



Antioxidant potential of *Murraya koenigii*'s (L.) Spreng polysaccharide

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ABSTRACT

In the present study, various fractions of plant polysaccharides (Hot water extracted polysaccharide, Cold water extracted polysaccharide, Alkali extracted polysaccharide A and Alkali extracted polysaccharide B) from leaf powder of Murraya koenigii were screened for their antioxidant effects using different antioxidant potential assays to evaluate the maximum potential. Total polysaccharide content was measured by alcohol insoluble hexose test method and total protein content was estimated. Acid hydrolysis of polysaccharides was performed using concentrated HCl. Standard chromatography procedure and negative DPPH staining were used. HPTLC is used for standardization. Six different tests were done to evaluate the antioxidant potential of four different fractions: The decreasing order in reducing power activity was as follows: AEPA > CWEP > HWEP > AEPB. The AEPA showed maximum antioxidant capacity among all the fractions by phosphomolybdenum method. Maximum ferric ion reduction was done by cold water extracted polysaccharides and minimum in AEPB by FRAP assay. The free radical scavenging potential of the fractions by DPPH assay were found to be in the order of HWEP > AEPB > AEPA > CWEP. The maximum metal chelating effect of iron with polysaccharide fractions of Murraya koenigii was evaluated by AEPA fraction. Colorless polysaccharide fractions of Murraya koenigii leaves show tremendous antioxidant potential. And metal chelating properties. HWEP fraction showed maximum results as potent antioxidant which gives an insight of antioxidants are not always present in colorful pigments.

Keywords: Polysaccharide fractions, Murraya Koenigii, DPPH.

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INTRODUCTION

It is known that hydroxyl radical is a powerful oxidant that can react with all biological molecules such as proteins, lipids, and carbohydrates [19] and that oxidative stress can mediate a wide variety of degenerative processes and diseases [2, 8, 18]. The damaging action of OH radical is very strong, oxidation of fatty acids in biological membranes leads to formation and propagation of lipid radicals, uptake of oxygen, rearrangement of the double bonds in unsaturated lipids, and eventual destruction of membrane lipids. Therefore, research on antioxidants, especially exploration of potent natural compounds with low cytotoxicity from plants, has become an important branch of biomedicine. Though the literature pertaining to the antioxidant activity of various low-molecular-weight compounds is voluminous, there are not many reports on antioxidant activity of polysaccharides. Rajasekaran *et al.* [14] found that the antioxidant activity of ethanolic extract from the gel of *Aloe barbadensis* leaf in the plasma and pancreas of diabetic rats was higher than that of the standard used, which supported the ethnopharmacological use of this plant in ameliorating the oxidative stress in diabetes. Water extracts from several medicinal plants, including *Arctium lappa* var. *hercules* were shown to have an inhibiting effect on lipid peroxidation in the rat brain homogenate [9]. Certain sulfated, acetylated, and phosphorylated derivatives of carrageenan oligosaccharides exhibited significant antioxidant activities in *in vitro* experiments, in some cases higher than the activity of polysaccharides and oligosaccharides. The effect of molecular mass was not obvious as both poly- and oligosaccharides showed similar antioxidant activity [21, 22].

An anti-ulcer pectic polysaccharide with antioxidant activity has been isolated from *Bupleurum falcatum* [11]. Liu and Ng *et al.* [9] demonstrated for the first time the presence of both gallic acid derivative and polysaccharides as major antioxidant principles of the aqueous extract of rose flowers, reflected in the

ability to inhibit lipid peroxidation in rat brain and kidney homogenates. Present study focuses on the isolation of polysaccharide fraction from curry leaves and establishes their antioxidant activity in these pigment-less fractions.

