Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:10.1016/S2221-1691(12)60269-X

Quantification of phytochemical constituents and *in-vitro* antioxidant activity of *Mesua ferrea* leaves

Narender Prasad D*, B Ganga Rao, E Sambasiva Rao, T Mallikarjuna Rao, VS Praneeth D

A.U College of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530003, Andhra Pradesh, India

ARTICLE INFO

Article history:
Received 30 June 2012
Received in revised form 4 July 2011
Accepted 15 August 2012
Available online 20 August 2012

Keywords:
Mesua ferrea leaves
Phenolic content
Alkaloid content
In-vitro
Antioxidant activity

ABSTRACT

Objective: To investigate the quantification of total phenolic, alkaloid content and *in-vitro* antioxidant activity of ethanol (70%), methanol, ethyl acetate and hexane extracts of *Mesua ferrea* (*M. ferrea*) leaves. **Methods:** The quantification of the total phenolic and alkaloid contents were estimated by taking gallic acid and atropine are as a standard; *In-vitro* antioxidant activity was evaluated for extracts by using different free radicals (superoxide, hydroxyl and DPPH). **Results:** *M. ferrea* leaves ethanol (70%) extract have more phenolic and alkaloidal content than other extracts. The selected plant extracts were produced concentration dependent percentage inhibition of different free radicals and produced maximum activity at a concentration of 1 280 μg and there after the percentage inhibition were raised gradually to its maximum level with higher concentrations. **Conclusion:** In the present study we found that the extracts of *M. ferrea* showed good antioxidant activity. Among the four extracts, the ethanol (70%) extract showed better activity than other extracts.

1. Introduction

Plant and plant products are being used as a source of medicine since long. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent against several disease, no side effects and economic viability. Several compounds widely distributed in plants which have been reported to exert multiple biological effect, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc[1-4]. Recently, natural plants have received much attention as sources of biological active substances including antioxidants. Numerous studies have been carried out on some plants, vegetables and fruits because they are rich sources of antioxidants, such as vitamin A, vitamin C, Vitamin E, carotenoids, polyphenolic compounds and flavonoids which prevent free radical damage, reducing risk of chronic diseases. Living cells may generate free radicals and other reactive oxygen species by-products as a results of physiological and biochemical processes. Free radicals can cause oxidative damage to lipids, proteins and DNA, eventually leading to many chronic diseases, such as cancer, diabetes, aging, and other degenerative diseases in humans[5].

Mesua ferrea (M. ferrea) (Ceylon ironwood, Indian rose chestnut is a species in the family Calophyllaceae, many parts having medicinal properties. It enhances the complexion. It leads to fragility transparency to the skin. The flowers are acrid, anodyne, digestive, constipating, and stomachache. They are useful in conditions like asthma, leprosy, cough, fever, vomiting and impotency. The seed oil is considered to be very useful in conditions like vata and skin diseases.

The objective of our research work was to investigate the total phenolic and alkaloid contents and the antioxidant properties of different fractions of the hydro alcoholic extract (70%) from *M. ferrea* leaves by using superoxide, hydroxyl, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radicals.

*Corresponding author: Narender Prasad Dasari, Research Scholar, A.U College of Pharmaceutical Sciences, Andhra University, Visakhapatnam- 530 003, A.P, India.
Tel: 9441940846
E-mail: narendraphd2011@gmail.com

2. Material and methods

2.1. Chemicals and drugs

All chemicals and solvents were of the analytical grade obtained from S.D. Fine Chemical Pvt. Ltd., Mumbai, Sigma Chemical Company, U.S.A., Loba Chemic, Mumbai.

2.2. Preparation of extracts from M. ferrea leaves

The plant material used in present study was collected from Araku valley, Visakhapatnam district, Andhra Pradesh and authenticated by the taxonomist Dr. Prayaga Murthy Pragada, Depart of Botany, Andhra University. Freshly collected plant material was dried under shade and the dried material was milled to obtain a coarse powder. The powdered material was separately extracted in a soxhlet apparatus for 6 h successively with hexane, ethyl acetate, Hydro-alcoholic (ethanol 70% v/v) and methanol was concentrated to dryness under vacuum by using Rota-vapor.

2.3. Quantification of total phenolic content

Total phenolic content was determined using the Folin-Ciocalteau reagent Singleton *et al*[6]. Folin-Ciocalteau colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue absorption with a maximum at 765 nm.The intensity of the light absorption at that wave length is proportional to the concentration of phenols. By using standard gallic acid calibration curve, measure the concentration of phenolic content in gallic acid total equivalents using unit's mg/g (GAE).

