



Research Article

ISOLATION AND CHARACTERIZATION OF *MURRAYA PANICULATA* ETHANOL SEED EXTRACT FOR THEIR ANTIOXIDANT COMPONENTS

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ABSTRACT

The objective of this study is isolation and characterization of *Murraya paniculata* ethanol seed extract for their antioxidant components. The 50 % ethanol seed extract of *Murraya paniculata* was screened for phytochemicals and subjected to qualitative and quantitative test for antioxidant activity with DPPH free radical and H_2O_2 scavenging activity. Total phenolic and flavonoid content was calculated and then extract was subjected to various isolation techniques Like TLC, Column chromatography and characterization of compound with IR, NMR and Mass spectroscopy. The extract was found to produce a profound antioxidant activity in a dose dependent manner with both methods. Isolated and characterized compound was flavones derivative. *Murraya paniculata* seed extract have potential source of antioxidant components which was isolated and characterized.

Keywords: *Murraya paniculata*, antioxidant, DPPH, Hydrogen peroxide.

INTRODUCTION

Oxidative stress is an imbalance between oxidants and antioxidants and it is caused by free radical damage¹. Abnormally high levels of free radicals which cause membrane damage due to per oxidation of membrane lipids and protein glycation and the simultaneous decline of antioxidant defense mechanism leads to cell damage^{2,3}. Oxidative stress is the causative factor in various chronic diseases such as cardiovascular diseases, cardiac failure⁴, alcohol induced liver disease, ulcerative colitis⁵, cancer, several neurodegenerative diseases, ageing process⁶. Due to the over production of ROS such as O_2 , H_2O_2 and OH induced by exposure to external oxidants substances or a failure in the defense mechanism, damages to the cells structure, DNA, lipids and proteins⁷. The concentration of ROS (reactive oxygen species) is modulated by the antioxidants enzymes and the non enzymatic scavengers⁸. To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system. It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals⁹. These components include:

- Nutrient-derived antioxidants like ascorbic acid (vitamin C), tocopherols and tocotrienols (vitamin E), carotenoids, and other low molecular weight compounds such as glutathione and lipoic acid.
- Antioxidant enzymes, e.g., superoxide dismutase, glutathione peroxidase, and glutathione reductase, which catalyze free radical quenching reactions.
- Metal binding proteins, such as ferritin, lactoferrin, albumin, and ceruloplasmin that sequester free iron and copper ions that are capable of catalyzing oxidative reactions.
- Numerous other antioxidant phytonutrients present in a wide variety of plant foods.

Normal levels of antioxidants defense mechanism are not sufficient for the eradication of the free radical induced

injury. Therefore administration from natural origins have a promising role to play several antioxidants of plant material are experimentally proved and widely used as a more effective agents against oxidative stress¹⁰⁻¹². *Murraya paniculata* Linn. (Synonyms: *Chalcas paniculata* L., *Chalcas exotica* L. and *Murraya exotica* L.) belongs to the family Rutaceae and is commonly known as orange jasmine, mock orange, satin wood, honey bush, China box, café de la India, mirto azahar, naranjo, jasmine, limonera, bun. The genus *Murraya* belongs to the orange subfamily (Aurantioideae) in Rutaceae and comprises two sections: *Murraya* and *Bergera*. Section *Murraya* contains 4 species and 3 varieties, all of which are closely related each other from taxonomical viewpoints. *Murraya paniculata* is geographically the most wide-spread species of Section *Murraya* occurring in either the tropics or subtropics of Asia and Oceania. It is distributed over the greater part of India and the Andaman Islands to an altitude of 1500 m. Native to tropical Asia from India and Sri Lanka to Myanmar (Burma), southern China and Taiwan, Thailand and eastwards throughout the Malayan region to northeastern Australia and Caledonia. This species has found wide medicinal value throughout the area of distribution¹³. It is an evergreen shrub or occasionally a small tree, usually 2 to 3 m in height but reaching 7.5 m and 13 cm in stem diameter. The leaves are alternately arranged along the stems and borne on stalks (i.e. petioles). They have entire margins, wedge-shaped (i.e. cuneate) bases and pointed tips (i.e. acuminate apices). Older orange jasmine normally has multiple stems from the ground level. The stems are supported by taproots with lateral roots and abundant fine roots. The fragrant flowers are borne in clusters, containing up to eight flowers, at the tips of the branches or in the upper leaf forks (i.e. terminal or upper axillary cymes). Each flower has five green sepals and five white petals (10-18 mm long) that are curved backwards (i.e. re curved). Fruits are Shiny, red elliptic fruits about 1 cm long develop. One or two light green seeds are embedded in the bitter, watery pulp^{14,15}. Various chemical constituent isolated from different parts of plant including roots, stem, bark, leaves and fruits were:

