Journal of Medicinal Plants Research Vol. 6(3), pp. 500-503, 23 January, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.1289
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Investigation of the effects of extraction solvent/technique on the antioxidant activity of *Cassia fistula L.*

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Accepted 30 November, 2011

Cheaper and safer antioxidants of natural origin is the focus of research in recent times due to increased in safety concerns about synthetic antioxidants. The effects of four extracting solvents that is, ethanol, methanol, n-Hexane and pet ether and two extraction techniques that is, simple maceration and hot percolation (Soxhlet apparatus) were investigated on the antioxidant activity of pods, leaves, barks and flowers of *Cassia fistula*. 1,1-diphenyl-2-picryl-hydrazyl (DPPH) was used as standard free radical while ascorbic acid (Vitamin C) and Quarcetin were used as standard anti oxidants. Experiments revealed that extracts have solvent-dependent and technique-dependent antioxidant effects. Using the simple maceration technique, 70% methanolic v/v leaf extract showed 89% DPPH scavenging activity when ascorbic acid was taken as standard and 84.7% when quercetin was taken as standard. However, the percentage inhibition of a similar concentration of pods, barks and flowers extract were 66, 81 and 83.4%, respectively, using simple maceration and ascorbic acid as a standard. However, extraction carried out by Soxhlets apparatus showed less free radical scavenging activities.

Key words: *Cassia fistula,* soxhlet apparatus, antioxidant activity.

INTRODUCTION

The most frequently used technique for the isolation of plant antioxidants is solvent extraction that is, maceration and percolation. Conversely, the extract yields and resulting antioxidant activities of the plant materials are strongly reliant on the type of extracting solvent, due to the presence of different antioxidant compounds of diverse chemical characteristics and polarities that may or may not be soluble in a particular solvent. Usually polar solvents are used for the recovery of polyphenols from a plant matrix. The most frequently used solvents include Acetone, Ethanol, Methanol, n-Hexane, Pet Ether, Ethyl acetate and Hydroalcoholic mixtures (Peschel et al., 2006; Bushra et al., 2009). Recently, there is a great interest in evaluating antioxidants and distribution prototype of fruits and vegetables. It occurred when it was known that plant botanicals, carotenoid and phenolics are important for the sensory properties of food, have pharmacological activities and shielding effects against a variety of degenerative diseases (Yigit et al., 2009). Several studies have revealed that many plant botanicals are used in the prevention of several diseases (Chu et al., 2002). The antioxidant system in plants is very complex, with antioxidants having different targets, sizes and interactions with each other.

Biological antioxidants as "molecules which, when present in small concentrations compared to the biomolecules they are supposed to protect, can prevent or reduce the extent of oxidative destruction of biomolecules (Kerchev, 2009). Indian Laburnum is distributed in various countries including Asia, Mauritius, South Africa, Mexico, China, West Indies, East Africa and

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Table 1. Conditions used to compare soxhlet and simple maceration extractions.

S/N	Parameter	Soxhlet extraction	Simple maceration
1	Sample size (g)	40	150
2	Extraction solvent	80% ethanol, 70% methanol, 40% methanol (v/v) and n-Hexane and pet ether	80% ethanol, 70% methanol, 40% methanol (v/v) and n-Hexane and pet ether
3	Temperature (℃)	70	Ambient
4	Flow-rate	35 min/cycle	N.A
5	Time	48 h	72 h
6	Solvent volume (ml)	1000 ml	1500 ml