MATERIAL AND METHODS

Extraction and purification of polysaccharides of *Murraya koenigii*:

For the extraction of polysaccharides from the leaves of *Murraya koenigii*, the method described by Ramesh *et al.* [16] was applied (figure). Leaves of *Murraya koenigii* were collected and sun dried for 2 days. The dried leaves were finely powdered for polysaccharide extraction. The curry leaf powder (100 g) was extracted with n-hexane (3X-300 ml) at room temperature for 2 hours and centrifuged. The residue was treated with 70% ethanol (3X volume) for 2 hours. The residue obtained was thoroughly extracted with water at an ambient temperature (27°C) for one hour each and centrifuged. From the supernatant, the cold water extracted polysaccharide (CWEP) was precipitated by three volumes of ethanol. The residue was subjected to aqueous extraction (3X) at boiling water temperature for one hour. After centrifugation, from the supernatant the hot water extracted polysaccharide (HWEP) was precipitated by three volumes of ethanol. The above residue was subjected to alkaline extraction by three volumes of 1N NaOH for one hour. After centrifugation, the supernatant was adjusted to pH 4.5 with diluted acetic acid (3X) at 4°C to get the alkali extracted polysaccharides A (AEPA). By adding ethanol to the supernatant, the alkali extracted polysaccharide B (AEPB) was precipitated. All the centrifugation operations were done at 10000 rpm.

Total polysaccharide content was measured by alcohol insoluble hexose test method [2] and total protein content was estimated by Bradford method as described earlier. Acid hydrolysis of polysaccharides was performed using concentrated HCl. For this all polysaccharide fractions were placed in boiling tube with ten drops of concentrated HCl and boiled gently. Neutralization was done by NaOH, and neutralized hydrolysates were used for TLC analysis using TLC aluminum sheets (20x20cm) precoated with silica gel 60 (Merck Co., Germany). Sugar were separated by running in the solvent system of 1-propanol, ethanol and water (7:1:2). Chromatograms were developed by spraying with a Aniline-diphenylamine solution [10] and anisaldehyde-H₂SO₄.

For antioxidant activity, reducing power activity, DPPH assay [22], FRAP assay [20], phosphomolybdenum method [13] and metal chelating effect [12] as described earlier were done on all the four polysaccharide fractions.

Statistical analysis of data

Statistical analysis of data included: one way analysis of variance (ANOVA), Multiple comparisons and Standard error of mean (SE). Observations made for all the methods were subjected to one-way analysis of variance (ANOVA) at 5% significance level to evaluate the significance of the observed differences in the values recorded for different parameters taken in the present investigation. Analysis of variance was carried out using Microsoft Excel software. The differences in the mean values of a given parameter for different groups were tested for significance (P<0.05) using multiple comparison procedures. All the pair wise multiple comparisons were carried out following Tukey's Test using Sigmastat statistical software. Mean and standard errors were calculated.

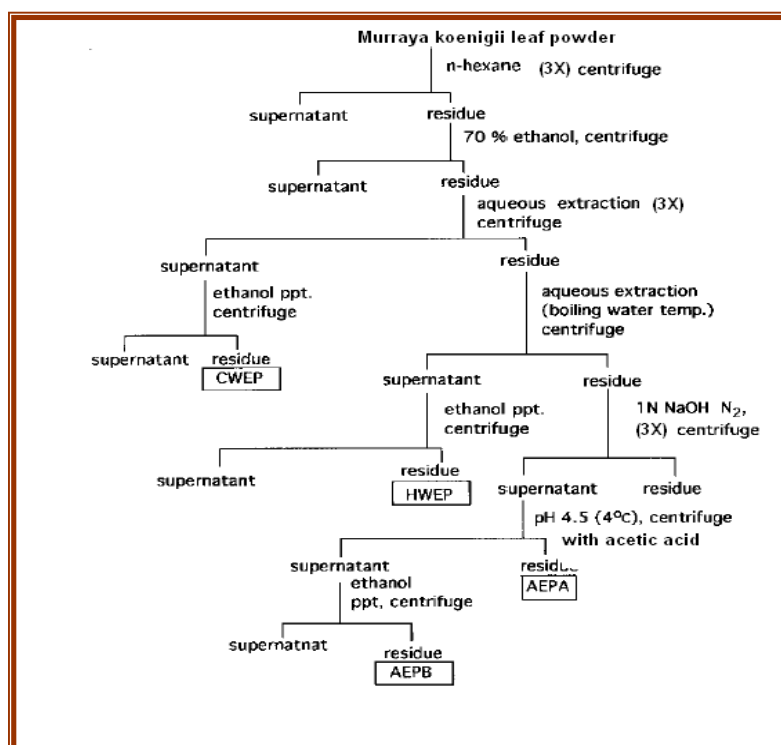


Figure 1: Scheme of extraction and purification of polysaccharides from *Murraya koenigii*

