DOI: 10.5138/09750185.2198

AR JOURNALS ADVANCED RESEARCH

International Journal of Phytomedicine
2018;10(1):39-44

Original Research Article

# Evaluation of antioxidant potential of methanolic extracts of bark and leaf of *Quassia indica* (gaertn.) Nooteb.

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**Abstract**

Plants are a natural source of secondary metabolites having antioxidant property and these compounds protect cells against adverse effects of reactive oxygen species. In the present study the methanolic extracts of bark and leaf of medicinal plant - Quassia indica (Gaertn.) Nooteb. Were evaluated and compared employing ABTS cation decolonization test, DPPH scavenging assay, reducing power and scavenging potency towards hydrogen peroxide, nitric oxide and super oxide. The results suggested that the bark extract has better antioxidant efficacy than leaf for all in vitro models compared. The $IC_{50}$ metrics (mg/g) of bark and leaf for the assays were ABTS (bark 552.36; leaf 1917); DPPH (bark 572.30; leaf 1782.31); $H_2O_2$ (bark 566.89; leaf 1904.32); Nitric Oxide (bark 956.75; leaf 1711.74) and Super oxide (bark 235; leaf 506.68) respectively. Results of the study concluded that Q. indica is a promo sing source of antioxidants and could be exploited as a safe and stable natural agent for pharmaceutical preparations.

**Keywords:** Quassia indica; Antioxidant; ABTS; DPPH; H2O2; NO; Reducing power assay; Superoxide scavenging

## Introduction

Quassia indica (Gaertn.) Nooteb. A medicinal plant is mainly used in Ayurvedic formulations and by tribal people for curing various diseases. The plant parts yield terpenoids and flavonoids in bitter taste which exerts medicinal properties against various disorders and skin diseases [1]. The plant parts used are: bark, wood chips, flowers, leaves, roots and seeds. Quassia indica (Gaertn.) Nooteb. Belongs to the family Simaroubaceae. This family is characterized by the presence of quassinoids — secondary metabolites responsible of a wide spectrum of biological activities such as anti tumor, antimalarial, antiviral, insecticidal, feeding deterrent, amoebicidal, anti parasitic and herbicidal [2] . Antioxidants have been much highlighted in the current scenario as they have the ability to remove free radicals which are produced during the degenerative diseases. Oxidation in living organisms is essential for the generation of energy during catabolism but these metabolic processes result in the continuous production of free radicals and reactive oxygen species (ROS) in vivo. Free radicals or more generally ROS are highly reactive species that are generated by cells during respiration and cell-mediated immune functions [3]. Free radicals formation is also triggered through the influence of environmental pollutants, cigarette smoke, automobile exhausts, radiations, and pesticides [4]. The free radicals are highly unstable and display high levels of reactivity due to the lone electron in the outer shell. As a consequence they can react with specific bio molecules in the body such as protein and lipids. The inherent antioxidant mechanism of our body can normally maintain a balance between the quantity of free radicals generated in the body and the level of antioxidant processes which scavenge/quench these free

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Received: 15 Oct 2017, Accepted: 24 Jan 2018

radicals to prevent them from causing deleterious effects in the body. The antioxidant mechanisms include endogenous and exogenous systems such as catalase and vitamin antioxidants etc. When the generation of free radicals exceeds the scavenging capacity of the cell's endogenous systems, the excess free radicals attain stability through electron pairing with biological macro molecules of healthy cells such as proteins, lipids, and DNA. The pairing of the free radicals with bio molecules can subsequently lead to the induction of lipid per oxidation which enhances the risks to cancer, atherosclerosis, cardiovascular diseases, ageing, and inflammatory diseases [4] [5].

In plants the antioxidants serve as a defense system by detoxifying harmful reactive oxygen species (ROS) that are generated by electron-transfer chemical reactions. ROS in plants serve as key regulators of growth, development, and defense pathways, as well as at excessive levels they cause oxidative damage to bio molecules leading to toxicity in the cell [6]. Antioxidants from natural sources are considered superior to synthetic as they are safe for use in foods or medicinal purposes. Q. indica a bitter plant widely used in traditional healing is a rich source of a wide spectrum of secondary metabolites with anti oxidative potency [1]. Literature showed the presence of Quassinoids in Simaroubaceae and therefore, the plant may show the antioxidant activity which needs to be evaluated in detail. Hence, the present study was focused out to assess the antioxidant activity of Q. indica by employing different in-vitro models.

