml sodium carbonate (7% w/v) and shaken. The solution could stand for 30 min in the dark at room temperature, after which absorbance was measured at 765 nm using UV-VISIBLE parkin elmer Lambda 23 with win lab N6.0 software. The phenolic content was calculated from the standard curve of gallic acid [26].

Total Flavonoid Content

A known volume of extract was placed in a 10 ml volumetric flask add distilled water to make final volume 5 ml followed by adding 0.3 ml $NaNO_2$ (1:20). Add 3 ml $AlCl_3$ (10%) 5 min later. After 6 min, 2 ml 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well again, and the absorbance was measured against a blank at 510 nm. The flavonoid content was calculated with quercetin as standard [27].

Metal Chelating Activity

Metal chelating activity was determined by method described by Chew et al., 2009 [28]. 0.2 ml plant extracts at different concentration, 0.2 ml $FeSO_4$ (0.1 mM) and 0.4 ml ferrozine (0.25 mM). After incubating at room temperature for 10 min, absorbance was read at 562 nm. Metal chelating activity was calculated using the following equation:

(Acontrol-Asample)/Acontrol × 100 (1)

1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Activity

DPPH free radical scavenging activity of plant extracts in different fraction was examined by Blois method [29] with minor modification. Various Concentration (20-100 µg/ml) of plant extracts dissolved in distilled water and 1 ml of DPPH solution (0.1 mM in methanol) was added, shaken well and incubated at 37°C for 30 min in dark. Decrease in absorbance was measured at 517 nm against suitable blank. The DPPH radical scavenging activity was calculated by using equation 1.

Hydroxyl Radical (OH) Scavenging Activity

The hydroxyl radical scavenging activity was based on the deoxyribose method [30]. Prepared reaction mixture 200 µl $FeSO_4$ (10 mM), 200 µl EDTA (10 mM), 200 µl H_2O_2 (10 mM), 200 µl deoxyribose (10 mM) was mixed with

1.2 ml phosphate buffer (100 mM, pH 7.4) containing 200 µl plant extract. The reaction mixture was incubated for 4 h at 37°C in a water bath. After incubation, 1 ml of 1% thiobarbituric acid and 1 ml of ice cold 2.8% trichloroacetic acid were added. The resultant reaction mixture was incubated for 10 min in a boiling water bath (95°C-100°C). After cooling down to room temperature and being centrifuged at 395 x g for 5 min. the absorbance was measured at 532 nm. Vitamin E was used as the positive control. The hydroxyl radical scavenging activity was calculated by using equation 1.

Ferric Reducing Antioxidant Power (FRAP)

Total FRAP were assessed by Benzie and Strain [31] with slight modification. The 1 ml extract (10-50 mg/ml), freshly prepared 2.8 ml FRAP reagent (25 ml acetate buffer (30 Mm; pH 3.6), 2.5 ml 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ) (10 Mm) and 2.5 ml ferric chloride (20 Mm) was added. The reaction cocktail was incubated for 30 min. in dark condition. The absorbance was taken at 593 nm. The standard curve was calibrated by ascorbic acid at 10 to 50 mg/ml concentration. The results were reported as ascorbic acid equivalent (AAE) per ml.

Statistical Analysis

All data were reported as mean+SD of three measurements. Graphs were calculated by using Graph Pad Prism 7 ink software.

RESULTS

Total Phenolic Contents

Table 1 represent the total phenolic content of petroleum ether, ethyl acetate, n-butanol and aqueous extract in *T. aestivum*, *P. betel*, *C. papaya* respectively. The results we revealed that, aqueous extract of *T.aestivum* contain high phenolic content were 445 followed by n-butanol extract of *P. betel* and *C. papaya* were 324.27 and 233.33 respectively.

Table 1: Total phenolic content (mg gallic acid equivalent/g)

Extracts	***T.aestivum* Grass**	***P.betel* Leaves**	***C.papaya* Leaves**
Petroleum ether	136.9 ± 2.9	300.36 ± 2.5	186 ± 1.5
Ethyl acetate	172 ± 0.7	171.38 ± 0.4	192.02 ± 1.7
n-butanol	184.05 ± 1.1	324.27 ± 5.3	233.33 ± 0.7
Aqueous	445 ± 12.3	144.2 ± 0.7	226.81 ± 2.9

Total Flavonoid Content

Table 2 represents the total flavonoid content of petroleum ether, ethyl acetate, n-butanol and aqueous extract in *T. aestivum*, *P. betel*, *C. papaya*. The flavonoid content of extracts calculated as quercetin equivalent. Highest flavonoid content was present in aqueous extract and n-butanol extract as compared to petroleum ether and ethyl acetate extract. Aqueous extract of P. bêtel and *T.aestivum* revealed flavonoid content to be 255.3 and 209.9 mg quercetin equivalent/g respectively. The flavonoid content in petroleum ether *T. aestivum* was relatively very low as compared to aqueous extract.

