



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 2579-2582
Received: 24-03-2019
Accepted: 26-04-2019

HN Krishna Kumar
Department of Studies in
Biotechnology, Pooja Bhagavat
Memorial Mahajana Education
Centre, PG wing of SBRR
Mahajana First Grade College,
K.R.S. Road, Metagalli, Mysore,
Karnataka, India

Jyoti Bala Chauha
Department of Studies in
Biotechnology, Pooja Bhagavat
Memorial Mahajana Education
Centre, PG wing of SBRR
Mahajana First Grade College,
K.R.S. Road, Metagalli, Mysore,
Karnataka, India

Correspondence
HN Krishna Kumar
Department of Studies in
Biotechnology, Pooja Bhagavat
Memorial Mahajana Education
Centre, PG wing of SBRR
Mahajana First Grade College,
K.R.S. Road, Metagalli, Mysore,
Karnataka, India

Assessment of anti-arthritic, anti-inflammatory and antioxidant activity of *Melia dubia*

HN Krishna Kumar and Jyoti Bala Chauha

Abstract

The present investigation deals with the phytochemical screening, anti-arthritic, anti-inflammatory and antioxidant potentials of ethanolic fruit extract of *Melia dubia*. Phytochemical screening studies of the extract showed the presence of flavonoids, steroids, saponins, alkaloids, glycosides and tannins. Evaluation of total phenolic contents revealed that the extract contains 142.8 mg/g of phenolic compounds. The antioxidant activity of the extract was evaluated through DPPH assay and reducing power assay. The free radical scavenging activity of the extract was confirmed in a DPPH assay. The extract showed the stronger radical scavenging effect with IC₅₀ value of 55.4 µg/ml. The reducing power of the extract observed was concentration dependent which is comparable with the standard antioxidant butylated hydroxyanisole (BHA). The plant exhibited good anti-arthritic activity in a dose dependent manner. The extract showed highest inhibitory activity of 81.2% with an IC₅₀ value of 58.2 µg/ml. Likewise, the fruit extract of *Melia dubia* showed strong anti-inflammatory activity in a concentration dependent manner with an IC₅₀ value of 52.6 µg/ml.

Keywords: Antioxidant activity, free radicals, phytochemicals, anti-arthritic and anti-inflammatory activity

Introduction

The free radicals derived from the oxygen such as superoxide anion and hydroxyl radicals are cytotoxic and promote tissue injury^[1]. Antioxidants are the substances used by the body to protect itself from the damage caused by free radicals which causes lipid peroxidation, oxidation of DNA, proteins etc. that damage cells^[2]. Oxidative stress is one of the key factors for several diseases like cancer, diabetes, arthritis, inflammation etc^[3]. Hence, research has been focused on use of antioxidants with particular emphasis on naturally derived antioxidants. The phenolic compounds derived from the plants viz., tannins, flavonoids, alkaloids, terpenoids etc. are known to be potent antioxidants^[4]. According to World Health Organization, a large proportion of the world uses Phytomedicines for their primary health care^[5]. In a country like India, different existing medicinal usage systems like Unani, Ayurveda, Siddha and local health traditions are focused on using plant based chemicals for treating several human and animal diseases^[6]. Rheumatoid arthritis is an autoimmune disease in which inflammation of joints, destruction of articular synovial proliferation^[7]. Inflammatory diseases are becoming common in aging society throughout the world. Recent studies indicate that the mediators and cellular effectors of inflammation are important constituents of the local environment of tumors^[8]. Natural products in general and medicinal plants in particular are believed to be an important source of new chemical substances with potential therapeutic efficacy^[9]. The prolonged usage of presently available drugs to treat these types of disorders may cause severe side effects. Hence, there is an urge to develop new therapeutic agents from plant source with minimum side effects.

Melia dubia also called as a Maha neem or forest neem is belonging to the family Meliaceae. It is found in deciduous forests of India, Sri Lanka and Tropical Asia from plains to 750m above the sea level. *Melia dubia* is a perennial tree growing up to 30 metres height. The plants start to produce flowers and fruits between March-April. Fruit is a globose drupe, dark green with 3 to 4 seeds. The plant possesses several therapeutic properties. It has been used traditionally in the treatment of various ailments and it contains alkaloids, carbohydrates, steroids, tannins, flavonoids, saponins and glycosides. The plant exhibited strong antioxidant activity^[10]. The ethanolic and aqueous extracts of the bark of *Melia dubia* were found to possess significant antibacterial activity against *Staphylococcus aureus*^[11]. The plant is reported for anti-inflammatory activity^[12]. Fruit extract of *Melia dubia* found to be an effective hypoglycaemic agent^[13]. Silver nanoparticles synthesized from *Melia dubia* plant extract showed remarkable cytotoxicity activity against KB cell line with evidence of high

therapeutic index value [14]. The aim of this study was to evaluate the antioxidative activity, antiarthritis and antiinflammatory activity of *Melia dubia* using different *in vitro* methods.

