Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(4): 1724-1728
Received: 25-05-2019
Accepted: 27-06-2019

Parveen Kumari
Department of Chemistry, CCS Haryana Agricultural University, Hisar, Haryana, India

Sukriti Nehra
Department of Chemistry, CCS Haryana Agricultural University, Hisar, Haryana, India

MK Deen
Department of Chemistry, CCS Haryana Agricultural University, Hisar, Haryana, India

Correspondence
Parveen Kumari
Department of Chemistry, CCS Haryana Agricultural University, Hisar, Haryana, India

Screening of Chinese rain tree (*Koelreuteria elegans*) leaves, bark and defatted seed cake for study of their total phenolic content, flavonoids and antioxidant properties

Parveen Kumari, Sukriti Nehra and MK Deen

Abstract
The aim of the study was to analyse methanolic crude extract of leaves, bark and defatted seed cake of *Koelreuteria elegans* for total phenolics, flavonoids and antioxidant properties. Total phenolic contents were determined using Folin-Ciocalteau reagent and calculated as Gallic Acid equivalents per gram of extract. Aluminium chloride colorimeteric method was used for flavonoids determination. The antioxidant activities of the extracts were measured by 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) method. The bark extract showed maximum antioxidant activity of 96 % at a conc. of 0.06 mg/mL with EC_{50} value 0.023±0.002 mg/mL of extract followed by leaves extract 95 % at a conc. of 0.07 mg/mL with EC_{50} value 0.022±0.002 and the seed cake extract 93 % at a conc. of 0.07 mg/mL with EC_{50} value 0.025±0.001 by DPPH method. Based on observations, it can be concluded that Chinese rain tree bark, leaves and the seed cake have a potential source of antioxidants of natural origin.

Keywords: Antioxidant activity, flavonoids, phenolics, gallic acid

1. Introduction
Antioxidants are compounds that scavenge the active radicals to suppress oxidation of other molecules by inhibiting the initiation or propagation of oxidative chain reactions [17]. Free radicals and reactive oxygen species (ROS) like superoxide, hydroxyl radical, peroxyl radical are highly reactive substances [12, 13]. These may cause reversible or irreversible damage to biological molecules such as lipids, proteins [5]. Antioxidants have been used to prevent oxidative deterioration and preservation of food. Synthetic antioxidant compounds such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate, tertiary butylhydroquinone are often added to various foodstuffs to prevent oxidative degradation but recent studies revealed the safety and health issues regarding the use of synthetic antioxidants in foods and also negatively sensed by consumers [8, 10]. Hence, with this awareness much interest has been taken in searching plant-derived antioxidants which provide health benefits.
Crude extracts of plant origin are rich in phenolics are increasingly of interest in food industry because they delay oxidative degradation of lipids thereby improve the quality and nutritional value of food. The preservative effect of extract of many plant species and herbs suggests the presence of antioxidative and antimicrobial constituents in their tissue [7]. Many food plants contain large amounts of antioxidants other than vitamin C, vitamin E and carotenoids [16]. The approach of phytochemicals in medicinal plants is mainly concentrated on their role in preventing diseases caused as a result of oxidative stress. Antioxidant compounds derived from plant sources such as phenols, flavonoids etc. are recommended as nutraceuticals/ functional foods [3, 4].
Koelreuteria elegans (Chinese rain tree) is an ornamental landscape tree belonging to family *Sapindaceae*. It is native tree of Taiwan. It is a fast growing species and tolerant of a wide range of environmental conditions. The other species *K. paniculata*, *K. bipinnata*, *K. henryi* are widely distributed in Northern China. Local people use the seeds of *K. elegans* as insecticides and the leaves as anti-fungal and anti-bacterial agent. Roots, bark, twigs and leaves of *K. henryi* have been used for the treatment of diarrhea, malaria and urethritis in traditional folk medicine [9]. It also exhibits significant anti-proliferation activity against cancer cell lines [15].
The high content of polyphenolic compounds and flavonoids reported in many species of the genus *Koelreuteria* act as antioxidant against oxidative stress and scavenge free radicals. Hence, in the absence of any report regarding antioxidant activity of *Koelreuteria elegans*, the

present investigation was to estimate total phenols, flavonoids and antioxidant activity of methanolic extracts of leaves, bark and seed cake of chinese rain tree.