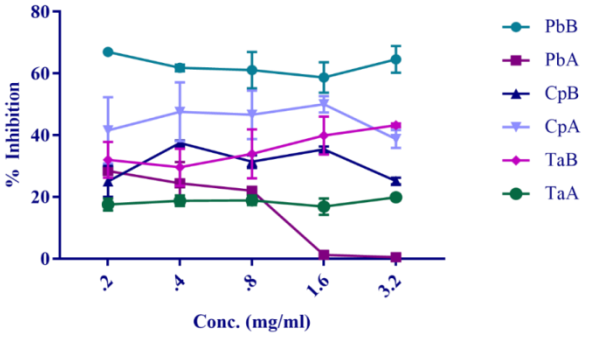
Table 2: Total flavonoid content (mg quercetin equivalent/g)

Extracts	*T.aestivum* Grass	*P.betel* Leaves	*C.papaya* Leaves
Petroleum ether	7.67 ± 1.4	43.46 ± 1.3	9.3 ± 0.3
Ethyl acetate	19.88 ± 1.1	131.82 ± 0.3	14.7 ± 4.3
n-butanol	85.79 ± 10.7	144.89 ± 0.6	24.93 ± 0.3
Aqueous	209.94 ± 4.2	255.39 ± 1.3	58.17 ± 1.7

Data presented as mean+SD with n=3

Metal Chelating Activity

Figure 1 illustrated the metal chelating activity. Butanolic extract of *P. betel* and *T. aestivum* shows maximum activity (64.6% and 43.3% at 3.2 mg/ml concentration). Minimum chelating activity was observed in *C. papaya* aqueous extract (38.8% at 3.2 mg/ml).

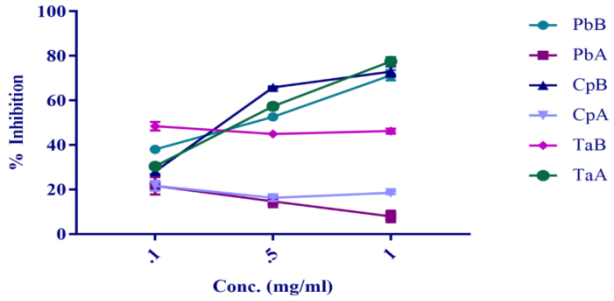


PbB-*Piper betel* n-Butanol	CpA-*Carica papaya* Aqueous
PbA-*Piper betel* Aqueous	TaB-*Triticum aestivum* n-Butanol
CpB-*Carica papaya* n-Butanol	TaA-*Triticum aestivum* Aqueous

Figure 1: Metal chelating activity of *T. aestivum* grass, *P. betel* leaves, *C. papaya* leaves

Hydroxyl Radical Scavenging Activity

Figure 2 illustrated the hydroxyl scavenging activity. Highest radical scavenging activity was shown *T.aestivum* aqueous extract (77.5%) followed by *C. papaya* and *P. betel* butanolic extract (72.9% and 71.3%) respectively at 1 mg/ml concentration.

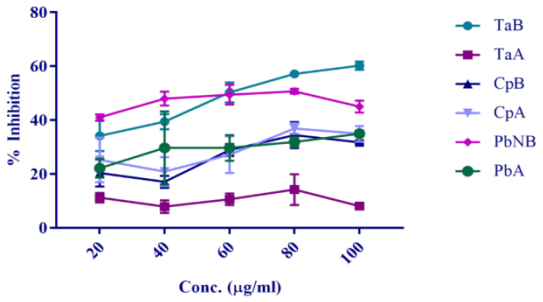


PbB-*Piper betel* n-Butanol	CpA-*Carica papaya* Aqueous
PbA-*Piper betel* Aqueous	TaB-*Triticum aestivum* n-Butanol
CpB-*Carica papaya* n-Butanol	TaA-*Triticum aestivum* Aqueous

Figure 2: Hydroxyl radical scavenging activity of *T. aestivum* grass, *P. betel* leaves, *C. papaya* leaves

1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Radical Scavenging Activity

Figure 3 illustrated the DPPH activity of *T. aestivum* grass, *P. betel* leaves and *C. papaya* leaves in n-butanol extract. *T. aestivum* n-butanol showed highest DPPH radical scavenging activity (60.3%) followed by *P. betel* and *C. papaya* was 45% and 31.7% respectively at 100 µg/ml concentration.



