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ISOLATION OF *MANILKARA HEXANDRA* SEEDS FOR ITS PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY

Amandeep Kaur* and Dr. Naresh Singh Gill

Department of Pharmaceutical Chemistry, Rayat Institute of Pharmacy, Railmajra.

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***Corresponding Author**
Amandeep Kaur
Department of Pharmaceutical Chemistry, Rayat Institute of Pharmacy, Railmajra.

ABSTRACT

Manilkara hexandra seed extract were investigated for its potential of natural antioxidant properties. In vitro antioxidant activity of methanol extract was evaluated by Hydrogen peroxide (H_2O_2) and Nitric Oxide (NO) method. The aim of the present study was to investigate the presence of phytochemicals of the selected medicinal plant and also to check its Antioxidant activity. Cold maceration was used for the organic solvent extraction. Solvent used was methanol. Cardiac glycosides and sterols were detected in the plant tested. With the increase in concentration of seed extract, the antioxidant activity increased proportionally to the maximum activity of 81.61 % at 100µg/mL and 60.5% at 100µg/mL with H_2O_2 and NO respectively. Our outcomes presented evidence that crude methanolic extract of *Manilkara* encompass medicinally essential bioactive compounds and it validates their use in the traditional medicines for the medication of several diseases.

KEYWORDS: Phytochemicals, sterols, cardiac glycosides, Antioxidant activity, *Manilkara hexandra*.

INTRODUCTION

Phytochemicals usually stem from the plant source are not anything but the bioactive compounds also recognized as secondary metabolites. There are two forms of metabolites produced in plants viz. Primary metabolites and Secondary metabolites.

Primary metabolites are crucial for the plants regular metabolism for instance growth and development. Secondary metabolites generated by plants may have minute need for them. These are produced in nearly all parts of the plant like bark, leaves, stem, root, flower, fruits, seeds, etc. In the course of past several years, phytochemicals have been employed worldwide as the customary herbal medicine. Because of this pharmaceutical industries as well as researchers put a greater emphasis on the phytochemical studies. Moreover, these phytochemicals existing in the various plant parts are utilized by the local people for healing of several disorders.[1] These are also broadly used in the field of agriculture.

Secondary metabolites are reasonably significant in the making of drugs, flavor and fragrances, dye and pigments, pesticides and food additives. Many of the drugs that are derived from the secondary metabolites are simple synthetic modifications or copies of these naturally obtained substances.[2]

The secondary metabolites of plant origins are gaining immense consideration recently due to their wide range of biological activities. Thus intake of the functional foods for their benefits has markedly increased with the awareness of their safety and nil side-effects.

The majority of pathological conditions like hyperglycemia, cancer, atherosclerosis, rheumatism, cataracts, acquired immune deficiency syndrome, and many other old age and auto-immune diseases are associated with oxidative stress.[3] There are different groups of free radical scavengers, reducing agents/antioxidants, as vitamins C and E, thiols, polyphenols, tri-peptide like glutathione, enzymes such as peroxidase, catalase and superoxide dismutase that act to prevent oxidative damages to deoxyribonucleic acid (DNA), proteins and lipids.[4-6] Antioxidants can delay, prevent or inhibit the oxidation of other oxidisable substances by scavenging free radicals, reactive oxygen species (ROS) and retreating oxidative stress.[7] Antioxidants are studied as vital constituents of nutraceuticals for the reason that these have many health benefits and are considerably used in the foodstuff industry as inhibitors of lipid peroxidation. The interest on the protective biochemical functions of natural antioxidants became increased as they have lesser side effects conversely synthetic antioxidants cause liver damage and carcinogenesis.[8] Vegetables, fruits, seeds, woods, barks, roots, leaf spices and herbs are the potential source of antioxidants.[9]

Manilkara hexandra (Roxb.) Dubard [Synonym: *Mimusops hexandra* (Roxb.)] has Sapotaceae family. It is a socio-economically essential underutilized fruit species illustrated by the occurrence of sticky, commonly white latex in the cuts of bark, branches, leaves and fruit. The small to medium sized evergreen trees are mostly found in western and central Indian states of Rajasthan, Gujarat, Madhya Pradesh and Maharashtra.[10] The matured fresh fruits are ovoid-oblong to ellipsoid in shape, measuring about 1–1.5 cm wide, one or two seeded, shining yellow colored which is soft and sweet in taste, being a good source of minerals and vitamins with low fat content.[11,12] It is locally known as 'Rayan' or 'Khirni'. The fruit and other parts of the tree (bark, stem bark, root, leaves and latex) are known for their various nutritional and medicinal properties used by the older and tribal peoples of India. It has been disclosed that, this fruit is nutritionally essential for a well-balanced diet as the methanolic extract of fruits are registered to have considerable hypoglycemic effect and can be use in the management or control of type II diabetes.[13] In addition, the acetone fraction of *M. hexandra* seed is reported to contain the crude saponin mixture with significant anti-inflammatory activity.[14] The seeds of *M. hexandra* contain approximately 25% edible oil, which can be used for cooking purposes.[15] It is known to have high remedial value as it is demulcent and emollient.[16] Parikh et al.[17] studied the nutritional profile of an underutilized Indian fruit of Rayan.

