Bionano Frontier, Vol.(2) July to December 2014, 231-234

EVALUATION OF PRELIMINARY PHYTOCHEMICAL PROPERTIES AND ANTIOXIDANT ACTIVITY: *LAWSONIA INERMIS* L.

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Received on : 10.12.2013 and Accepted Revised on 26.06.2014

ABSTRACT: The antioxidant activity of the aqueous extracts of *Lawsonia inermis* L. leaves were evaluated by several *in vitro* systems of assay, namely, reducing power assay, nitric oxide scavenging activity and total antioxidant activity. In the present study the total phenolic content was evaluated. The free radical scavenging and antioxidant activity was attributed to the presence of phenolic and flavonoid compounds in the leaf extract. Preliminary phytochemical analysis performed showed the presence of alkaloids, carbohydrate, tannins, phenols, flavonoids, essential oil, saponins, etc. *Lawsonia inermis* L. procured from Jodhpur (Rajasthan) showed higher phenolic content and antioxidant activity than leaf procured from Kalyan (Maharashtra).

KEY WORDS: *Lawsonia inermis* L., nitric oxide scavenging activity, ferric reducing power, antioxidant activity, phenolic content.

INTRODUCTION :

Antioxidants protect against free radicals and they are therefore essential in obtaining and preserving good health. Much attention has been given to polyphenols with strong antioxidant activities, which are ubiquitously present in a broad range of medicinal plants and dietary products. The antioxidants may reconcile their upshot by directly reacting with ROS, quenching them and/or chelating the catalytic metal ions (Robak and Marcinkiewicz, 1995). Several synthetic antioxidants, e.g., butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commercially accessible but are quite perilous and their toxicity is a problem of disquiet (Madhavi and Salunkhe, 1995). The use of these synthetic antioxidants, have been restricted in foods as they are suspected to be carcinogenic. Therefore, the importance of search of natural antioxidants has greatly increased in the recent years (Jayaprakasha *et al*., 2003).

Lawsonia inermis L. (Henna) belongs to the family Lytheracae. It is known for its healing attributes thus a subject of intense scientific study. The leaves are used in the treatments of wounds, ulcers, cough, bronchitis, lumbago, rheumatagia, inflammations, diarrhoea, dysentery, leucoderma, scabies, boils, anemia, hemorrhages, fever, falling of hair and greyness hair (Kidanemariam, 2013). Henna has a wide range of medicinal properties and there is an increasing awareness among people towards this plant due to their non-toxic properties, fewer side effects, more medicinal value. This versatile plant is the source of various types of chemical compounds (Phirke and Saha, 2013). Therefore, the purpose of the present study is to evaluate preliminary phytochemical screening and antioxidant activity of aqueous extracts of *Lawsonia inermis* L.

MATERIALS AND METHODS:

Collection of plant material: Leaf material of field grown plant of *Lawsonia inermis* L. from Kalyan (Maharashtra) and Jodhpur (Rajasthan) were collected, dried, powdered and stored in air tight containers separately. Authentication of the plant (S.H.-1533) was done at Blatter Herberium, St. Xavier's College, Mumbai and the specimen voucher was deposited there.

Phytochemical Screening: Qualitative chemical examination of the dried leaf powder of *Lawsonia inermis* L. revealed the presence or absence of various plant constituents in different chemical extracts. The observations were recorded in + (present) or – (absent). The tests were performed according to Khandelwal (1998) and Kokate (2007).

Preparation of crude extract: 500 mg of *Lawsonia inermis* L. leaf powder (Kalyan and Jodhpur) was extracted separately in 100 ml of distilled-water overnight. The content was filtered through Whatman filter paper No. 1. The filtrate was evaporated on boiling water bath until dry. The extracts were then stored for further use.

Total Phenolic Content: The soluble phenolic of *Lawsonia inermis* L. was estimated by Folin-Ciocltеau reagent method (Slinkard and Singleton, 1977) using gallic acid as a standard phenolic compound. The total phenolic content of different extracts was measured using colorimetric Folin - Ciocalteu method. The reaction mixture consisted 0.5ml of diluted sample to which 0.5 ml of distilled water and 0.5 ml Folin - Ciocalteu reagent was added. After 3 minutes, add 2 ml of 20% Na_2CO_3 solution and place the tubes in boiling water bath for one min, cooled and the absorbance was measured at 760 nm. Standard graph was prepared by using different concentration of gallic acid.

Total Antioxidant Activity: 0.1ml of extract was combined in Eppendorf tube with 1 ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped and incubated in thermal block at 95°C for 90 minutes. After cooling to room temperature; the absorbance of the aqueous solution of each was measured at 695 nm against blank (Shirwaikar, *et al*., 2006).

Reducing power assay: The reducing power of aqueous extracts was determined according to the method of Oyaizu.

Different concentrations of leaf extract of *Lawsonia inermis* L. (50– 1000 µg ml^{-1}) in 1ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferrocyanide (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm and compared with standard Ascorbic Acid. Increased absorbance of the reaction mixture indicated increased reducing power.