## Material and Methods

***Preparation of Plant extracts***

The bark and leaf of Q. indica (Gaertn.) Nooteb. Were collected, washed thoroughly in tap water, shade dried and ground into fine powder. The dried powder (25 g) samples were extracted with 100 ml of methanol. The samples were placed in a water bath at a temperature of $45^0$C for 24 hours and then kept in a gyratory shaker at 120 rpm for 48 hours. Each type of extract was filtered using what man No.1 filter paper, dried to attain constant weight. The final residues were stored in a refrigerator at $4^0$C for further use.

***Evaluation of antioxidant activity***

***ABTS Radical scavenging assay***

The ability of the extracts to scavenge ABTS i.e. 2, 2'-azinobis (ethylbenzthiazoline-6-sulfonic acid) cation radical was analyzed following the protocol of [7]

The radical cation was prepared as follows: Solution I

— 20 mm ABTS in distilled water

Solution II — 17 MM potassium per sulfate in distilled water

About 0.3 ml of solution II was added to 50 ml of solution I. The reaction mixture was allowed to stand at room temperature overnight in dark before use. After completion of reaction, the solution was diluted with ethanol to get an absorbance of 0.700 + 0.05 at 734 nm. The photo metric assay was carried out by adding 0.16 ml of ABTS solution to 0.2 ml of test solution containing various concentrations (125 – 2000 mg/ml) of extracts. After mixing the samples were kept for 20 minutes and the absorbance was recorded at 734 nm.

The antioxidant activity of the samples was calculated on basis of decrease in absorbance as follows:

ABTS scavenged (%) = (A control — A Test)/A control x 100

Where "A control" is the absorbance of the control reaction and "A test" is the absorbance of the sample of the extracts.

***DPPH (2,2-Diphenyl-l-Picryl Hydrazyl) radical scavenging assay***

The antioxidant activity of the plant extracts was estimated using the DPPH radical scavenging protocol [8] . DPPH solution (0.004% w/v) was prepared in methanol. A stock solution of DPPH solution — 0.004% (w/v) prepared in 95 % methanol. Methanolic extract and standard ascorbic acid were prepared in the concentration of mg/ml. From stock (methanolic extract and ascorbic acid) solution different aliquots were taken in test tubes. With solvent made the final volume of each test tube up to 1 ml to get the required concentration ranges (100 – 1000 mg/ml for bark; 200 – 4000 mg/ml for leaf). 2 ml of freshly prepared DPPH solution (working solution) (0.004% w/v) was added in each of these test tubes. The reaction mixture was incubated in the dark for 30 min and thereafter the optical density was recorded at 517 nm against the blank. For the control, 2 ml of DPPH solution in methanol was mixed with 1ml of methanol and the optical density of the solution was recorded after 30 min. The assay was carried out in triplicates. The decrease in optical density of DPPH on addition of test samples in relation to the control was used to calculate the antioxidant activity, as percentage inhibition (%IP) of DPPH radical. The capability of scavenging DPPH radical was calculated using the following equation.

DPPH scavenged (%) =(A control — A Test) / A control x 100

Where "A control" is the absorbance of the 'control' reaction and "A test" is the absorbance of the sample of the extracts. $IC_{50}$ values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

***Hydrogen peroxide scavenging assay***

The hydrogen peroxide scavenging assay was carried out according to the method of Ruch et al. [9]. A solution of hydrogen peroxide (43 mm) was prepared in phosphate buffer (0.1 M, pH 7.4). The extract at different concentrations (125 –2000 mg/ml) in 3.4 ml phosphate buffer was added to 0.6 ml of $H_2O_2$solution (43 mm). The absorbance of the reaction mixture was recorded at 230 nm.

Calculation: $H_2O_2$ scavenging (%) = (A control — A Test) / A control x 100

***Nitric oxide scavenging activity***

This assay is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generate nitric oxide which interacts with oxygen to produce nitrite ions that can be quantified using Griess reagent [10]. Griess reagent was prepared by mixing equal amounts of 1% sulfanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use. A volume of 1 ml of test solutions each containing varying concentrations (125 – 2000 mg/ml) of the extracts. The mixture was incubated at $25^0$C for 180 minutes. The extract was mixed with an equal quantity of freshly prepared Griess reagent. Control samples without the extracts but with an equal volume of buffer were prepared in a similar manner as was done for the test samples. After 30 minutes, 1.5 ml of the incubated solution was taken and diluted with 1.5 ml of Griess reagent. Absorbance of the chromophore formed during diazotization of the nitrate with sulfanilamide and subsequent coupling with N-1 naphthyl ethylene diamine dihydrochloride was measured spectrophotometrically at 546 nm. The percentage scavenging activity was measured with reference to the standard Gallic acid.