At the present state of knowledge the free radical scavenging and reducing potentials of seed extracts of *M. hexandra* are not well studied. Therefore, this study was performed to evaluate *in vitro* free radical scavenging and reducing potentials of leaf extract fractions of *M hexandra*.

MATERIAL AND METHOD

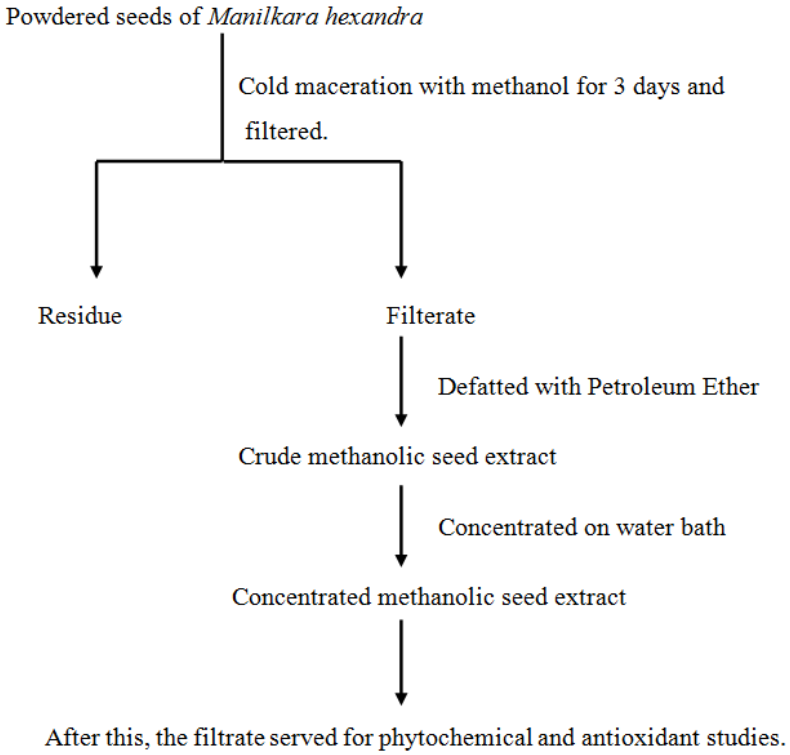
Collection of Plant Material

The fruits of *Manilkara hexandra* were collected in the month of June and July from the village Mehatpur of district Hoshiarpur, Punjab (India). After that, seeds were separated from the fruits by removing their pulp. The healthy seeds were selected from them for future procedure.

Extraction

The healthy seeds of *Manilkara hexandra* were selected and shade dried. The dried seeds were weighed and subjected to cold maceration for 3 days with methanol.

The extraction procedure is given below:



Phytochemical Screening of the Extract

Phytochemical tests were Carried out for various constituents like Flavonoids, Tannins, Alkaloids, Sterols, Triterpenoids, Carbohydrates, Cardiac Glycosides, Saponins, Volatile Oil and Proteins.

Antioxidant Activity

In vitro evaluation of free radical scavenging activities is done by following 2 methods:

H_2O_2 Method

NO Method

4.4.1 Quantitative Evaluation of H_2O_2 Free Radical Scavenging Activity

Scavenging activity of hydrogen peroxide was calculated by the following explained method. A solution of hydrogen peroxide (40mM) was prepared in 0.1 M phosphate buffer (pH 7.4). 1mL of each methanolic extract of *Manilkara hexandra* at different concentrations (25-400 µg/mL) was added to 0.6 mL of 40 mM hydrogen peroxide solution. Absorbance of hydrogen peroxide at 230 nm was revealed after 10 min against a blank comprising phosphate buffer without hydrogen peroxide. The percentage scavenging of hydrogen peroxide of plant extract and reference standard ascorbic acid was computed using the following formula:

% scavenging [H_2O_2] = Absorbanceof control-Absorbance of test sampleAbsorbance of control×100

Mixture of 0.1 M Dipotassium hydrogen phosphate and Potassium Dihydrogen phosphate were used as buffer.

Requirements

Chemicals and Reagents: H_2O_2 (40mM), Phosphate buffer (0.1 M), Ascorbic acid (0.05mM).