Materials and Methods

Chemicals

All chemicals and solvents used in the study were of analytical grade. 1, 1-diphenyl-2-picryl hydrazyl (DPPH) was purchased from Sigma Aldrich Co. St. Louis, USA. Ethanol, Trichloroacetic acid, Ascorbic acid, Potassium ferric cyanide, ferric chloride, butylated hydroxy anisole (BHA), Folin-Ciocalteu reagent, Sodium carbonate, Gallic acid, Bovine Serum Albumin, Dimethyl sulfoxide (DMSO) etc. were procured from Sd Fine chem. Ltd, India.

Plant material collection and Preparation of extract

The plant material consisting of mature fruits of *Melia dubia* Cav. was collected from forest areas of Mysore, Karnataka, India. The materials were identified and authenticated by Department of Studies in Botany, University of Mysore. The fruits were cleaned and washed under running tap water then dried at 40° C in an oven for 3 days. The dried fruits were powdered using a grinder and extracted with ethanol using Soxhlet apparatus. Extract was further concentrated using rotary vacuum evaporator at 45-50°C and stored at 4°C.

Phytochemical Screening

The extract was analyzed for the active phytoconstituents such as flavonoids, alkaloids, tannins, saponins, terpenoids, steroids, glycosides etc according to the standard protocol [15].

Determination of the total phenolic content

The amount of total soluble phenolic content present in the extract was evaluated according to Folin-Ciocalteu method [16]. Briefly, the extract (1mg/ml) was mixed with 20 µl of Folin Ciocalteu reagent (1:10) and 50 µl of aqueous 2.5% Na₂CO₃. The mixtures were allowed to stand for an hour at room temperature. Absorbance was measured at 765 nm using spectrophotometer. The standard graph was plotted using different concentrations of gallic acid. Total phenolic content was expressed as mg gallic acid equivalent/gram of dry weight of extract.

DPPH radical scavenging assay

DPPH radical scavenging activity was measured using the method described by Oktay *et al* [17]. The reaction mixture contained 0.1 ml of fruit extract at different concentrations and 5 mL of 0.004% solution of DPPH in ethanol was incubated for 30 minutes in dark. After incubation, discoloration was measured at 517 nm. Ascorbic acid was used as a positive control. The percentage inhibition was calculated using the following formula,

$$\% \text{ inhibition} = [(Ac - Ae)/Ac] \times 100$$

Where, 'Ac' denote the absorbance of control and 'As' denote the absorbance of sample (extract).

Reducing Power assay

The reducing power of the extract was measured according to the method of Oyaizu [18]. Different concentrations of plant extract and standard BHA solutions were mixed with 2.5 ml of 0.2M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at

50°C for 20 min. Following incubation, 2.5 ml of 10% trichloro acetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. After centrifugation, 2.5ml upper layer solution was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1 % FeCl₃ solution. The absorbance was measured at 700 nm.

Anti-arthritis assay

The anti-arthritis activity of the extract was evaluated by protein denaturation method [19]. The experiment was carried out using bovine serum albumin. The reaction mixture consists of 5% aqueous solution of bovine serum albumin (0.45ml) and 0.05ml of the extract at different concentrations. pH was adjusted to 6.3. Samples were incubated at 37°C for 20 minutes then the temperature was increased to 57°C for 3 minutes. After incubation, 2.5ml of Phosphate buffer was added to all the samples. Absorbance was measured at 416 nm using UV visible spectrophotometer. Diclofenac sodium was used as reference drug. The Percentage inhibition of protein denaturation was calculated using the following formula,

$$\% \text{ inhibition} = [(Ac - Ae)/Ac] \times 100$$

Where, 'Ac' denote the absorbance of control and 'As' denote the absorbance of sample (extract).