PbB-*Piper betel* n-Butanol	CpA-*Carica papaya* Aqueous
PbA-*Piper betel* Aqueous	TaB-*Triticum aestivum* n-Butanol
CpB-*Carica papaya* n-Butanol	TaA-*Triticum aestivum* Aqueous

Figure 3: DPPH radical scavenging activity of *T. aestivum* grass, *P. betel* leaves, *C. papaya* leaves in two extracts n-butanol and aqueous

Ferric Reducing Antioxidant Power (FRAP)

Table 3 represents the butanolic and aqueous extract of *T. aestivum*, *P. betel* and C.papaya highest FRAP was revealed by butanolic extracts of *T. aestivum* grass and *P. betel* leaves followed by *C. papaya* leaves.

Table 3: Ferric reducing antioxidant power (mg ascorbic acid equivalent/ml)

Extract	***T.aestivum* Grass**	***P.betel* Leaves**	***C.papaya* Leaves**
n-butanol	19.9 ± 1.4	19.1 ± 0.2	13.5 ± 0.6
Aqueous	11.0 ± 0.6	1.0 ± 0.1	2.7 ± 0.1

DISCUSSION

The high antioxidant activity may be due to natural antioxidants that are present in the plants. Phenolics and flavonoids possess the antioxidant power due to their free radical scavenging activity, which may be due to electron donor, metal chelating and scavenging of singlet oxygen [32]. In the present study highest phenolic as well as flavonoid content was present in n-butanol and aqueous extract in comparison to petroleum ether and ethyl acetate extract (Table 1 and Table 2). Thus the study was narrowed down to only two extracts. To prevent ROS generation, the strategy adopted is redox active metal catalysis which involves chelating of the metal ions and capture of ferrous ions before ferrozine. Highest metal chelating activity was shown by n-butanol extract of P.betel and followed by butanolic extract of *T. aestivum* (Figure. 1). It may be due to the redox active catalysis, which involves chelating of the metal ions High phenolic and flavonoid content can be correlated to metal chelating activity. The extract may consist of dihydroxy group which can conjugate with transition metal. Thereby, preventing metal induced free radical. Presence of chelating agent in the extract disrupts the ferrozine-Fe^{2+} complex formation and decreased the red colour [33]. It has been found chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion.

Hydrogen peroxide is one of the most important ROS formed from superoxide. It could be transformed to the hydroxyl radical by Fenton reaction where transition metal ions (Fe^{2+}) reduced H_2O_2 to the hydroxyl radical. Highest hydroxyl scavenging activity was shown by *T. aestivum* aqueous extract followed by butanolic extract of *P. betel*

and *C. papaya* (Figure 2). Thus, the chelation of Fe^{2+} ion and/or the reduction of Fe^{3+} is an important occurrence in depleting the oxidative stress [34,35].

DPPH has been extensively used as a free radical to evaluate antioxidant substances that reduce DPPH by donating hydrogen to form the non-radical DPPH-H [36]. It is a marker of free radicals originating in lipids [37]. DPPH has an intense violet colour, but turns colourless as unpaired electrons are scavenged by antioxidant [36]. Highest DPPH activity was present in n-butanol extract of *T. aestivum*. *C. papaya* shows minimum activity as compared to *P. betel* (Figure 3). The FRAP method is based on the reduction of a Fe^{3+}-TPTZ complex to its Fe^{2+} coloured form in the presence of antioxidants [38]. Total antioxidant power of each extract was calculated as the FRAP value and represented as ascorbic acid equivalent per ml. The highest FRAP value was observed in n-butanol extract of all three plants as compared to aqueous extract (Table 3).

DPPH and FRAP assays showed no differences among determinations, which revealed the fact that they may be used to determine the antioxidant activity. Both the parameters revealed a high level of reproducibility. The antioxidant capacity of the extract was assessed by the ability of antioxidant compound present in extracts to quench the peroxyl radical and to reduce DPPH free radicals and ferric ion *in vitro* system.

CONCLUSION

The present investigation gives a comparative evaluation of the secondary metabolites, metal chelating and antioxidant activity of the various extracts of *T. aestivum, P. betel* and *C. papaya*. n-butanol fraction of *T. aestivum* followed by *P. betel* prove to a better scavenging activity as compared to *C. papaya*. The results of high antioxidant and metal chelating activity may be correlated to the high phenolic and flavonoid content in n-butanol extract of *T. aestivum* and *P. betel*. Antioxidant molecules are reported to be potent inhibitors of sickle cell haemoglobin polymerization. Phenolics which contribute to antioxidant properties scavenge and neutralize the damage caused by free radicals. Thus the increase intake of dietary antioxidants from *T. aestivum* grass and P.betel leaves may contribute to the management of oxidative stress induced diseases.

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CONFLICT OF INTEREST

None.

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