RESULTS AND DISCUSSIONS

In the present study, various fractions of plant polysaccharides (Hot water extracted polysaccharide, Cold water extracted polysaccharide, Alkali extracted polysaccharide A and Alkali extracted polysaccharide B) from leaf powder of *Murraya koenigii* were screened for their antioxidative effects using different antioxidant potential assays. The yield of HWEP was maximum to an extent of 52% in present study while it was only 18.81% in fenugreek [15]. The minimum yield was in AEPB with 0.24% while it was only 7.48% of AEPA in fenugreek. In the present study two alkali extracted polysaccharide fractions (AEPA and AEPB) were obtained as done in some of previous reports. In fenugreek (*Trigonella foenum-graecum* L.) similar fractions were obtained [16]. NaOH extractions for polysaccharides of gum is well known. Similarly *Fucus vesiculosus* was sequentially extracted with water at 22 °C (fraction 1 (F₁)) and 60 °C (F₂), and with 0.1 M HCl (F₃) and 2 M KOH (F₄) at 37 °C [17].

The total polysaccharide content was measured by the phenol-sulphuric acid method and protein by Bradford method (Bradford, 1976). The sugar composition of crude polysaccharides was analyzed by TLC [1]. The purified polysaccharides were white to off-white fluffy powders with <7 % protein and 87% carbohydrates. Soluble fractions (42.3% yield) from *Fucus vesiculosus* were composed of neutral sugars (18.9-48 g/100 g), uronic acids (8.8-52.8 g/100 g), sulfate (2.4-11.5 g/100 g), small amounts of protein (<1-6.1 g/100 g), and nondialyzable polyphenols (0.1-2.7 g/100 g) [17]. The HWEP fraction of *Murraya koenigii* contained a maximum of 73.258% v/v sugar and 6.65%v/v protein. Similar reports in other plant polysaccharides were obtained like Aloe polysaccharide (APS) consists of ~0.5% protein and 85.1% polysaccharides (73% being mannose) (Kim *et al.*, 1999). The AEPB fraction of fenugreek contained a maximum of 12.09% protein [16].

The observed traces of glucose and other sugars in TLC of acid hydrolysates of extracts were expected in all four fraction, as previous study revealed. In *Fucus vesiculosus*, the main neutral sugars were fucose, glucose, galactose, and xylose [1, 18, 16].

In the present study, all four fractions were studied for their antioxidant activity with six different methods. No single fraction showed higher antioxidant capacity in all the methods tested. The AEPA extract showed maximum antioxidant activity by phosphomolybdenum and reducing power ability. It also showed maximum chelating effect of iron. The CWEP extract exhibited highest radical potential by FRAP assay. This observation is contradictory to the previous report in *Fucus vesiculosus* where alkali extracted polysaccharide A fraction showed the highest antioxidant potential by the ferric reducing antioxidant power (FRAP) assay, followed by the alkali- and water-soluble fractions [7]. The HWEP fraction showed highest scavenging potential for DPPH radicals. Similar report is available for *Asparagus racemosus* where an active fraction consisting of polysaccharides (termed as P3) was effective even at a

low concentration of 10mg/ml [4]. The sulfated polysaccharide fraction F₂ from *Porphyra haitanesis* could be used to compensate the decline in trolox equivalent antioxidant capacity and the activities of antioxidant enzymes and thereby to reduce the risks of lipid peroxidation [23, 24] but in the present study results were negative for lipid peroxidation assay. Non of the fractions showed lipid peroxidation activity.

(b) Antioxidant potential of polysaccharide fractions of *Murraya koenigii*

The results of antioxidant capacity of *Murraya koenigii* polysaccharides fractions were summarized (figure 1-6; table 1). Six different test were done to evaluate the antioxidant capacity of four different fractions: reducing power activity, phosphomolybdenum method, FRAP assay, DPPH assay, metal chelating effect and MDA content or lipid peroxidation test.

The reducing activity of all fractions was expressed as L-ascorbic acid equivalents (Figure 2). The maximum reducing ability was found in the AEPA fraction. The decreasing order in reducing power activity was as follows: AEPA > CWEP > HWEP > AEPB.