Anti-inflammatory assay

The Human red blood cell membrane stabilization method was used to predict the anti-inflammatory activity of the extract [20]. The blood sample was collected from healthy human volunteers. Collected blood sample was mixed with equal volume of Alsever solution and centrifuge at 3000 rpm. The packed cells were washed with isosaline and 10% suspension was made. Different concentrations of the extract were prepared using DMSO then 1ml Phosphate buffer, 2ml of hyposaline and 0.5ml of human red blood cell (HRBC) Suspension was added to all the solutions. The samples were incubated at 37°C for 30 minutes and centrifuge at 3000 rpm for 20 minutes. The absorbance of clear supernatant liquid was measured using UV visible spectrophotometer at 560 nm. Diclofenec sodium was used as reference drug. The percentage inhibition of membrane stabilization was calculated by using the following formula,

$$\% \text{ inhibition} = [(Ac - Ae)/Ac] \times 100$$

Where, 'Ac' denote the absorbance of control and 'As' denote the absorbance of sample (extract).

Statistical analysis

All the analyses were carried out in triplicate and the results were expressed in mean ± SD.

Results and Discussion

Phytochemical Screening and Determination of the total phenolic content

The phytochemical analysis of the extract revealed the presence of flavonoids, steroids, saponins, alkaloids, glycosides and tannins (Table 1). Determination of total phenolic contents of the the extract exhibited 142.8 mg/g of phenolic compounds. The phenolic concentration of the extract was expressed as milligram of gallic acid equivalents per gram of extract. Phenols are very important plant constituents because of their free radical scavenging ability

due to their hydroxyl groups [21]. It has been reported that phenolic compounds are associated with antioxidant activity and play a crucial role in stabilizing lipid peroxidation [22]. Consumption of polyphenolic compounds up to 1g daily from diet has remarkable inhibitory effects on mutagenesis and carcinogenesis in humans [23]. The result of the present work strongly suggests that phenolic compounds are important components of this plant and some of their pharmacological effects could be attributed to the presence of these valuable constituents.

Table 1: To show phytochemical constituents of the ethanolic fruit extract of *Melia dubia*

Terpenoid	Saponin	Steroid	Alkaloid	Glycoside	Tanin	Flavanoid
s	s	s	s	s	s	s
--	+	+	--	--	+	+

+ indicates presence of constituents

-- indicates absence of constituents

DPPH Radical scavenging assay

Evaluation of free radical scavenging activity revealed that the extract showed highest radical scavenging potential with IC₅₀ value of 55.4 µg/ml. It is comparable to standard antioxidant ascorbic acid (Table 2). Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants through their scavenging power are useful for the management of those diseases. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts [24]. Hydrogen-donating ability of the antioxidant molecule contributes to its free radical scavenging nature [25].

Table 2: Showing DPPH radical scavenging activity of the ethanolic fruit extract of *Melia dubia* and Ascorbic acid

Concentration of the extract and ascorbic acid (µg)	% inhibition of the extract	% inhibition of ascorbic acid
20	35 ± 0.9	81.2 ± 1.1
40	49 ± 1.1	92.1 ± 0.9
60	65 ± 0.8	95.8 ± 0.6
80	81 ± 1.1	97.4 ± 1.2
100	92 ± 1.2	98.5 ± 1.3

Values are shown in mean ± SE

Reducing power assay

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [26]. Reducing power is the measure of reductive ability of antioxidant and it is evaluated by the transformation of Fe³⁺ to Fe²⁺ in the presence of extracts [27]. In the present investigation, the reducing power of the extract increased with increasing concentration. The extract showed potent ferric reducing power in a dose dependent manner. The reducing power of the extract is comparable to standard antioxidant BHA (Table 3).

Table 3: Reducing power activity of the ethanolic fruit extract of *Melia dubia* and BHA

Concentration of the extract and BHA (µg)	Reducing property (absorbance) of the extract	Reducing property (absorbance) of BHA
20	0.31 ± 0.03	0.51 ± 0.04
40	0.56 ± 0.05	0.85 ± 0.06
60	0.88 ± 0.07	1.19 ± 0.08
80	0.97 ± 0.1	1.62 ± 0.11
100	1.12 ± 0.09	1.82 ± 0.14

Values are shown in mean ± SE

Anti-arthritic assay

In rheumatoid arthritis the denaturation of protein is one of the causes [28-29]. Production of auto-antigens in certain rheumatic diseases may be due to *in vivo* denaturation of proteins. Mechanism of denaturation probably involves alteration in hydrogen, hydrophobic electrostatic and disulphide bonding. In the present investigation, ethanolic fruit extract of *Melia dubia* was evaluated against denaturation of bovine serum albumin protein. The result showed concentration dependent inhibition of protein denaturation by the extract. The maximum inhibition of protein denaturation observed was 81.2% with an IC₅₀ value of 58.2 µg/ml (Table 4). Various anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation [30].