% inhibition = (A control — A Test) / A control x 100

***Superoxide scavenging assay***

Super oxide anion are generated in riboflavin-NADH system by the oxidation of NADH and assayed by the reduction of NBT (Nitro-blue tetrazolium) resulting in the formation of blue formazan product [11]. Different concentrations of extracts (125-2000$\mu$g/ml), 0.05ml of Riboflavin solution (0.12mM), 0.2 ml of EDTA solution (0.1M), and 0.1 ml NBT solution (1.5mM) were mixed in test tube and reaction mixture was diluted up to 2.64ml with phosphate buffer (0.067M). The absorbance of solution was measured at 560 nm using DMSO as blank after illumination for 5 min and difference in OD was determined after 30 minutes incubation in fluorescent light. Absorbance was measured after illumination for 30 min. at 560 nm on UV visible spectrophotometer.

Calculation : (A control — A Test) / A control x 100

***Reducing power assay***

Methanolic and aqueous extracts of bark and leaf samples were compared for their Ferric reducing power [12] using ascorbic acid as standard.

In ferric reducing antioxidant power assay, 1 ml of test sample of extract in different concentrations were mixed with 1 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide in separate test tubes. The reaction mixtures were incubated in a temperature-controlled water bath at $50^0$C for 20 min, followed by addition of 1 ml of 10% trichloroacetic acid. The mixtures were then centrifuged for 10 min at room temperature. The supernatant obtained (1 ml) was added with 1 ml of deionised water and 200 $\mu$l of 0.1% $FeCl_3$. The blank was prepared in the same manner as the samples except that 1% (w/v) potassium ferricyanide was replaced by distilled water. The absorbance of the reaction mixture was measured at 700 nm. The reducing power was expressed as an increase in A700 after blank subtraction.

## Results & Discussion

The methanolic extract of bark and leaf of Q. indica were analyzed for antioxidant activity by various in vitro methods such as ABTS cation decolorization test, DPPH quenching assay, reducing power efficiency, scavenging capacity towards hydrogen peroxide ($H_2O_2$) radical, Nitric oxide (NO) radical inhibition activity and Super oxide assays. The percentage scavenging and $IC_{50}$ values were calculated for all in vitro models and comparisons made.

***ABTS assay***

The antioxidant activity of the bark and leaf extract samples determined for a concentration range 125 – 2000 mg/ml is illustrated in Table 1. At 1000 mg/ml, the inhibition by the extracts were 64.76% for bark and 41.40% for leaf and the potency of the bark was detected as equivalent to the standard ascorbic acid (64.09%). The ABTS quenching ability with $IC_{50}$ values were determined as 526.09 mg/ml (ascorbic acid), 552.36 mg/ml (Bark) and 1917 mg/ml (leaf) respectively.

The result on ABTS Assay suggested that there is a posi- tive correlation between extract concentration and percentage of ABTS cation scavenging. Among the extract, the highest scavenging activity was recorded — 78.10% for bark extract of 2000 $\mu$g/ml concentration.

**Table 1** Results of ABTS assay of methanolicextract of leaf and bark of Q. indica (Gaertn.) Nooteb.

| | | Percentage of inhibition | |
|---|---|---|---|
| Conc. (μg) | Ascorbic acid | Leaf | Bark |
| 125 | 15.08 | 8.86 | 12.62 |
| 250 | 24.62 | 14.78 | 22.66 |
| 500 | 47.52 | 34.05 | 45.26 |
| 1000 | 64.09 | 41.40 | 64.76 |
| 2000 | 83.49 | 52.16 | 78.10 |

### *DPPH antioxidant activity*

The DPPH antioxidant activity of methanolic extract of bark was determined for the concentration range 100 – 1000 mg/ml, as more than 70% inhibition was attained (Table 2a). However, for leaf extracts, much higher levels of concentrations were required to decrease the DPPH* radicals by $IC_{50}$. Therefore, a concentration range of 200 – 4000 mg/ml was selected in order to attain a same pattern of inhibition (Table 2b). The $IC_{50}$ values were estimated as 572.30 (bark) and 1782.21 (leaf).