Brazil as an ornamental tree for its beautiful bunches of yellow flowers. Recognize by the British pharmacopoeia (Mukhopadhyay et al., 1998). The main constituents present in seeds are tannins, fatty acids isoflavonoids, flavonoids, glycosides, anthraquinones, and phenolic compounds (Nayan et al., 2011). The seeds are reported to have demulcent and lubricating effect, bitter, acrid, cooling, emollient and useful in skin diseases, pruritus, burning sensation, dry cough and bronchitis (Sharma et al., 2005). The whole plant possesses medicinal properties useful in the treatment of skin diseases, inflammatory diseases, rheumatism, anorexia and jaundice. The root is useful in skin diseases, leprosy, tuberculosis, and glands' cures, burning sensations. The leaves are laxative, antipyretic; heal ulcers, used in rheumatism (Kirtikar and Basu, 1991). It also possesses some other important pharmacological properties such as antibacterial, Antifungal and anticandidal (Panda et al., 2011). The present study was conducted with the main objective of investigating the most effective solvent/technique for exploring the most potent antioxidant part of *Cassia fistula*.

MATERIALS AND METHODS

Chemicals

1, 1-Diphenyl-2-picrylhydrazyl (Sigma Germany), Ethanol and Methanol (Merck KGaA Darmstadt, Germany), Petroleum Ether (BDH England), and n-Hexane (Franken Chemicals, Germany) were chemical used in this study.

Apparatus

Elisa Plate Reader (Biotek Synergy HT), Soxhlet Assembly (Pyrex England), Electrical Balance (Precisa BJ-210, Switzerland), Refrigerator Dawlance, Pakistan), Rotary evaporator (Eyela Company Limited Japan), ultraviolet (UV) Spectrophotometer (Shimadzu Japan).

Plant material

The choice of plant materials in the present study was based on their prospective folk medicinal uses. Medicinal plant parts that is, barks, fresh leaves, ripped pods and flowers were collected from Abbasia Campus, the Islamia University of Bahawalpur, Pakistan. The identification of this plant was performed at Cholistan Institute of Desert studies, The Islamia University of Bahawalpur. A voucher specimen was preserved at the herbarium of Pharmacognosy Section, Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur for future reference.

Preparation of plant extracts

Simple maceration

150 g shade-dried ground plant material for each sample was extracted with each of the solvents - 80% ethanol, 70% methanol, 40% methanol (v/v), n-Hexane and pet ether (1500 ml) – for 72 h at room temperature in a in 5 liter beaker in separate experiments. The residues were extracted twice with the same fresh solvent and extracts combined.

Soxhlet extraction

40 g shade-dried ground plant material for each sample was extracted with each of the solvents – 80% ethanol, 70% methanol, 40% methanol (v/v), n-Hexane and pet ether (1000 ml) for 48 h at 70℃ temperature in a soxhlet apparatus under reflux on a water bath in separate experiments. Conditions used to compare soxhlet and simple maceration extractions are shown in Table 1.

Filtration

The extracts were separated from the residues by filtering 1st through several layers of muslin cloth for coarse filtration and then through Whatman No. 1 filter paper.

Evaporation

The filtered extracts were concentrated and solvents were evaporated under reduced pressure at 40℃, using a rotary evaporator (EYELA, CA-1111, Rikakikai Company Limited Tokyo, Japan). The dried crude concentrated extracts were weighed to calculate the yield and stored in a refrigerator (- 8°C), until used for analyses.

DPPH scavenging activity of different plant parts

DPPH scavenging activities were performed in accordance to Blois method (Blois, 1958). A 0.5 mM 1, 1-diphenyl-2-picrylhydrazyl

Table 2. DPPH scavenging activity for various parts of *C. fistula* while using the simple maceration.

Plant part	*DPPH scavenging activity in % (simple maceration)				
	Solvent				
	70% methanol	40% methanol	80% ethanol	n-Hexane	Petroleum ether
Leaves	89.0	76.6	80.1	71.5	70.7
Flowers	83.4	76.3	72.0	68.6	66.7
Bark	81.0	80.5	67.4	69.0	70.6
Pods	77.0	65.0	74.5**	71.2	70.8

** 66% for quercetin standard.

Table 3. DPPH scavenging activity for various parts of *C. fistula* while using the hot percolation (soxhlet apparatus).