2.4. Quantification of total alkaloid content

Total alkaloid content was determined by the Fazel et al method[7]. The plant extract (1 mg/mL) was dissolved in 2 N HCl and then filtered. The pH of phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. One mL of this solution was transferred to a separating funnel and then 5 mL of BCG solution along with 5 mL of phosphate buffer were added. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extracts were collected in a 10 mL volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. All experiments were performed thrice; the results were averaged and reported in the form of Mean ± S.E.M.

2.5. In-vitro anti oxidant activity

For the assessment of free radicals scavenging activity, the hexane, ethyl acetate, Ethanol (70%v/v) and methanol extracts were dissolved in water and 5% dimethyl sulphoxide (DMSO) respectively.

2.5.1. Superoxide radical scavenging activity

Superoxide scavenging activity of the plant extract was determined by McCord & Fridovich method, which depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitroblue tetrazolium.

2.5.2. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity is commonly used to evaluate the free radical scavenging effectiveness of various antioxidant substances[8]. Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe^{2+}/EDTA/H_2O_2 system (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances (TBARS).

2.5.3. DPPH radical scavenging activity

The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca *et al*[9]. In DPPH assay method is based on the reduction of alcoholic DPPH solution (dark blue in colour) in the presence of a hydrogen donating antioxidant converted to the non radical form of yellow colored diphenyl-picrylhydrazine. Lower the absorbance higher the free radical scavenging activity[10].

3. Results

3.1. Quantification of phytochemical constituents

The Quantified phenolic contents of *M. ferrea* leaves extracts were ranging from 40.14±0.72 to 3.38±0.63 (mg/g). The ethanol (70%) extract have more phenolic content 40.14 ±0.72 (mg/g) than other extracts and the alkaloidal content was ranging from 42.64±0.26 to 12.91±0.1 (mg/g). The ethanol (70%) extract has more alkaloidal content 42.64±0.26 (mg/g) than other extracts. Results of quantified phenolic and alkaloidal contents were showed in Table 1.

3.2. In-vitro antioxidant activity

The Ethanol (70% v/v), methanolic, ethyl acetate and hexane extracts *M. ferrea* leaves were found to possess concentration dependent scavenging activity on DPPH radicals and the results were given in Figure 1. The mean IC_{50} values for DPPH radical of alcoholic crude extract, methanolic, ethyl acetate and hexane extracts *M. ferrea* leaves were found to be 122 μg, 147 μg, 150 μg and 438 μg respectively. The mean IC_{50} value of ascorbic acid was found to be 16 μg. The results were given in Table 2.

Table 1.
Total phenolic and alkaloid content (mg/g) of *M. ferrea* leaves extracts.

Name of the extract	Total phenolic content (mg/g)	Total alkaloid content (mg/g)
Hexane	3.21±0.63	12.91±0.10
Ethyl acetate	26.63±0.25	21.45±0.53
Methanol	10.10±0.45	14.68±0.19
Ethanol (70%v/v)	40.14±0.72	42.64±0.26

Table 2.
In vitro 50% inhibition concentration (IC_{50}) of different extracts of *M. ferrea* leaves on DPPH, superoxide and hydroxyl free radicals.

Name of the extract	IC_{50} value (μg)		
	DPPH radical	Superoxide radical	Hydroxyl radical
Alc ext. *M. ferrea*	122	167	211
Methanol Ext. *M. ferrea*	147	243	215
Ethyl acetate ext. *M. ferrea*	150	191	240
Hex ext. *M. ferrea*	438	328	375
Ascorbic acid	16	59	66

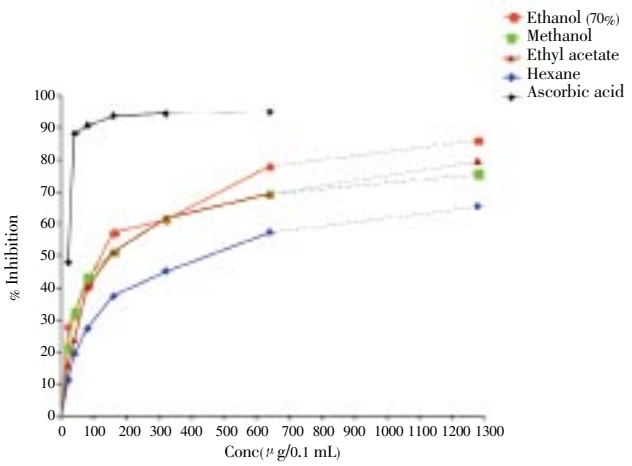

Figure 1. Concentration dependent percent inhibition of DPPH radical by different extracts of *M. ferrea* leaves and ascorbic acid in *in-vitro* studies.