flavonoids, Indole alkaloids, Spiroquinazoline alkaloid, Coumarins, Isoflavonoids, Essential oils, Polysaccharides and fatty acid. *Murraya paniculata* (L). Plant has been evaluated for various its pharmacological activities i.e. anti implantation, antidiarrhoeal, antinociceptive, antiinflammatory, immunoreactivity, infertility, antioxidant, antimicrobial, antifungal, antifeedant, dysentery and stimulant¹⁶⁻¹⁸. The present study was undertaken to investigate the free radical scavenging potential of the ethanolic seed extract of *Murraya paniculata*.

MATERIALS AND METHODS

Murraya paniculata seeds were purchased from chhaged garden in Maharashtra (Pune), India. The healthy looking seeds were selected and authenticate from the Botanical and Environmental Science Department, Guru Nanak Dev University, Amritsar (Punjab) and deposited in GNDU Amritsar (Punjab), India.

Extraction and Phytochemical Screening

Murraya paniculata seeds were cleaned, washed and dried in shade. Seeds were powdered (below 20°C) and weighed 100 g. Powdered seeds were subjected to cold extraction with solvent (200 ml ethanol + 200 ml distilled water) for 3 days. Extract was filtered and partially concentrated. Defatting of concentrate was carried out 3 times by Hexane in separating funnel. The ethanolic fraction was separated and concentrated on water bath. The crude extract thus obtained was weighed (8 g). Extract was screened for following phytochemicals Components: flavonoids, tannins, alkaloids, sterols, triterpenoids, carbohydrates, anthraquinone, glycosides, coumarin glycosides, Proteins according to standard procedure.

In vitro Antioxidant Activity of *Murraya paniculata* Ethanol Seed Extract

Qualitative DPPH Scavenging Activity

DPPH (1, 1-diphenyl-2-picrylhydrazyl) is a free radical. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color. The extract solution 1 ml was spotted on to coated TLC plate. The plate was dried in air and developed in a tank containing mobile phase Hexane : ethyl acetate (5:5). After developing, the plate was sprayed with 1, 1-diphenyl-2-picrylhydrazyl (DPPH) reagent (0.2 %) in methanol and dried. The color changes (Yellowish color development on pinkish background on the TLC plate) were noted as an indicator of the presence of antioxidant substance¹⁹.

Quantitative DPPH Scavenging Activity

The diluted working solution of the extract was prepared in 50 % ethanol. Ascorbic acid was used as standard. 0.05 mM of DPPH was prepared in ethanol and 2 ml of this solution was mixed with 2 ml of sample solution ranging from 25-200 µg/ml and standard solution separately. These solution mixtures were kept in dark for 30 minutes (incubation period) and optical density was measured at 517 nm. Ethanol was taken as blank and DPPH (0.05 mM) solution as control solution.

In vitro Hydrogen Peroxide (H₂O₂) Scavenging Activity

H₂O₂ itself is not particularly reactive with most biological components but it is a precursor for hydroxyl radicals which are very toxic to the cell. For *in vitro* study, 1 ml of various

drug concentrations (25-200 µg/ml) was prepared. To every drug dilution, 0.6 ml of 40 mM H₂O₂ already prepared in 0.1 M phosphate buffer (pH 7.4) was added. After 10 minutes absorbance was measured at 230 nm. Ethanol was taken as blank and H₂O₂ (40 mM) solution as control solution²⁰.

Estimation of Total Flavonoid and Phenolic Content

Total Flavonoid Content

In a 10 ml test tube, 0.3 ml of extract, 3.4 ml of 30 % ethanol, 0.15 ml of sodium nitrite (0.5 M) and 0.15 ml of aluminum chloride hexahydrate (0.3M) were mixed. After 5 minutes 1 ml of sodium hydroxide (1 M) was added. The solution was mixed well and absorbance was measured against the reagent blank at 506 nm. The standard curve for total flavonoids was made using rutin standard solution (20 µg/ml – 100 µg/ml) under the same procedure as earlier described. The total flavonoids were expressed as milligrams rutin equivalents per gram of dried fraction²¹.

Total Phenolic Content

The reaction mixture was prepared by mixing 0.5 ml of ethanolic solution (1 g/ml) of extract, 2.5 ml of 10 % Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5 ml sodium bicarbonate. The samples were incubated at 45°C for 15 minutes. The samples were prepared in triplicate and the mean value of absorbance was obtained. Blank was concomitantly prepared, with ethanol instead of extract solution. The same procedure was repeated for the gallic acid and the calibration line was constructed. The total phenolic content was expressed in terms of gallic acid equivalent²².