The antioxidant capacity of the fractions was measured spectrophotometrically by phosphomolybdenum method (Figure 3), which is based on the reduction of Mo (IV) to Mo (V) by the sample analyte and subsequent formation of green phosphate/ Mo (V) compounds with a absorption maxima at 695 nm. The antioxidant capacity of polysaccharide fraction of *Murraya koenigii* was found to decrease in order, AEPA > CWEP > HWEP > AEPB. The antioxidant capacity by AEPB was very less.

Values for FRAP assay of different fraction of *Murraya koenigii* have been presented in figure 4. Significant variations have been recorded between all the fractions of *Murraya koenigii* ($F_{4,16} = 1427.35$, $P < 0.0001$). Maximum ferric ion reduction was done by cold water extracted polysaccharides. Alkali extracted polysaccharides A also showed higher FRAP values, but very less and negligible amount of reduction was observed by hot water extracted polysaccharides (HWEP) and alkali extracted polysaccharides B (AEPB). The decreasing order of FRAP assay values of different polysaccharide fraction of *Murraya koenigii* was as follows: CWEP > AEPA > HWEP > AEPB.

The free radical scavenging activity of the leaves of *Murraya koenigii*'s polysaccharides fractions were tested through DPPH method and the results are presented in the figure 5; table 1. The free radical scavenging potentials of all the fractions were significantly different with each other ($F_{4,16} = 4826.09$, $P < 0.0001$). The decreasing order of DPPH assay of different polysaccharide fraction of *Murraya koenigii* was as follows: HWEP > AEPB > AEPA > CWEP.

The HWEP, AEPB, AEPA and CWEP fractions did not show malondialdehyde (MDA) content formation by the TBARS method.

The metal chelating effect of iron with polysaccharide fractions of *Murraya koenigii* was evaluated by absorbance change and/or spectral shift after incubation (figure 6). The Fe²⁺-CWEP fraction complex had peaks at 198.50 nm and 288 nm and the Fe²⁺-HWCP fraction had peak at 199nm and 286 nm. Metal ions caused a spectral shift and absorbance change of FeSO₄. The FeSO₄ solution had peaks at 289 nm while the Fe²⁺-EDTA complex has peaks at 209 nm and 284 nm. The Fe²⁺-AEPA fraction complex had peaks at 192 nm and 275 nm and the Fe²⁺-AEPB fraction had peak at 216 nm and 317 nm.

Table 1: Yield /compositional analysis and antioxidant capacity of polysaccharide fractions of *Murraya koenigii*.

Name of leaf fraction of <i>Murraya koenigii</i>	Cold water extracted polysaccharides (CWEP)	Hot water extracted polysaccharides (HWEP)	Alkali extracted polysaccharides A (AEPA))	Alkali extracted polysaccharides B (AEPB)
Parameters				
Yield (%w/w)	10	52	1.96	0.24
Total sugars (%v/v)	73.258	4.94	8.93	0.502
Total protein (%v/v)	6.65	0.162	0.253	0.01
TLC profile	+++	+	+++	++
Reducing power (abs (nm) of fraction 100µg/ml)	1.216	1.004	1.473	1.002
Phosphomolybdenum method	1.286	0.468	1.661	0.416
FRAP Value	188.33±1.8	23.33±0.8	78.33±1.4	13.8±0.6
DPPH SC ₅₀ value	167.41±1.12	367.46±1.15	260.55±1.14	280.18±1.28
MDA content	nil	nil	nil	nil

+++ = Higher intensity spots; ++ = Average intensity spots; + = Lower intensity spots