DPPH is a stable free radical and it can accept an electron or hydrogen radical to become a stable diamagnetic molecule[13]. A freshly prepared DPPH solution is of deep purple color with l max 517 nm and in the presence of an antioxidant, the purple color disappears due to quenching of DPPH* free radicals and converting them into a colorless product- 2,2-diphenyl-1-picryl hydrazine [14].

In the present study, the result on DPPH scavenging activity suggested that there is increase in percentage of radical scavenging activity with increase in concentration of the extract for both bark and leaf. Among the plant parts compared, bark contained phyto ingredients to effectively scavenge DPPH than leaf. This could be due to greater amounts of phenolics and flavonoids in bark.

**Table 2** A. Results of DPPH assayof methanolic extract of bark of Q. indica (Gaertn.) Nooteb.

| Conc. (μg/ml) | % Inhibition |
|---|---|
| 100 | 15.03 |
| 200 | 24.73 |
| 300 | 31.12 |
| 400 | 41.06 |
| 500 | 46.27 |
| 600 | 52.42 |
| 700 | 57.16 |
| 800 | 63.55 |
| 900 | 70.06 |
| 1000 | 73.25 |

### *Reducing power assay*

The bark and leaf extracts of Q. indica (Gaertn.) Nooteb. indicated concentration dependent reducing power and a comparative assessment is depicted in Table 3. The higher absorbance value indicated a stronger reducing power of the extract. The extracts, being a source of antioxidant, can donate electron to free radicals. Reducing power was measured on basis of the reduction of $Fe^{3+}(CN^{-})_6$to $Fe^{2+}(CN^{-})_6$ and the reaction product was monitored by means of the intense Prussian blue color of the complex that is measured at 700 nm. The reducing power potential of both leaf and bark extracts at 100 – 200 μg/ml was detected as higher than that of standard Ascorbic acid.

**Table 3** Table 2b. Results of DPPH assay of methanolicextract of leaf of Q. indica (Gaertn.) Nooteb.

| Conc.(μg/ml) | % Inhibition |
|---|---|
| 400 | 7.05 |
| 800 | 15.05 |
| 1200 | 32.56 |
| 1600 | 46.46 |
| 2000 | 56.11 |
| 2400 | 64.86 |
| 2800 | 67.28 |
| 3200 | 74.51 |
| 3600 | 75.29 |
| 4000 | 77.09 |

**Table 4** Resultsof FRAP Assay of Methanolic extract of Leaf and Bark of Q. indica (Gaertn.) Nooteb.

| | | Absorbance | |
|---|---|---|---|
| Conc. (μg/ml) | Ascorbic acid | Leaf | Bark |
| 100 | 0.089 | 0.157 | 0.182 |
| 200 | 0.167 | 0.194 | 0.231 |
| 300 | 0.253 | 0.233 | 0.249 |
| 400 | 0.321 | 0.261 | 0.319 |
| 500 | 0.386 | 0.299 | 0.372 |
| 600 | 0.452 | 0.351 | 0.418 |
| 700 | 0.496 | 0.401 | 0.531 |
| 800 | 0.573 | 0.419 | 0.551 |
| 900 | 0.625 | 0.437 | 0.629 |
| 1000 | 0.712 | 0.511 | 0.664 |

### *Hydrogen peroxide scavenging assay*

The ability of extracts to scavenge hydrogen peroxide, monitored for a concentration range – 125–2000 μg/ml is illustrated in Table 4.

The results indicated a dose-dependent scavenging potential and the bark extract could scavenge more than 50 % at 500 μg/ml. The standard ascorbic acid exhibited much higher inhibition potency than the sample extracts.

Hydrogen peroxide may enter into the human body through inhalation of vapor or mist and through eye or skin contact. $H_2O_2$ is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals (-OH) that can initiate lipid peroxidation and cause DNA damage in the body [15].