Blank: Methanol

Control: H_2O_2 (40mM) solution

Percentage Inhibition (%) = $(A_0\text{-}A/A_0)$ 100

A_0 = Absorbance of control

A = Absorbance of test/ standard

4.4.2 Quantitative Evaluation of NO Free Radical Scavenging Activity

Reagents

10 mM sodium nitroprusside

Phosphate buffered saline pH 7.4

2 % sulphanilamide in ortho-phosphoric acid

0.1 % naphthylethylenediamine dihydrochloride

Procedure

To 1ml of sodium nitroprusside, 2.5 ml, phosphate buffered saline pH 7.4 was added and mixed with 1 ml of extract at various concentrations (µg/ml), then the mixture was incubated at 25˚ C for 30 minutes. From the incubated mixture 1.5 ml was taken. To it, 1ml of sulphanilamide in phosphoric acid and 0.5 ml of naphthyl ethylenediamine dihydrochloride were added and the absorbance was measured at 546 nm. Ascorbic acid was used as a standard. The percentage inhibition of nitric oxide radical generated was calculated by using the following formula:

% scavenging [NO] =Absorbanceof control-Absorbance of test sampleAbsorbance of control×100

RESULT AND DISCUSSIONS

The phytochemical characteristics of medicinal plant tested were summarized in the table. The results revealed the presence of medically active compounds in the plant studied. From the table, it could be seen that, proteins, carbohydrates, sterols, cardiac glycosides and steroids were present in the plant. Cardiac glycosides are effective in treatment of cardiac failure, also investigated for their antitumor activity.[18,19] Steroids are used as anti-hormones,[20] contraceptive drugs,[21] cardiovascular agents,[22] anti-asthmatics, anti-inflammatories.[23]

Table: Phytochemical screening of methanolic extract.

S. No.	Phytoconstituents	Intensity
1.	Alkaloids	-
2.	Steroids and triterpenoids	+++
3.	Flavonoids	+
4.	Amino acid	-
5.	Cardiac glycosides	+++
6.	Saponin glycosides	-
7.	Carbohydrates	+
8.	Proteins	+
9.	Sterols	+
10.	Tannins	-
11.	Volatile oil	++

(-) indicates absence of chemical constituents / (+) indicates presence of chemical constituents / (+++) indicates higher content of chemical constituents

In Vitro Antioxidant Activity

M. hexandra seeds show antioxidant activity by H_2O_2 method and NO method.

1. Quantitative Estimation of Antioxidant Activity Using Hydrogen Peroxide Method

Hydrogen peroxide (H_2O_2) is an oxidant that is being formed continuously in living tissues as a result of several metabolic processes. It reacts with Fe^{2+} (Feton type reaction) and generate extremely reactive oxygen species, including hydroxyl free radical, thus, its detoxification is necessary. Therefore, measurement of H_2O_2 scavenging activity is one of the useful methods for determining the ability of antioxidants to decrease the level of such pro- oxidants.

$$Fe^{2+} + H_2O_2 \quad Fe^{3+} + HO^- + OH^+$$

Table: H_2O_2 Scavenging activity of *Manilkara hexandra* seeds.

S. No.	Concentration	Mean Absorbance (Test)	% Inhibition (Test)	%Inhibition (Standard)
1.	25	0.077	58.35±0.001	61.62±0.002
2.	50	0.055	70.25 ±0.003	75.67±0.001
3.	75	0.044	76.20±0.002	80.54±0.004
4.	100	0.034	81.61±0.001	84.46±0.002

Values are taken as an average triplicate experiment and represented as Mean ± SEM.

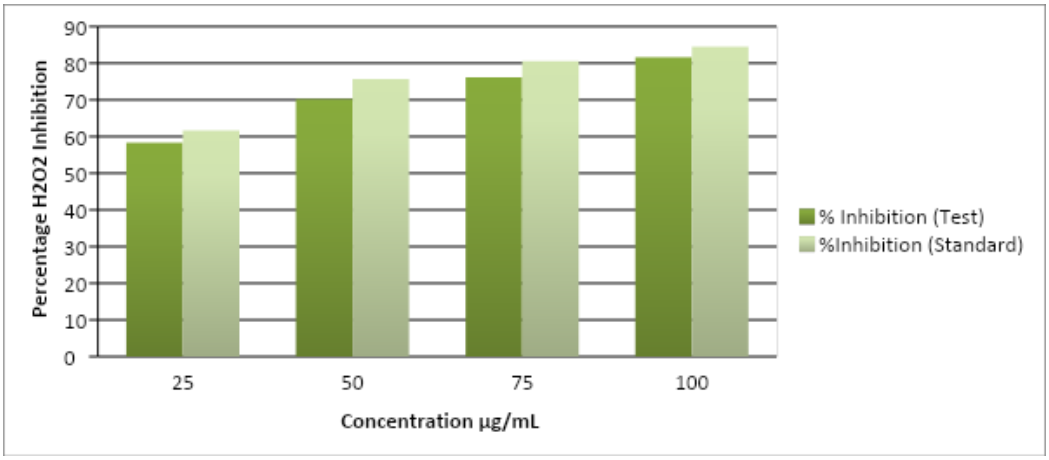


Fig. Graphical representation of H_2O_2 scavenging activity.