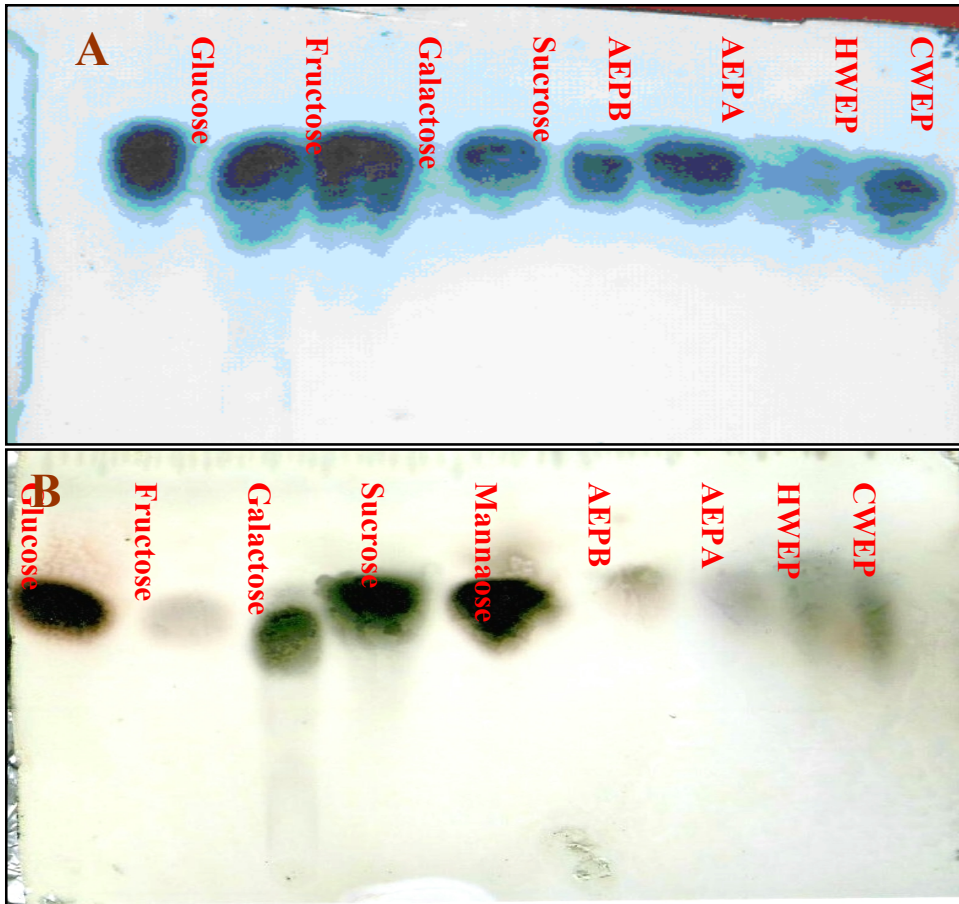


Figure 2: (A) TLC staining by aniline-diphenylamine method of polysaccharide fractions of leaves of *Murraya koenigii*. (B) TLC staining by anisaldehyde-H₂SO₄ method of polysaccharide fractions of leaves of *Murraya koenigii*. CWEP: Cold water extracted polysaccharides; HWEP: Hot water extracted polysaccharides; AEPA: Alkali extracted polysaccharides A; AEPB: Alkali extracted polysaccharides B.

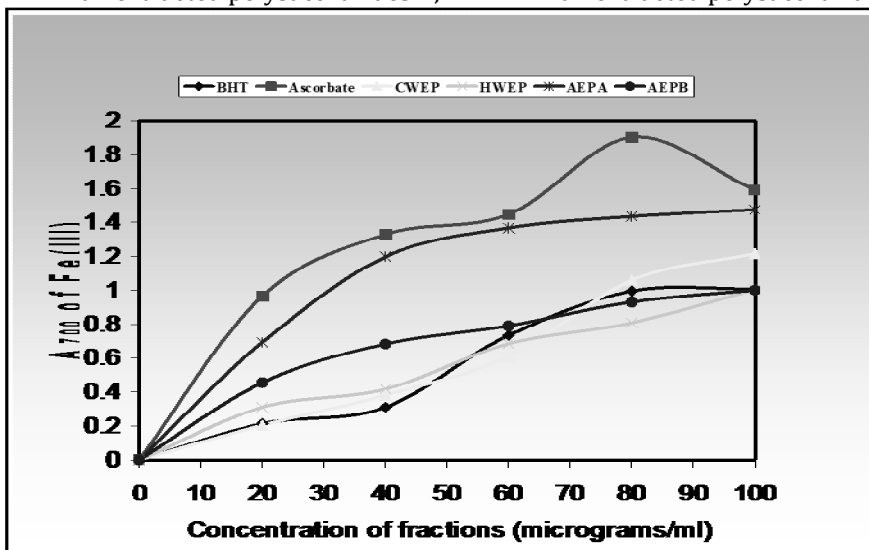


Figure 3: Antioxidant activity of polysaccharide fractions of leaf of *Murraya koenigii* by reducing power activity.