Table 4: Showing anti-arthritic activity of the ethanolic fruit extract of *Melia dubia* and Diclofenac sodium

Concentration of the extract and diclofenac sodium (µg)	% of inhibition of the extract	% of inhibition of diclofenac sodium
20	43.40 ± 0.31	62.5 ± 0.53
40	53.20 ± 0.42	73.4 ± 0.68
60	62.1 ± 1.2	80.5 ± 0.25
80	69.4 ± 0.18	84.4 ± 0.48
100	81.2 ± 0.09	93.21 ± 0.81

Values are shown in mean ± SE

Anti-inflammatory assay

This assay is based on the stabilization of human red blood cell (HRBC) membrane. In the present study, maximum percentage protection of the extract observed was 80.1% at 100 µg/ml with an IC₅₀ value of 52.6 µg/ml (Table 5). It is a well-known fact that hydrolytic enzymes released during inflammation generate different disorders. The extra cellular activity of these enzymes is said to acute or chronic inflammation [31]. The extract of the assessed plant in the present investigation showed high membrane stabilization property in concentration dependent manner.

Table 5: To show anti-inflammatory activity of the ethanolic fruit extract of *Melia dubia* and Diclofenac sodium

Concentration of the extract and diclofenac sodium (µg)	% of inhibition of the extract	% of inhibition of diclofenac sodium
20	39.30 ± 0.22	59.4 ± 0.61
40	48.70 ± 0.63	68.3 ± 0.54
60	63.53 ± 1.1	76.5 ± 0.21
80	70.34 ± 1.18	83.7 ± 0.52
100	80.1 ± 1.24	94.63 ± 1.01

Values are shown in mean ± SE

Conclusions

The results obtained in the *in vitro* models such as DPPH radical scavenging assay, reducing power assay, anti-arthritic assay and anti-inflammatory assay clearly indicated that, the ethanolic fruit extract of *Melia dubia* exhibited strong biological activity when compared with standards. The anti-arthritic, anti-inflammatory and free radical scavenging activity of the extract may be due to the presence of phenolic compounds such as flavonoids, steroids, saponins, alkaloids, glycosides and tannins. Protein denaturation is one of the main causes of rheumatoid arthritis due to the production of auto antigens. The results of the present study showed that the extract may control the production of auto antigens by

preventing protein denaturation. Lysosomal membrane lysis prevention is one of the contributions to anti-inflammatory activity. In the present study, plant extract exhibited protective effect against heat and hypotonicity induced RBC membrane lysis. The results of this study revealed that the extract can be used as easily accessible source of natural antioxidants and as a possible food supplement. The fruits of *Melia dubia* could be served as a new source of nutraceuticals with potential applications to reduce the level of oxidative stress and related health benefits. This study established a significant scope to develop a broad spectrum use of *Melia dubia* in herbal medicine and as a base for the development of novel potent drugs against the oxidative stress related health disorders in human beings.

Acknowledgements

The authors are thankful to Prof. C.K. Renukarya, Director, Pooja Bhagavat Memorial Mahajana Education Centre, PG Wing, Mysore for facilities to carry out this research work.