Nitric oxide radical scavenging activity: Nitric oxide was generated from sodium nitroprusside and measured by the Griess reaction. Sodium nitroprusside in aqueous solution at physiological *p*H spontaneously generates nitric oxide, which interacts with oxygen to produce nitric ions that can be estimated by use of Griess reagent. Scavenger of nitric oxide competes with oxygen leading to reduced production of nitric oxide (Sreejayan, 1997). Sodium nitroprusside (5 mM) in phosphate–buffered saline (PBS) was mixed with 3.0 ml of different concentrations (1000-5000 µg ml^{-1}) of the drugs dissolved in the suitable solvent systems and incubated at 25°C for 150 min. The samples from the above were reacted with Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm and referred to the absorbance at standard solutions of potassium nitrite, treated in the same way with Griess reagent. The percentage scavenging of nitric oxide of *Lawsonia inermis* L. and standard Ascorbic acid was calculated using the following formula:

NO Scavenged (%) = (A cont - A test)/A cont × 100

Where A cont is the absorbance of the control reaction and A test is the absorbance in the presence of the sample of the extracts.

RESULTS AND DISCUSSION:

Preliminary phytochemical analysis of leaf extracts from *Lawsonia inermis* L. showed the presence of alkaloids, anthraquinone, carbohydrates glycosides, saponins, flavonoids, tannins and essential oils (Table 1).

Total phenolic content: The total phenolic contents in the examined leaf extracts using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalent (the standard curve equation: y = 0.019– 0.028, r^2 = 0.985). The total phenolic contents of *Lawsonia inermis* L. were 2.91 (Kalyan) and 4.75 (Jodhpur) µg gallic acid equivalent/mg of sample, respectively (Table 2). Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities (Van Acker *et al*., 1996).

Total antioxidant capacity: Total Antioxidant capacity of *Lawsonia inermis* L. is shown in Table 2. In this assay aqueous extract of *Lawsonia inermis* L. from Jodhpur was found to have higher activity than Kalyan. The phosphormolybdenum method was based on reduction of MO (VI) to MO (V) by the antioxidant compound and the formation of green phosphate/ MO (V) complex at acidic pH. The extracts demonstrated electron donating capacity and thus they may act as radical chain terminators, transforming reactive free radical species into stable non reactive products (Dorman *et al.*, 2003).

Reducing power ability: The results showed that the reducing power of the aqueous extract of leaf powder (Kalyan) was less than leaf powder (Jodhpur) (Table 3). As shown in Figure 2, a higher absorbance value indicates a stronger reducing power of the samples. Leaf extract of *Lawsonia inermis* L. showed concentration-dependent reducing power. However, its reducing power was weaker than that of ascorbic acid, which exhibited the strongest reducing power. In the reducing power assay, the antioxidant compounds convert the oxidation form of iron (Fe+3) in ferric chloride to ferrous (Fe+2). The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. The reducing power increased with increasing amount of the extract. Increased absorbance of the reaction mixture indicated increased reducing power (Gupta *et al.*, 2007).

Nitric oxide scavenging method: Nitric oxide scavenging activity of aqueous extract of leaf powder (Kalyan and Jodhpur) was determined. The IC_{50} value of aqueous extract of leaf powder of *Lawsonia inermis* L. procured from Kalyan and Jodhpur was found to be 4.01 mg and 3.68 mg/ml respectively (Figure 3, Table 4). Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and is involved in the regulation of various physiological processes. Excess concentration of NO is associated with several diseases. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions, which act as free radicals. The nitric oxide scavenging method showed moderate scavenging activity compare to standard, ascorbic acid (Kumaran and Karunakaran, 2007).

CONCLUSION:

Preliminary phytochemical analysis of leaf extracts from *Lawsonia inermis* L. shows presence of alkaloids, anthraquinone, carbohydrates glycosides, saponins, flavonoids, tannins and essential oils. Aqueous extract of leaf from *Lawsonia inermis* L. (Kalyan and Jodhpur) showed potent antioxidant activity, nitric oxide scavenging radicals and reducing power activities when compared with standard ascorbic acid. In addition, the aqueous extract of leaf from *Lawsonia inermis* L. (Kalyan and Jodhpur) found to contain a noticeable amount of total phenols, which play major role in controlling antioxidant. The results of this study show that the leaf from *Lawsonia inermis* L. (Kalyan and Jodhpur) can be used as easily accessible source of natural antioxidant.

ACKNOWLEDGEMENT:

Authors are thankful to Botany Department, B. N. Bandodkar College of Science, Thane for providing the laboratory facilities.

Table 1: Preliminary Phytochemical analysis of leaf from *Lawsonia inermis* L.