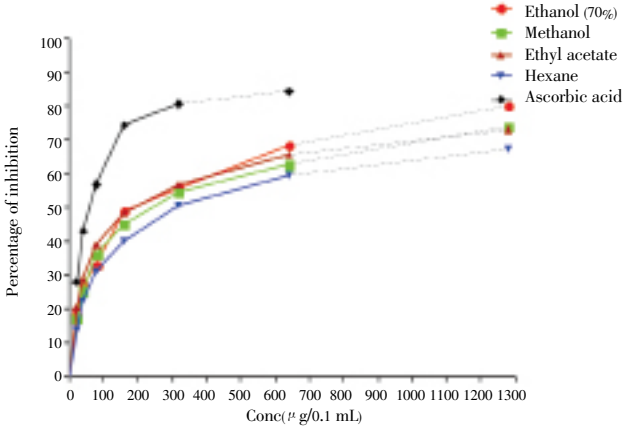

Figure 2. Concentration dependent percent inhibition of superoxide radical by different extracts of *M. ferrea* leaves and ascorbic acid in *in-vitro* studies.

In the present study, the Ethanol (70% v/v), methanolic, ethyl acetate and hexane extracts *M. ferrea* leaves were found to possess concentration dependent scavenging activity on superoxide generated by photoreduction of riboflavin and the results are given in Figure 2. The mean IC_{50} values for superoxide radical of Ethanol (70% v/v), methanolic, ethyl acetate and hexane extracts *M. ferrea* leaves were found to be 167 μg, 243 μg, 191 μg and 328 μg respectively. The mean IC_{50} value of ascorbic acid was found to be 59.3 μg. The results were given in Table 2.

The Ethanol (70% v/v), methanolic, ethyl acetate and hexane extracts *M. ferrea* leaves were found to possess concentration dependent scavenging activity on hydroxyl radicals and the results were given Figure 3. The mean IC_{50} values for hydroxyl radical of ethanol (70%), methanolic, ethyl acetate and hexane extracts *M. ferrea* leaves were found to be 211 μg, 215 μg, 240 μg and 375 μg respectively. The mean IC_{50} value of ascorbic acid was found to be 66 μg. The results were given in Table 2.

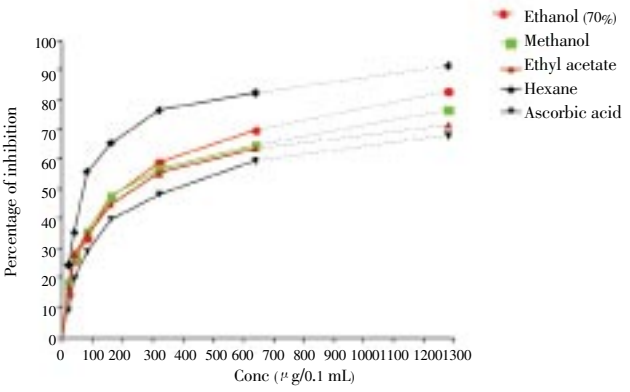

Figure 3. Concentration dependent percent inhibition of hydroxyl radical by different extracts of *M. ferrea* leaves and ascorbic acid in *in-vitro* studies.

4. Discussion

The phytochemical analysis conducted on *M. ferrea* leaves extracts revealed the presence of carbohydrates, alkaloids, steroids, phenols, glycosides *etc*. Chemical compounds from plants are known to be useful in the treatment of many diseases[11-13]. Thus, *M. ferrea* leaves containing these compounds may serve as a potential source of bioactive compounds in the treatment of diseases. In traditional

medicine, different parts of the plant were used for treating many diseases. The flowers were used to treat asthma, leprosy, cough, fever, vomiting and impotency. The seeds oil is considered for healing purpose as in sores, wounds and rheumatism. Plants with antioxidant activities have been reported to possess free radical scavenging activity. Free radicals are known as major contributors to several clinical disorders such as diabetes mellitus, cancer, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defense mechanism.

The plant extracts give positive for steroids and alkaloids which are very important compounds especially due to their medicinal uses in recent years. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity[14]. The presence of phenolic compounds in this plant may contribute to its antioxidative properties and thus the usefulness of these plants in herbal medicament. Phenols have been found to be useful in the preparation of some antimicrobial and antioxidant compounds[15,16].