Isolation and Characterization of Extract

Compounds were isolated using different chromatographic techniques like Thin layer chromatography and Column chromatography. Ethyl acetate : glacial acetic acid : formic acid : water (100 : 11 : 11 : 26) was used as solvent system in both procedures. All the fractions eluted from column were first screened for phytochemical component and then individually spotted on Silica gel G plates, developed in the solvent system and analyzed in the UV Chamber. The fractions which produced single spots were dried and further characterized by NMR and IR Spectroscopic techniques.

RESULT AND DISCUSSION

Murraya paniculata ethanolic seeds extract exhibited DPPH scavenging activity. With the increase in the concentration of seeds extract, the anti oxidant activity increases proportionally with maximum activity 81.80 % at 200 µg/ml. IC₅₀ was found to be 23.32 µg/ml as shown in Figure 1. Hydroxyl radical (HO•) is the most powerful oxidant, it indiscriminately reacts with almost all biological compounds. *Murraya paniculata* ethanol seeds extract scavenged hydrogen peroxide in a dose dependent manner with maximum activity²³ of 95.81 % at 200 µg/ml. The IC₅₀ value was found to be 20.29 µg/ml as shown in Figure 2.

Total Flavonoid and Phenolic Content

The ethanolic seeds extract *Murraya paniculata* was evaluated for total flavonoid and phenolic content and they were calculated from the standard plot of Rutin and gallic acid. It was expressed as milligrams of standard equivalents per gram of dried fraction as shown in Table 1 and Figure 3 and 4.

Table 1: Total Flavonoid and Phenolic content of *Murraya paniculata* seeds extract

Plant	Total Flavonoid content	Total Phenolic content
<i>Murraya paniculata</i> seed extract	65 ± 0.0005 mg equivalents of rutin/g of dried extract.	55 ± 0.0005 mg equivalents of gallic acid/g of dried extract.

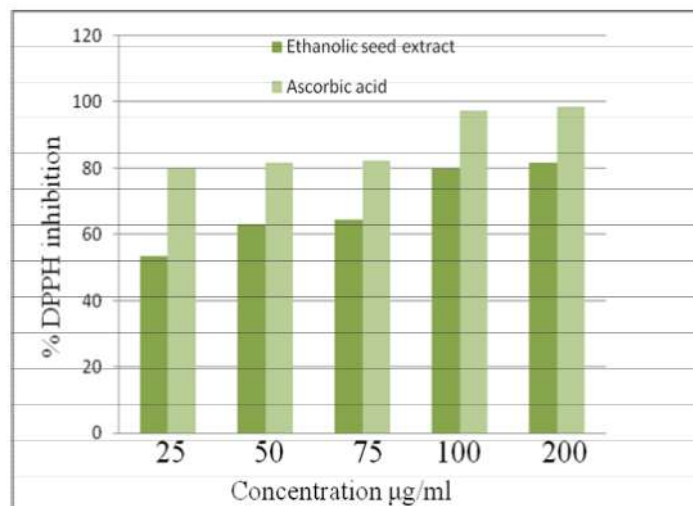


Figure 1: DPPH scavenging activity

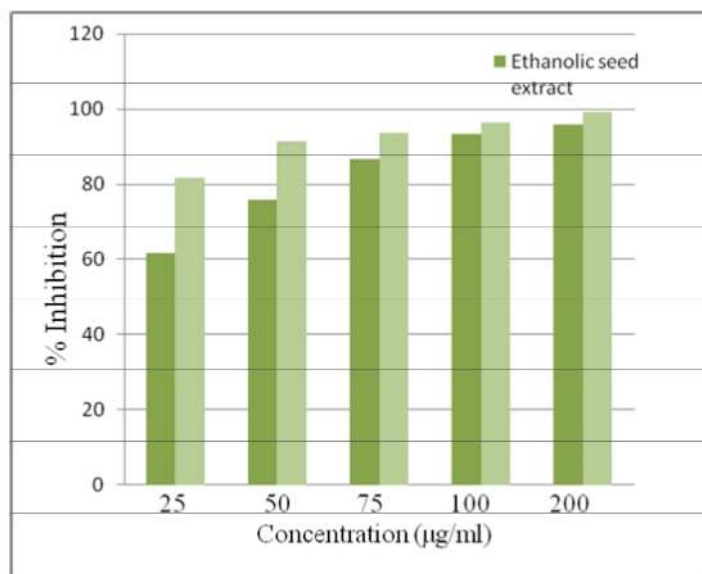
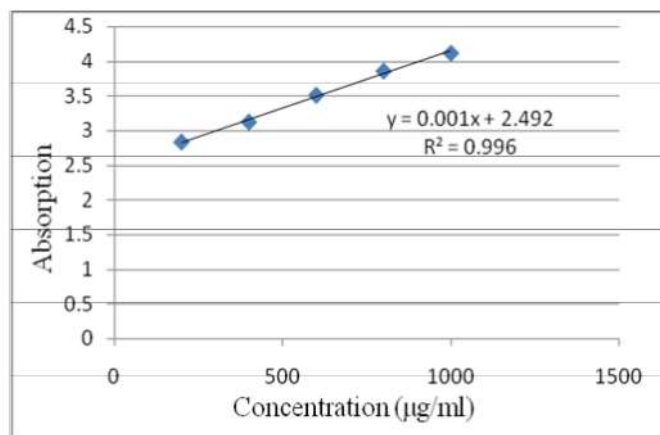
Figure 2: H₂O₂ scavenging activity