Test	Kalyan		Jodhpur	
	Aqueous	Alcoholic	Aqueous	Alcoholic
Alkaloids				
Mayer	-	-	-	-
Dragendroff	+	+	+	+
Wagner	+	+	+	+
Anthraquinone	+	+	+	+
Proteins	-	+	-	+
Carbohydrates	+	+	+	+
Glycosides (Killer-Kinliani Test)	+	+	+	+
Phenols	+	-	+	-
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Saponins	+	-	+	-
Essential oils	-	+	-	+
Amino acids	-	-	-	-
Starch	-	-	-	-

+ = Present; - = Absent

Table 2: Total Antioxidant Activity and Total Phenolic content of *Lawsonia inermis* L.

Extracts	Total Phenolic content (µg/mg)	Total Antioxidant Activity (µg/mg)
Kalyan	2.91 ± 0.25	8.206 ± 0.15
Jodhpur	4.75 ± 0.66	10.03 ± 0.57

Values are mean ± S.E. (n=3)

Table 3: Reducing power of *Lawsonia inermis* L.

Concentration (µg ml^{-1})	Absorbance at 700 nm		
	Ascorbic Acid	Kalyan	Jodhpur
20	0.18 ± 0.004	0.17 ±0.01	0.11 ±0.005
40	0.31 ±0.03	0.30 ±0.01	0.4 ±0.008
60	0.5 ±0.02	0.48 ±0.03	0.51 ±0.01
80	0.65 ±0.01	0.62 ±0.01	0.59 ±0.01
100	0.95 ±0.01	0.69 ±0.01	0.78 ±0.01

Values are mean ± S.E. (n=3)

Table 4: *Nitric oxide* scavenging activity of *Lawsonia inermis* L.

Concentration (µg ml^{-1})	% Scavenging activity		
	Ascorbic Acid	Kalyan	Jodhpur
1000	35.29	3.92	5.88
2000	43.13	21.56	31.37
3000	56.86	35.29	41.17
4000	62.74	52.94	56.86
5000	68.62	64.7	70.58
Ic_{50} value		4.01 mg/ml	3.68 mg/ml

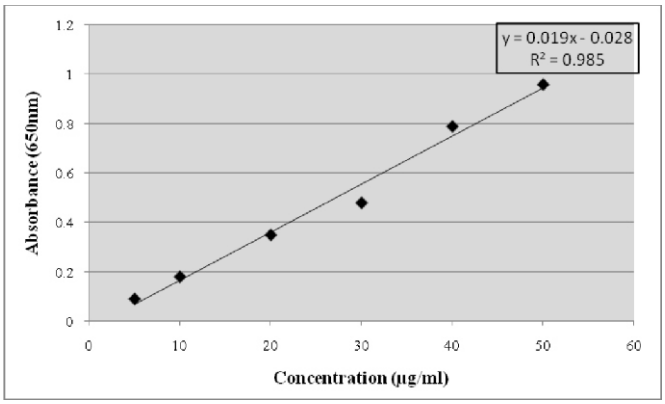

Figure 1: Total Phenolic content of *Lawsonia inermis* L.

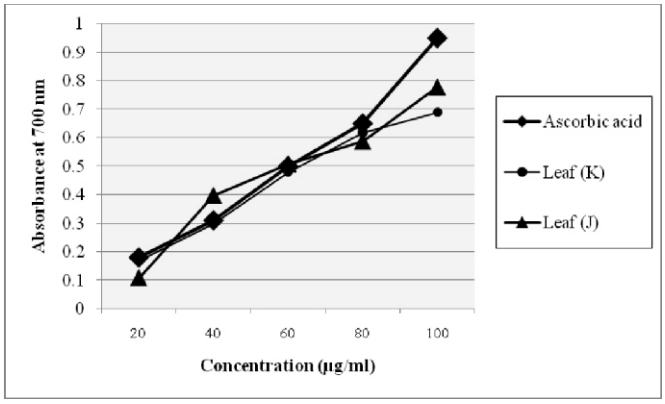

Figure 2: Reducing power activity of aqueous extract of leaf from *Lawsonia inermis* L. compared with ascorbic acid as standard

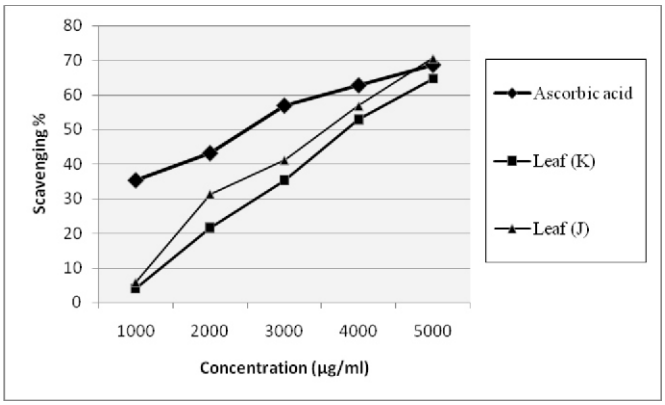

Figure 3: Nitric oxide scavenging activity of aqueous extract of leaf from *Lawsonia inermis* L. compared with ascorbic acid as standard

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