2. Materials and Methods

2.1 Material and Chemicals

The seeds, leaves, and bark of *Koelreuteria elegans* were collected from the university campus CCS, Haryana Agricultural University, Hisar. After cleaning, the seeds, leaves and bark were dried in shade and ground to fine powdered form. Commercially available chemicals from Sigma-Aldrich, Qualigens, Merk and Ranbaxy, of highest purity, were used for various experimental procedures.

2.2 Solvent extraction

The powdered sample of leaves, bark and seed cake were extracted with petroleum ether (60-80°) using soxhlet method. The dried defatted powdered sample of each were then extracted separately with methanol by refluxing 8h. These extracts were analysed of total phenols, flavonoids, and DPPH free radical scavenging activity.

2.3 Determination of total phenolics content

The total phenolics were determined by the Folin-Ciocalteu reagent method using gallic acid as standard [14]. Diluted extracts (1.0 mL) were added to test tube containing 1.0 mL of (1:1) Folin-Ciocalteu reagent and 2.0 mL of Na_2CO_3 (20% w/v) mixed and volume was made up to 10 mL with double distilled water. After 30 minutes, the mixture was centrifuged at 6000 rpm for 10 minutes and the absorbance of supernatant solution was measured at 730 nm using spectrophotometer against a blank prepared similarly with the same solvent but omitting the extract. The amount of total phenolics present in the extracts was calculated from the standard curve and the results was expressed as milligrams of gallic acid equivalent per gram (mgGAE/g).

2.4 Determination of flavonoids

The aluminium chloride colorimetric method for estimation of flavonoids was used [18]. Diluted extract (1.0 mL) was added to test tubes containing 4mL of double distilled water. To the mixture 0.3 mL 5% $NaNO_2$ was added. After 5 minute, 0.3 mL 10% $AlCl_3$ was added. Immediately, 2.0 mL 1M NaOH was added and the total volume was made upto 10.0 mL with double distilled water. The solution was mixed thoroughly and the absorbance of both the samples and blank (Reagent blank using double distilled water instead of sample was prepared) was read at 510 nm using UV visible spectrophotometer. Total flavonoids of samples were expressed as mg catechin equivalent per gram of the extract (mg CAE/g).

2.5 2, 2'-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

The antioxidant activity of the extracts was evaluated by DPPH free radical scavenging method [6]. Briefly 0.01, 0.02, 0.03, 0.04, 0.05,0.06,0.07,0.08,0.09 mg of methanol extracts of bark, leaves and seed cake were added to flasks containing 1.0 ml of 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH: $0.025gL^{-1}$ in methanol) final volume was made to 10 mL with methanol and mixed for 5 minute. The absorbance of the sample was measured at 517 nm in every 10 minutes till a steady state is reached using the spectrophotometer Spectronic 20 (Milton Roy Company). For each sample separate determinations were carried out. Similarly, a control sample was also prepared. The antioxidant activity was expressed as the percentage of decline of the absorbance after 2 hrs relative to the control, corresponding to the percentage of DPPH that was scavenged.

Calculation

The percentage of DPPH, which was scavenged (% $DPPH_{sc}$) was calculated using:

$$\% DPPH_{sc} = \{(A_{cont} - A_{samp}) / A_{cont}\} \times 100$$

Where A_{cont} is the absorbance of control and A_{samp} is the absorbance of sample.