The result of scavenging activity assay in this study indicates that the plant was potently active. This suggests that the plant extract contain compounds that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. The plant extracts were capable of scavenging super oxide, hydroxyl and DPPH in a concentration dependent manner.

The data clearly indicated that the extracts ethanol (70%), hexane, ethyl acetate and methanol of M. ferrea showed good antioxidant activity. Among the all the ethanol (70%) extract showed better activity.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgment

The authors were thankful to A.U College of Pharmaceutical Sciences, Andhra University for providing necessary laboratory facilities to carry out present research work.

References

[1] Ganga Rao B, P Rajeswararao, P Prayaga Murty, E Sambasiva Rao, P Madhukiran, T Mallikarjuna Rao, et al. Investigation on regional variation in total phenolic, alkaloid content and *in-vitro* antioxidant activity of *Leucas aspera*. *IJPSR* 2011; **2**(10): 2699-2703.

[2] B Ganga rao, N Jaya raju. Investigation of hepatoprotective activity of *Spondias pinnata*. *Int J Pharm Sci Res* 2010; **1**(3): 193-198.

[3] Abhishek Bhanot, Rohini Sharma, Malleshappa. Natural sources as potential anti-cancer agents: A review. *Int J Phytomed* 2011; **3**: 9-26.

[4] Ganga Rao Battu, Sambasiva Rao Ethadi, Veda Priya G, Swathi Priya K, Chandrika K, Venkateswara Rao A, et al. Evaluation of antioxidant and anti-inflammatory activity of *Euphorbia heyneana* Spreng. *Asian Pac J Trop Biomed* 2011; S191-S194.

[5] Olayinka A Aiyegoro, Anthony I Okoh. Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. *BMC Complement Altern Med* 2010; **10**: 21.

[6] Ganga Rao B, P Rajeswararao, P Prayaga Murty, E Sambasiva Rao, P Madhukiran, T Mallikarjuna Rao, et al. Investigation on regional variation in total phenolic, alkaloid content and *in-vitro* antioxidant activity of *Cleome chelidonii* L. *Int J Pharm Pharm Sci* 2011; **3**(4): 416-418

[7] Fazel Shamsa, Hamidreza Monsef, Rouhollah Ghamooshi, Mohammadreza Verdian-rizi. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. *Thai J Pharm Sci* 2008; **32**: 17-20.

[8] S Kalidas, B Kameswari, P Devi, B Madhumitha, R Meera, NJ Merlin. Phyto-Physico chemical evaluation, antioxidant activities and diuretic activity of leaves of *Lagerstroemia reginae*. *Asian J Res Chem* 2008; **1**(2): 83-87.

[9] Nguelefack TB, Mbakam FH, Tapondjou LA, Watcho P, Nguelefack-Mbuyo EP, Ponou BK, et al. A dimeric triterpenoid glycoside and flavonoid glycosides with free radical-scavenging activity isolated from *Rubus rigidus* var. camerunensis. *Arch Pharm Res* 2011; **34**(3): 543-550.

[10] Anita Murali, Purnima Ashok, Madhavan V. *In vitro* antioxidant activity and HPTLC studies on the roots and rhizomes of *Smilax zeylanica* l. (smilacaceae). *Int J Pharm Pharm Sci* 2011; **3**(1): 192-195.

[11] Gordian C Obute, Godswill O Adubor. Chemicals detected in plants used for folk medicine in South Eastern Nigeria. *Ethnobotanical Leaflets* 2007; **11**: 173-194.

[12] Ha Sook Chung, Jin Chul Shin. Characterization of antioxidant alkaloids and phenolic acids from anthocyanin-pigmented rice (*Oryza sativa* cv. Heugjinjubyeo). *Food Chem* 2007; **104**(4): 1670-1677.

[13] Cheenpracha S, Park EJ, Yoshida WY, Barit C, Wall M, Pezzuto JM, et al. Potential anti-inflammatory phenolic glycosides from the medicinal plant *Moringa oleifera* fruits. *Bioorg Med Chem* 2010; **18**(17): 6598-6602.

[14] H De Wet, G Fouche, FR Van Heerden. In vitro cytotoxicity of crude alkaloidal extracts of South African Menispermaceae against three cancer cell lines. *Afr J Biotechnol* 2009; **8**(14): 3332-3335.

[15] MS Brewer. Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. *Compre Rev Food Sc Food Saf* 2011; **10**: 221-245.

[16] Jin Dai, Russell J Mumper. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules* 2010; **15**: 7313-7352.