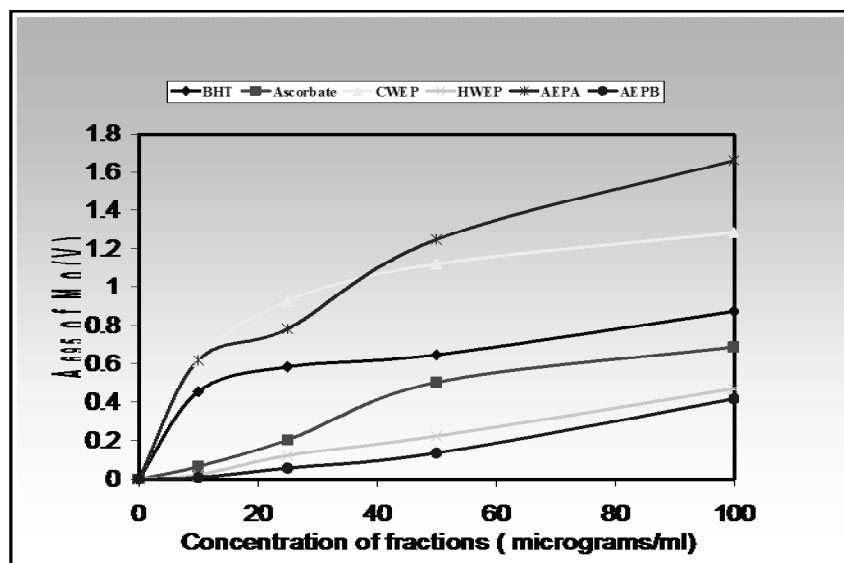


Figure 4: Antioxidant activity of polysaccharide fractions of leaf of *Murraya koenigii* by Phosphomolybdenum method.

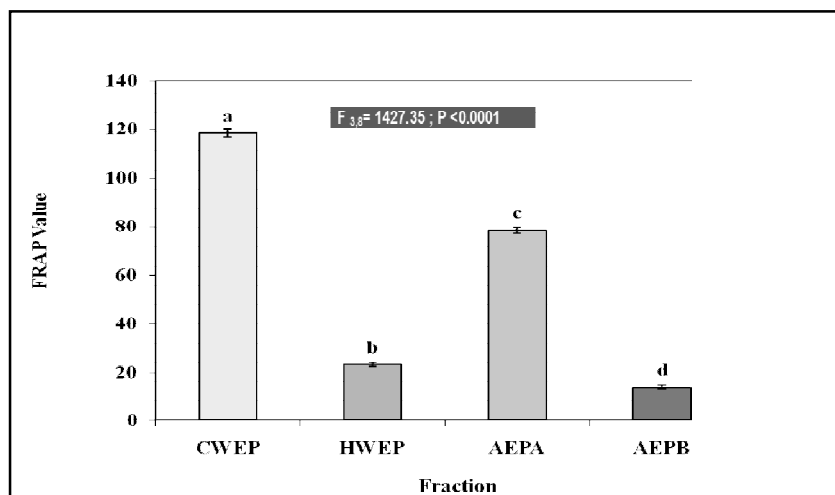


Figure 5: FRAP values in different polysaccharide fractions from *Murraya koenigii*.