2.6 Stastical analysis

Three replicates of each sample were used for stastical analysis and resulting values are expressed as mean±S.D. Correlation analyses of antioxidant activity, flavonoids and total phenolic content were carried out using pearson correlation programme in Online Stastical Analysis (OPSTAT www.hau.ernet.in)

3. Results and Discussion

3.1 Yield percentage, total phenols, flavonoids and free radical scavenging activity

Extracts yield, total phenols, flavonoids, free radical scavenging activity by DPPH method, of all methanolic extracts are presented in the Table 1. Extract yield (%) was found to be highest in leaves extract ie 22.9±0.2 followed by bark extract (19.5±0.4) and then seed cake extract (17.0±0.2). Total phenolic content (mg GAE/g) was reported to be highest in leaves extract ie 109.1±2.5 followed by bark (99.3±1.2) and seed cake (60.0±0.1) extracts. Similar trend was observed in flavonoids content. It was highest in leaves extract (36.3±0.2 mgCAE/g) followed by bark extract (26.9±0.4 mgCAE/g) and seed cake extract (10.0±0.06 mgCAE/g). Phenolics are secondary plant metabolites, aromatic in nature having high level antioxidant properties because of their ability to scavenge free radical.

Table 1: Phytochemical content and antioxidant activity of chinese rain tree extracts

Parameters	Bark extract	Leaves extract	Seed cake extract
Yield of methanol extract (%)	19.5±0.4	22.9±0.2	17.0±0.2
Total phenols mgGAE/g	99.3±1.2	109.1±2.5	60.0±0.1
Total flavonoids mgCAE/g	26.9±0.4	36.3±0.2	10.0±0.06
DPPH(EC_{50}) (mg/mL)	0.023±0.002	0.022±0.002	0.025±0.001
DPPH (Max. activity %) Conc. (mg/mL)	96 (0.06)	95 (0.07)	93 (0.07)

Values are mean of three replicates ± standard error
mg GAE/g- milligrams gallic acid equivalent/g of the extract
mg CAE/g- milligrams catechin equivalent/g of the extract

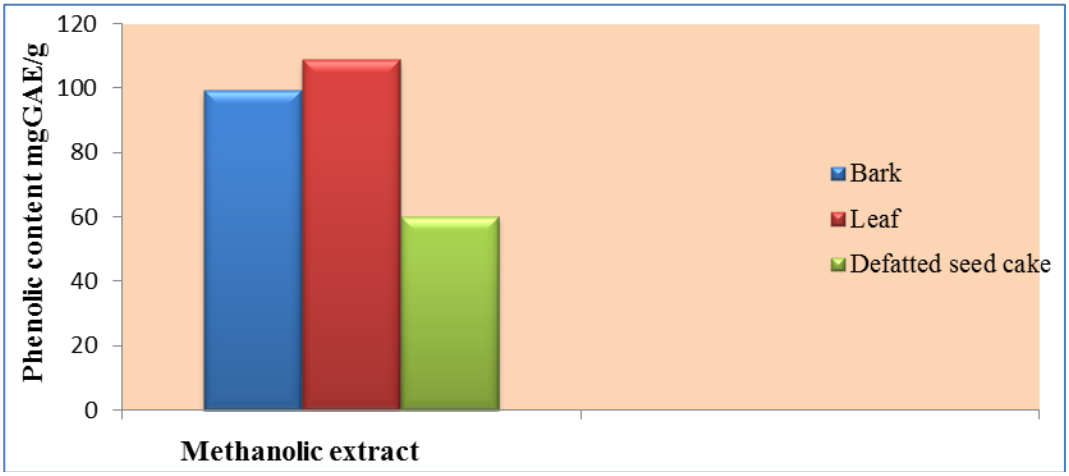

Fig 1: Total phenolic content of methanolic extract of bark, leaves and defatted seed cake of *Koelreuteria elegans*