Figure 3: Standard curve of Rutin (20-100 µg/ml)

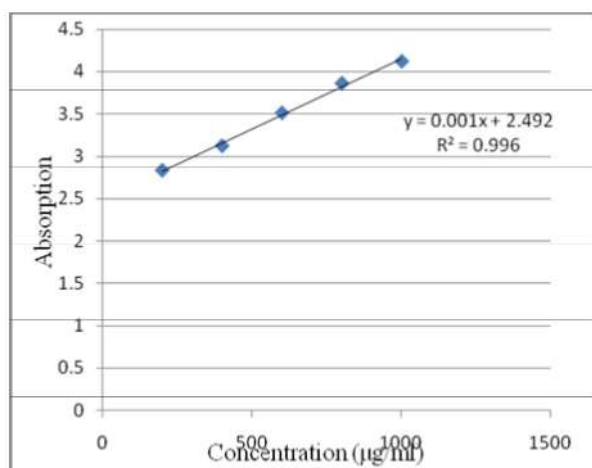


Figure 4: Standard curve of Gallic acid (200-1000 µg/ml)

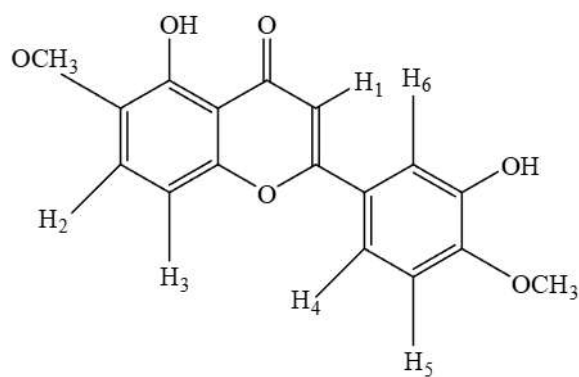


Figure 5: Hypothetical structure of the isolated compound



Figure 6: IR Spectra of isolated compound

Isolation and Characterization

As solvent system (Ethyl acetate: Glacial acetic acid: Formic acid: Water) was used to run through column for elution. All the 8 (F₁, F₂, F₃, F₄, F₅, F₆, F₇ and F₈) fractions eluted from column were individually spotted on Silica gel G plates, developed in the solvent system and analyzed in the UV Chamber. Out of these 8 fractions only 2 fractions (F₃ and F₄) single spot indicating isolated single compound. Further characterization of the single compound i.e. F₃ was carried out by IR and NMR spectroscopy.

Infrared Spectroscopy

IR of this compound shows various regions corresponds to ether group, ketone group, alcohol group, phenol groups etc. Various groups with various regions were shown in IR Spectra given below in Figure 6: C-H stretching (2929 cm⁻¹); ketone (1712 cm⁻¹); C-O-H bending (1385 cm⁻¹); Alcohol (1267 cm⁻¹); C-O stretch (ether, 1073 cm⁻¹); O-H (H-bonded alcohol, phenol, 3400 cm⁻¹); Aromatic C=CH stretch (618 – 670 cm⁻¹); C-O stretch (3° alcohol, 1153 cm⁻¹)

NMR spectroscopy

Calculated NMR shifts are: CH (H₂ - 7); CH (H₃ - 6.36); CH (H₄ - 6.75); CH (H₅ - 6.55); CH (H₆ - 6.66); OH (5.0); CH₃ (3.73); CH₃ (2.35); OH (5.0); OH (2.0); H₁ (6.71). After studying both IR peaks and NMR Shift expected structure was found to be as shown in Figure 5. The hypothetical structure of compound isolated from the *Murraya paniculata*

ethanol seed extract resembled to flavone derivative. For more refine structure further studies are needed.

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