Plant part	*DPPH scavenging activity in % (soxhlet extraction)				
	Solvent				
	70% methanol	40% methanol	80% ethanol	n-Hexane	Pet ether
Leaves	66.0	66.5	52.5	65.7	67.0
Flowers	56.5	62.5	64.5	54.0	59.5
Bark	68.8	66.5	66.5	10.8	49.0
Pods	70.8	71.0	69.8	27.0	66.5

(DPPH) radical solution was prepared in respective solvents, and 5 µL of this solution was mixed with 2.5 ml of the sample solution. Final concentrations of extracts were 100 and 300 µg/ml. The obtained mixtures were kept at room temperature for 20 min. Then, the absorption of the mixtures at 517 nm was taken, in comparison with the control solution (maximum absorption). A decreasing absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity. This activity is reported as a percent of DPPH radical scavenging according to the following relation:

$$\% \ Inhibition = \frac{(\text{A control} - \text{A test})}{\text{A control}} \times 100$$

DPPH radical scavenging activity was measured in duplicate, and the values are reported as the average.

RESULTS AND DISCUSSION

DPPH scavenging activity of different plant parts

Simple maceration

The DPPH radical scavenging activities of various plant extracts from *C. fistula* cultivars are shown in Tables 2 and 3. The extracts of all plant parts possessed free radical scavenging properties, but to varying degrees, up to 89%. Using the simple maceration technique, 70% methanolic v/v leaves extract showed 89% DPPH scavenging activity when ascorbic acid was taken as standard and 84.7% when quercetin was taken as standard. Pods, barks and flowers showed 66, 81 and 83.4% DPPH scavenging activity, respectively when ascorbic acid was taken as standard. Almost similar results were found for all these three parts when quercetin was used as standard. In case of 40% methanol, a maximum scavenging activity was offered by leaves extract (78.6%), followed by pods extract (77%) and flowers extract (76.3%). 80% ethanolic leaves extract showed 80.1%, bark 67.4%, flowers 72% and pods 74.5% scavenging activity for Vitamin C standard. Similar results were found when quercetin was used as standard expect for pods which showed 66% activity. DPPH scavenging activity for ethanol, ether and n-Hexane are shown in Table 1. Various researchers have screened aqueous extract of *C. fistula* for anti oxidant activity.

Manonmani et al. (2005) suggested that *C. fistula* (Linn.) flowers have got promising antioxidative activity in alloxan diabetic rats (Manonmani et al., 2005). Our findings are in good agreement with the previous findings of Nayan et al. (2011) who found that hydro alcoholic extracts seeds of *C. fistula* have significant radical scavenging activity when DPPH assay was compared to ascorbic acid (vitamin C), and ferric reducing power Oyaizu method. As the solvent is concerned our findings are in agreement with the study of Subramanion et al. (2011) who found that the methanolic extract exhibit a significant dose dependent inhibition of DPPH activity with 50% of inhibition (IC_{50}) at concentration of 11.07 mg/ml.

Soxhlet extraction

DPPH scavenging activity for various parts of *C. fistula* while using the hot percolation (Soxhlet apparatus) are shown in Table 2. The overall results are low for hot percolation as compared to the simple maceration technique. These results are in good agreement with the previous findings of Cheng et al. (2006) who found the effects of postharvest treatment and heat stress on availability of plant antioxidants (Cheng et al., 2006). It is well known that free radical scavenging activity of plant botanicals is chiefly due to polyphenols. The low radical scavenging activity of the *C. fistula* various parts extract, obtained by the Soxhlet apparatus might be attributed to the thermal decomposition of plant phenolic compounds (Bushra et al., 2009).

Conclusion

The results of the present investigation revealed that hydroalcoholic solvent (70% methanol) extracts of leaves, prepared by simple maceration techniques, exhibited better antioxidant activities so we sugesst that methanol (70%) will be the best solvent and simple maceration will be the best technique for the extraction of antioxidant activities of *C. fistula L*.

ACKNOWLEDGEMENTS

The authors thank the Dean and Chairman of the Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur for their constant encouragement and moral support.

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