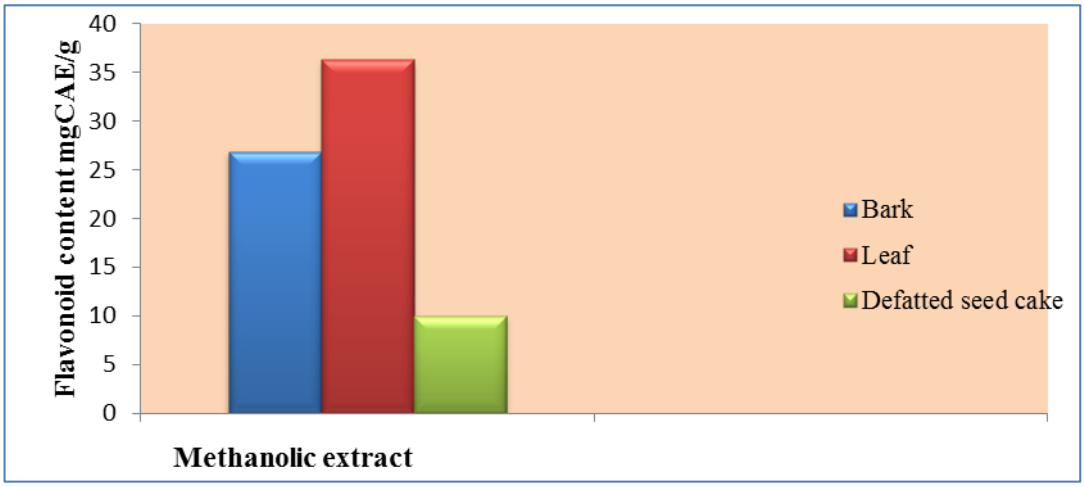

Fig 2: Flavonoid content in methanolic extract of bark, leaves and defatted seed cake

3.2 DPPH radical scavenging efficiency

2,2'- diphenyl-1-picrylhydrazyl radical (DPPH) was used to evaluate the antioxidant activity. It is one of the few stable radicals. Alcoholic solutions of DPPH show maximum absoption at 517 nm. When an antioxidant (AH) is added to DPPH solution a decrease in absorbance at 517 nm takes place due to the formation of the non-radical form DPPH-H which does not absorb at 517 nm. All the above described extracts were screened for radical scavenging activity against DPPH. The antioxidant activity exhibited by methanolic extracts of bark, leaves and seed cake was 96 % at a conc. of 0.06 mg/mL, 95 % at a conc. of 0.07 mg/mL and 93 % at a conc. of 0.07 mg/mL reapectively. The corresponding EC_{50} values to scavenge DPPH radical were 0.023±0.002 mg/mL, 0.022±0.002 mg/mL and 0.025±0.001 mg/mL respectively. Among all the methanolic extracts bark extract showed maximum antioxidant acticity in terms of radical scavenging capacity as well as inhibition percentage which is at par with BHA (standard) (figure 3).

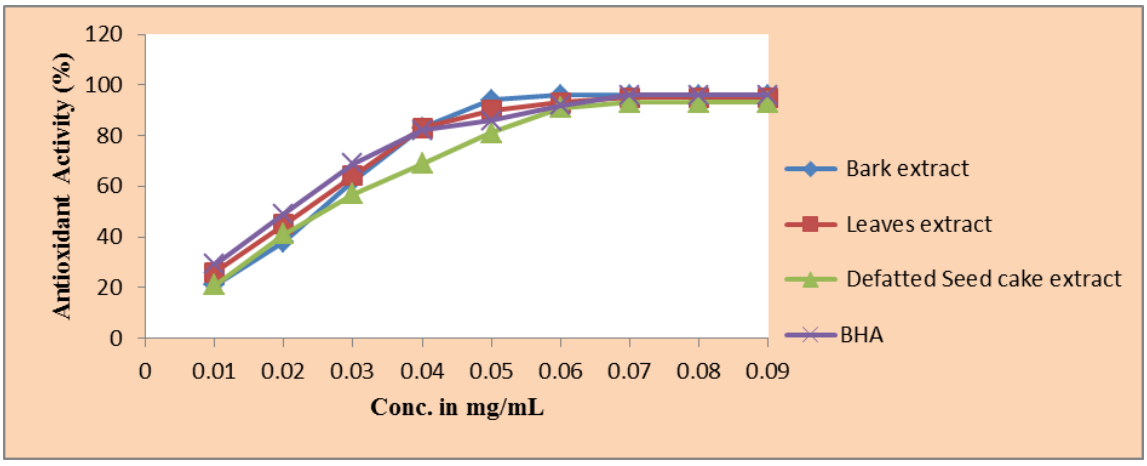

Fig 3: Antioxidant Activity (%) of all the extracts by DPPH method (BHA taken as standard)