**Table 5** Results of $H_2O_2$ ScavengingAssay of Methanolic extract of Leaf and Bark of Q. indica (Gaertn.) Nooteb.

| Conc. (μg/ml) | % Inhibition | | |
|---|---|---|---|
| | Ascorbic acid | Leaf | Bark |
| 125 | 16.66 | 6.38 | 16.20 |
| 250 | 28.97 | 15.80 | 33.90 |
| 500 | 44.10 | 22.68 | 56.93 |
| 1000 | 67.94 | 41.20 | 64.34 |
| 2000 | 80.76 | 52.52 | 72.44 |

Moreover, hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. $H_2O_2$ can cross cell membranes rapidly and once inside the cell, $H_2O_2$ can react with $Fe^{2+}$ to form hydroxyl radical and initiate toxic effects [16]. The percentage of hydrogen peroxide scavenging by the extracts indicates antioxidant potential and in the present study, the bark extract could scavenge $H_2O_2$ up to 72.44% at a concentration level of 2000 mg/ml. The $IC_{50}$ values were estimated as 439.32 (Ascorbic acid), 566.89 (Bark) and 1904.32 (leaf).

***Nitric oxide scavenging activity***

The results of the ability of extracts to scavenge nitric oxide are represented in Table 5. The scavenging potential increased in concentration dependent manner. A significant level of inhibition due to decrease in NO radical due to the presence of antioxidants in plant extract. A comparison with standard, Gallic acid suggested that the extracts were lesser efficient than standard.

**Table 6** Results of Nitric Oxide scavenging Assay ofMethanolic extract of Leaf and Bark of Q.indica (Gaertn.) Nooteb.

| Conc. (μg/ml) | | % Inhibition | |
|---|---|---|---|
| | Gallic acid | Leaf | Bark |
| 125 | 19.44 | 7.68 | 20.83 |
| 250 | 34.53 | 14.62 | 21.75 |
| 500 | 58.08 | 32.36 | 39.72 |
| 1000 | 71.63 | 40.36 | 52.26 |
| 2000 | 92.90 | 58.42 | 70.94 |

The $IC_{50}$values were found to be lower for bark (956.75) in comparison to leaf (1711.74) and concluded a better antioxidant potency of bark. Both the extracts were lesser efficient than the standard, gallic acid.

In vitro inhibition of nitric oxide radical is a measure of antioxidant activity of plant extracts. Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent [17]. The methanolic extract decreased the amount of nitrite generated from the decomposition of sodium nitroprusside which may be due to the presence of antioxidant principles in the extract.

***Superoxide radical scavenging activity($O_2$-)***

As monitored for all other in vitro assays, the superoxide radical scavenging ability was also dose- dependent and the bark proved to be a better source of antioxidant with much lower $IC_{50}$ of 235mg/ml.

Superoxide radical is a potent reactive oxygen species (ROS) and is highly toxic at fairly low concentrations [18] . Though this anion (O2-) is a weak oxidant, it can react with other molecules in tissues to form highly toxic hydroxyl radicals as well as single oxygen, both are significant contributors to oxidative stress.

**Table 7** Results of Superoxide Scavenging Assay ofMethanolic extract of Leaf and Bark of Q.indica (Gaertn.) Nooteb.

| Conc. (μg/ml) | | % Inhibition | |
|---|---|---|---|
| | Ascorbic acid | Leaf | Bark |
| 125 | 41.19 | 19.59 | 31.82 |
| 250 | 47.03 | 35.97 | 53.19 |
| 500 | 62.70 | 49.34 | 63.81 |
| 1000 | 76.32 | 63.25 | 76.86 |
| 2000 | 90.68 | 68.13 | 83.26 |

The data in Table 6 illustrates a comparative evaluation of the scavenging potential of the standard- Ascorbic acid in relation to the extracts. The values of bark and standard are in almost proximal levels at 500 and 1000 mg/ml.

## Conclusions

The results of the present study concluded that the bark of Quassia indica (Gaertn.) Nooteb. possesses more antioxidant activity than leaf in all in vitro assays compared. Observations from all antioxidant tests indicated a dose dependent increase in efficacy to either scavenge or to reduce the free radicals. The potential of bark as an antioxidant is almost comparable with the standard Ascorbic acid and Gallic acid in various assays. The leaf exhibits moderate activity.

## Authors' contributions

First author (Sudha Bai R): Conception and design of project, data interpretation and manuscript preparation.

Second author (Anusha P): Conducted experiments for data acquisition.

**Acknowledgements**

The authors acknowledge Kerala State Council for Science, Technology and Environment (KSCSTE) for providing financial

support under Student Project Scheme. The authors are thankful to the Principal, University College, Thiruvananthapuram for extending all Laboratory facilities.

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