References

- Peterhans E. Oxidants and antioxidants in viral diseases: disease mechanisms and metabolic regulation. *J Nutr.* 1997; 127:962-965.
- Sies H. Oxidative stress: Oxidant and antioxidants. *Exp Physiol.* 1997; 82:291-95.
- Rajan S, Gokila M, Jency P, Brindha P, Sujatha RK. Antioxidant and phytochemical properties of Aegle marmelos fruit pulp. *Int J Curr Pharm Res.* 2011; 3(2):65-70.
- Pratt DE, Hudson B. Natural antioxidants not exploited commercially- Food Antioxidant. Amsterdam: Elsevier, 1990, 222-45.
- Bandaranayake WM. In: I. Ahmad, F. Aqil, and M. Owais (Eds.), *Modern Phytomedicine. Turning Medicinal Plants into Drugs*, (Weinheim: Wiley-VCH GmbH & Co. KGaA), 2006, 25-57.
- Prakash P and Gupta N. *Ind J Physiol Pharmacol.* 2005; 49:125-131.
- Singh M, Soni P, Upmanyu N and Shivhare Y. In vitro Anti-arthritis Activity of Manilkarzapota Linn. *Asian J Pharm Tech.* 2011; 1(4):123-124.
- Mantovani A, Allavena P, Sica A *et al.* Cancer-related inflammation. *Nature.* 2008; 454:436-444.
- Ameh SJ, Obodozie OO, Afolabi EK, *et al.* Some basic requirements for preparing an antisickling herbal medicine - NIPRISAN. *Afr J Pharm Pharmacol.* 2009; 3(5):259-264.
- Valentina P, Ilango K, Kiruthiga B and Parimala MJ. Preliminary phytochemical analysis and biological screening of extracts of leaves of *Melia dubia* Cav. *International Journal of Research in Ayurveda and Pharmacy.* 2013; 4(3):417.
- Anonymous. Preliminary phytochemical and antimicrobial investigations on *Melia dubia* Bark. *Ancient Science of Life.* 1999; 19(1-2):1-6.
- Khadse CD and Kakde RB. Anti-Inflammatory activities of aqueous extract of fruits and their different fractions of *Melia dubia*. *Research Journal of Pharmaceutical, Biological and Chemical Sciences.* 2014; 5(4):780.
- Susheela T, Balaravi P, Theophilus J, Reddy TN, Reddy PUM. Evaluation of hypoglycaemic and antidiabetic effect of *Melia dubia* CAV fruits in mice. *Current Science.* 2008; 94(9):1191-1195.
- Karthikeyan J, Kamalanathan MN, George T, Elangovan V. Larvicidal and antibacterial efficacy of green synthesized silver nanoparticles using *Melia dubia*. *International Journal of Pharmacy and Pharmaceutical Sciences.* 2014; 6(7):395-399.
- Tiwari P, Kumar B, Kaur M. Phytochemical screening and extraction: A review. *International Pharmaceutical Sciencia.* 2011; 1:98-106.
- Jain AK, Paras S, Mukesh SS. Evaluation of anti-inflammatory and antipyretic activity of total alcoholic extract of *Croton sparsiflorus* and its fractions. *Journal of Pharmacy Research.* 2010; 3:1149-51.
- Oktay M, Gulcin I, Kufrevioglu OI. Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extract. *Lebensmittel-Wissenschaft und Technologie.* 2003; 36:263-271.
- Oyaizu M. Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn J Nutr.* 1986; 44:307-315.
- Vallabh deshpane, Varsha Jadav M, Kadam V J. *In-vitro* anti-arthritis activity of *Abutilon indicum* (Linn.) sweet. *J Pharm Res.* 2009; 2(4):644-645.
- Gandhudasan R, Thamaraihelvan A, Babraj S. Antiinflammatory action of *Lannea coromandelica* by HBRC membrane stabilization. *Fitoterapia.* 1991; 50(12):81-83.
- Hatano T, Edamaysu R, Mori A, Fujita Y, Yasuhara E. *Chem Pharm Bull.* 1989; 37:2016-21.
- Yen GC, Duh PD, Tsai CL. The relationship between antioxidant activity and maturity of peanut hulls. *Journal of Agricultural and Food Chemistry.* 1993; 41:67-70.
- Tanaka M, Kuei CW, Nagashima Y, Taguchi T. Application of antioxidative maillard reaction products from histidine and glucose to sardine products. *Nippon Suisan Gakkaishi.* 1998; 54:1409-1414.
- Koleva II, Van Beek TA, Linszen JPH, de Groot A and Evstatieva LN. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochemical Analysis.* 2002; 13:8-17.
- Chen CW and Ho CT. Antioxidant properties of polyphenols extracted from green tea and black tea. *Journal of Food Lipids.* 1995; 2:35-46.
- Blazovics A, Lugasi A, Szentmihalyi K and Kery A. *Acta Biologica Szegediensis.* 2003; 47:99-102.
- Gulcin I, Oktay M, Kirecci E, Kufrevioglu I. *Food Chem.* 2003; 83:371-382.
- Vane JR and Botting RM. New insights into the mode of action of anti-inflammatory drugs. *Inflammation Research.* 1995; 44(1):1-10.
- Mizushima Y. Screening test for anti-rheumatic drugs. *Lancet.* 1966; 288(7460):443.
- Grant NH, Alburn HE and Kryzanasuskas C. Stabilization of serum albumin by anti-inflammatory Drugs. *Biochemical pharmacology.* 1970; 19(3):715-722.
- Sadique J, Rqobah AI, Bughaiith NA, ElGindy AR. The bioactivity of certain medicinal plants on the stabilization of RBC membrane system. *Fitoterapia LX.* 1989; 525-532.