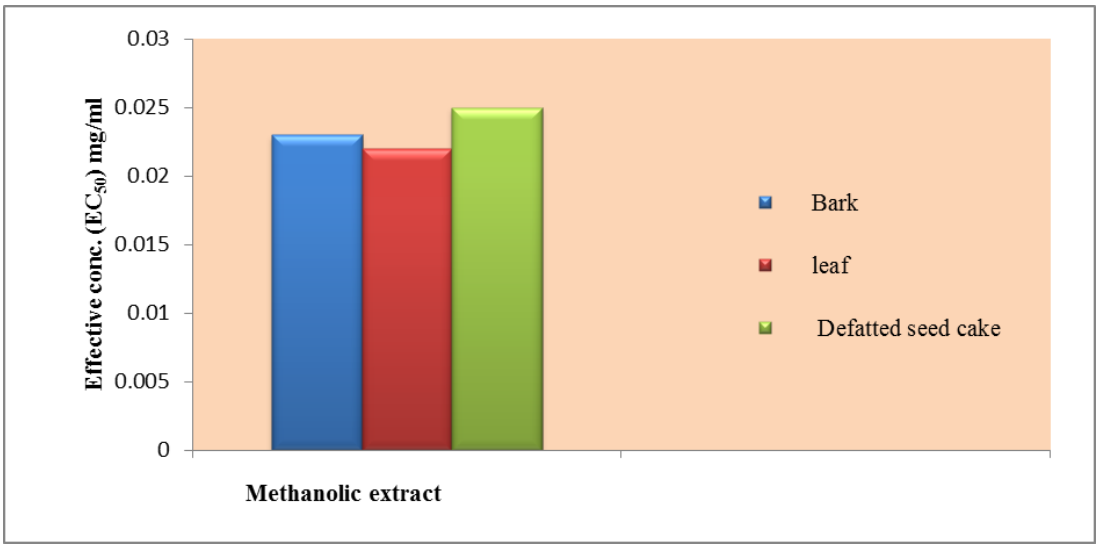

Fig. 4: Antioxidant activity (EC_{50}) of methanolic extract of bark, leaves and seed cake

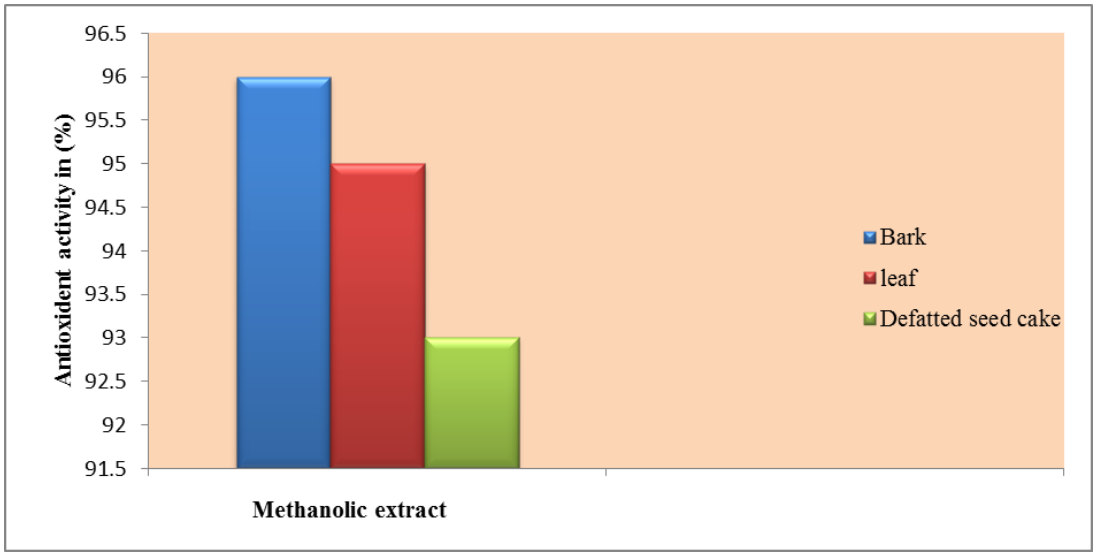

Fig. 5: Antioxidant activity (%) of methanolic extract of bark, leaves and the seed cake

Redox properties of phenolic compounds including reducing agents, hydrogen donars, and singlet oxygen quenchers account for their antioxidant nature [11]. Here, all the three methanolic extract exhibit high free radical scavenging activity. This might be due to hydroxyl groups in the structure of phenolic compound that can provide necessary component as a radical scavenger. Presence of hydroxyl substituents on flavonoid skeleton boosts activity [1, 2].