Methanolic extract of Manilkara hexandra seeds exhibited H_2O_2 scavenging activity. With the increase in concentration of seed extract, the antioxidant activity increased proportionally with the maximum activity of 81.61 % at 100µg/mL. The absorption decreased proportionally with the increase in the concentration of extract. The maximum absorption was found to be of control sample excluding drug. The percentage inhibition of test and standard samples was calculated by subtracting their absorptions from the absorption of control sample according to formula:

%inhibition = $(A_o – A/ A_o) \times 100$

Where A_o = Absorption of control

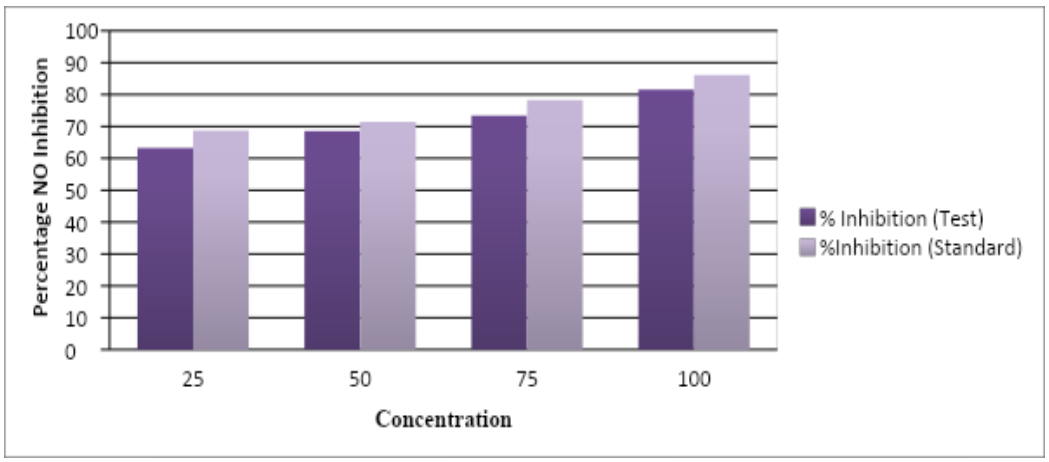
A = Absorption of test/ standard sample

5.4.2 Quantitative Estimation of Antioxidant Activity Using Nitric Oxide Scavenging Method

Nitric oxide scavenging activity was determined according to the method reported by Green et al., 1982. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interact with oxygen to produce nitrite ions, which can be determined by the use of the Griss Illosvoy reaction. The nitrite ions produced diazotizes sulphanilamide and then the diazonium salt reacts with N, N-Naphthyl ethylene diamine dihydrochloride to give a pink colour chromophore which has a maximum absorption at 546 nm.

Table Scavenging activity of *Manilkara hexandra* seeds.

S. No.	Concentration	Mean Absorbance (Test)	% Inhibition (Test)	%Inhibition (Standard)
1.	25	0. 048	63.27	68.72
2.	50	0.041	68.52	71.48
3.	75	0.035	73.45	78.29
4.	100	0.024	81.56	86.14



Graphical representation of NO scavenging activity

Methanolic extract of Manilkara hexandra seeds exhibited NO scavenging activity. With the increase in concentration of seed extract, the antioxidant activity increased proportionally with the maximum activity of 60.5% at 100µg/mL. The absorption decreased proportionally with the increase in the concentration of extract. The maximum absorption was found to be of control sample excluding drug.

CONCLUSION

The outcomes revealed the occurrence of medicinally essential constituents in the plants studied. Many confirmations collected in earlier studies which validated the identified phytochemicals to be bioactive. Several studies confirmed the presence of these phytochemicals contribute medicinal as well as physiological properties to the plant studied in the treatment of different ailments. The methanolic extract of seed of *M. hexandra* showed the most effective NO Scavenging and Hydrogen peroxide scavenging power and total antioxidant activity in terms of ascorbic acid equivalent. Therefore, it may be considered as a natural source of antioxidants though there is need for further *in vivo* antioxidant *an*d toxicity assessments.

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