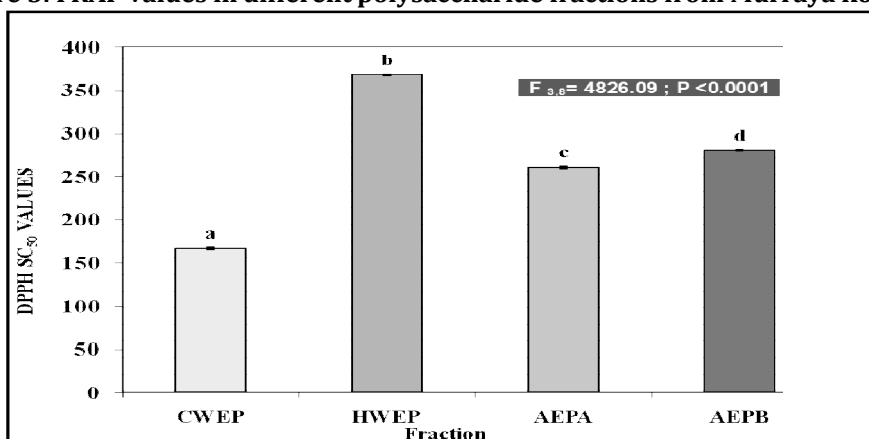
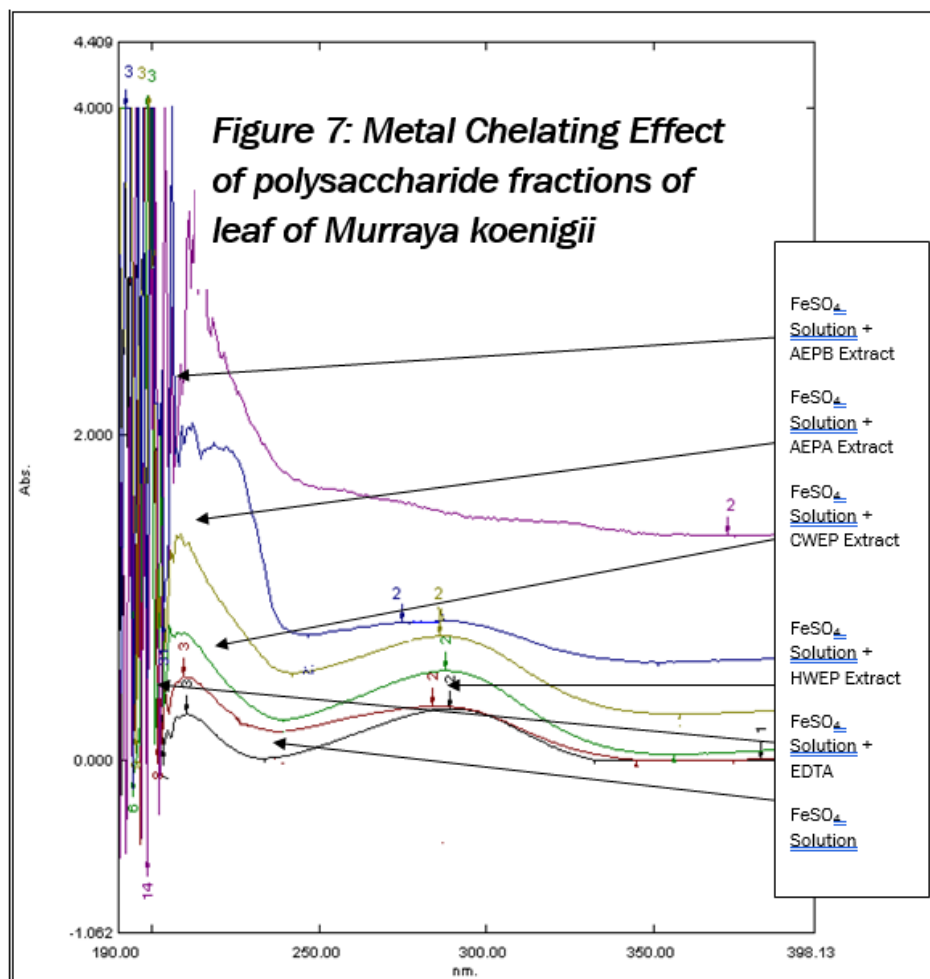


Figure 6: DPPH SC₅₀ value in different polysaccharide fractions from *Murraya koenigii*. Columns represent mean value and vertical bars indicate s.e. (n=3). Similar letters above bars of corresponding series represent no significant difference according to Tukey's multiple comparison procedure at P<0.05. Box in the chart area represents one-way analysis of variance (ANOVA) at P<0.05. CWEP: Cold water extracted polysaccharides; HWEP: Hot water extracted polysaccharides; AEPA: Alkali extracted polysaccharides A; AEPB: Alkali extracted polysaccharides B.



CONCLUSION

Thus the present study represents a novel approach for the importance of antioxidation potential of polysaccharides of *Murraya koenigii*. Polysaccharides from curry leaf potentially could be used as natural antioxidants by the food industry.

It has also been observed that plant polysaccharides (Hot water extracted polysaccharide, Cold water extracted polysaccharide, Alkali extracted polysaccharide A and Alkali extracted polysaccharide B) from leaf powder of *Murraya koenigii* were good source of antioxidants. Among various antioxidation potential assays, different polysaccharides showed different efficacy, which established that all polysaccharides from *Murraya koenigii* were equally important as natural antioxidants.

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