4. Conclusion

The methanolic extracts of bark, leaves and defatted seed cake contain remarkable amount of phenols and flavonoids and exhibit excellent antioxidant potential. So we can conclude that these extracts may provide potential natural antioxidant for nutraceutical industry and other fields. Present study could be an effective introduction to the antioxidant properties of bark, leaves and seed cake of Chinese rain tree and more work should be done to characterise individual phenolic compounds in order to assign their antioxidant properties.

5. References

1. Acker SABE, Van Van den Berg DJ, Tromp MNJL, Griffioen DH, Van Bennekom WP, Van der Vijgh WJ *et al*. Structural aspects of antioxidant activity of flavonoids. Free Radical Biology and Medicine. 1996; 20:331-342.
2. Arora A, Nair MG, Strasburg GM. Structure-activity relationships for antioxidant activities of a series of flavonoids in a liposomal system. Free Radical Biology and Medicine. 1998; 24:1355-1363.
3. Aruoma OI. Nutrition and health aspects of free radicals and antioxidants. Food Chemical and Toxicology. 1994; 32:671-683.
4. Ferguson LR. Role of plant polyphenols in genomic stability. Mutation Research. 2001; 475:89-111.
5. Goldberg G. Plants: Diet and health. The report of a British Nutrition Foundation Task Force. 2003; Blackwell Science. Oxford. U.K.
6. Hatano T, Kagawa H, Yasuhara T, Okuda T. Two new flavonoids and other constituents in licorice root; their relative astringency and radical scavenging effects. Chemical and Pharmaceutical Bulletin. 1988; 36:2090-2097.
7. Hirasa K, Takemasa M. Spice science and technology, Chemical characterisation of some plants found in local accession and used in traditional medicines in Iran. Journal of Agricultural Food Chemistry. 1998; 50:5878-5883.

8. Iqbal S, Bhanger MI, Anwer F. Antioxidant properties and components of bran extracts from selected wheat varieties commercially available in Pakistan. LWT-Food Science and Technology. 2007; 40:361-367.
9. Jeng YC. (ed). Chinese medicinal plants. Publisher's Reader's Digest Association Far East Ltd. Hong Kong, 1994, 207
10. Jeong SK, Kim SY, Kim DR, Jo SC, Nam KC, Ahan DU. Effect of heat treatment on the antioxidant activity of extracts from citrus peels. Journal of Agricultural Food Chemistry. 2004; 52:3389-3393.
11. Nakatani N. Antioxidants from species and herbs. In: Shahidi F. editor. Natural antioxidants: chemistry, health effects and applications, Champaign: IL, AOCS, 1997, 64-75.
12. Niki E. Free radical pathology and antioxidants. Journal of Nutritional Science Vitaminology. 1992; 38:538-540.
13. Niki E. Free radical in the 1900's from *in-vitro* to *in-vivo*. Free Radical Research. 2001; 33:693-704.
14. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. American Journal of Enology Viticulture. 1965; 16:144-158.
15. Song YN, Zhang HL, Chang CJ, Bollag DM. Cytotoxic cyclolignans from Koelreuteria henryi. Journal of Natural Products. 1994; 57:1670-1674.
16. Spanos GA, Wrolstad RE. Infuence of processing and storage on the phenolic composition of Thompson seedless grape juice. Journal Agricultural Food Chemistry. 1990; 38:1565-1571.
17. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agricultural Food Chemistry. 1998; 46:4113-4117.
18. Zhishen J, Mengcheng T, Jjianming W. Determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry. 1999; 64:555-559.