IN VITRO ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL SCREENING OF LEAF EXTRACTS OF GREWIA HETEROTRICHA MAST

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ABSTRACT

Objective: To investigate the presence of phytochemical components and to evaluate the in vitro antioxidant activity of pet. ether, chloroform, methanol and aqueous extracts of Grewia heterotricha mast leaves.

Methods: The leaves of Grewia heterotricha mast were dried and extracts were prepared using a pet. ether, chloroform, methanol by soxhlet extraction method. The aqueous extract was prepared using distilled water by cold extraction method. The preliminary phytochemical analysis was carried out on aqueous, methanol, chloroform and pet. ether leaf extracts of the plant using standard qualitative procedures. The total phenolic content (TPC) was estimated using modified Folin-Ciocalteau method, tannin content by Folin-Denis method and total flavonoids by aluminum chloride method.

Results: The preliminary phytochemical analysis revealed the presence of complex bioactive constituents like phenols, tannins, alkaloids, terpenoids, flavonoids, saponins, steroids, glycosides, coumarins, proteins and carbohydrates. Methanolic extract showed highest total phenolic content (87.58±2.52 mg CE/g) than aqueous extract (78.46±5.36 mg CE/g). Higher tannin content was found in the aqueous extract (148.0±8.96 mg TAE/g). Total flavonoids were highest in chloroform extract (314.9±25.06 mg QE/g) followed by aqueous (242.9±32.42 mg QE/g) and methanolic extract (217.0±18.32 mg QE/g) and lowest in a pet. ether extract (188.6±23.35 mg QE/g). The methanolic extract had shown very significant DPPH radical scavenging activity (IC50 98.95 µg/ml) and H2O2 scavenging activity (IC50 110.1µg/ml) compared to the standard ascorbic acid. Higher reducing ability was observed in methanol extract (131.0±11.67 mg AE/g).

Conclusion: The results obtained reveal that the leaves of Grewia heterotricha mast have potent antioxidant property. The observed activity may be associated with bioactive components like phenolics, flavonoids present in the leaf extracts and could have greater importance as therapeutic agents in oxidative stress-related degenerative diseases. Further studies are needed in order to purify bioactive compounds responsible for the antioxidant property.

Keywords: Grewia heterotricha mast, Antioxidant activity, Flavonoids, Phenolics, DPPH, Reducing power

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INTRODUCTION

A number of plants have been used in traditional medicine over many years. These medicinal plants have been considered as sources for new drugs to treat numerous diseases. Therapeutic properties of medicinal plants are due to the presence of a wide variety of bioactive components in them. The most important bioactive components are phenolic compounds, flavonoids, alkaloids, and tannins. These phytochemicals are secondary metabolites synthesized by the plants [1, 2].

The secondary metabolites like flavonoids and phenolic compounds are considered as strong antioxidants which have the ability to scavenge free radicals, inhibit the activity of lipoxigenase and prevent tissue damage [3-5]. Natural antioxidants can reduce the risks of various oxidative stress-related diseases such as cancer, neurodegenerative disorders, heart diseases and inflammation [6-8].

Grewia heterotricha mast belongs to the family Malvaceae, is a scendent shrub common in forests and along hedges in India. It is widely used as folk medicine in wound healing, fever, bronchitis, and to cure some skin and intestinal infections [9]. The pharmacological properties of this plant have not yet been evaluated. Hence, the present study was undertaken to identify the bioactive components and to evaluate antioxidant activity of leaves of Grewia heterotricha mast by in vitro methods.

MATERIALS AND METHODS

Collection of plant material

Grewia heterotricha mast plants were collected from Udipi, during monsoon and post-monsoon seasons. The plant was authenticated by Dr. K. Gopalakrishna Bhat, Botanist, Udipi. A voucher specimen (20/11/2013) has been kept in our laboratory for future reference. The leaves were washed, shade dried and finely powdered and stored in air-tight container.

Preparation of extract

The powdered leaves (75g) were extracted successively with 350 ml each of petroleum ether (40-60°C), chloroform, methanol using Soxhlet extractor for 24hr. The extracts were concentrated by evaporation using rotary vacuum evaporator to obtain dark viscous semi-solid. Similarly, water extract was also prepared by mixing leaf powder in distilled water and stirred continuously using magnetic stirrer for 4hr. The mixture was filtered and the filtrate was then concentrated. All the extracts were stored in a refrigerator and were used for further study.

Phytochemical screening

The leaf extracts were tested for the presence of bioactive compounds like flavonoids, terpenoids, alkaloids, glycosides, tannins, phenolics, saponins carbohydrates and proteins by using standard methods [10-12].

Quantitative analysis of antioxidant component

Determination of total phenolic content

The total phenolic content was estimated using the modified Folin-Ciocalteu method [13]. 0.5 ml of extract and 0.5 ml Folin-Ciocalteu’s reagent was mixed and the mixture was incubated at room temperature for 3 min. Then 2.0 ml 20% sodium carbonate solution was added and the mixture was incubated at room temperature for 10 min.
was added and further incubated in boiling water bath for 1 min and the absorbance was measured at 650 nm. Catechol was used as a standard. Total phenols were expressed in terms of catechol equivalent (mg CE/g of dry extract) (table 2).

**Determination of total flavonoids**

The total flavonoid content was determined by aluminium chloride method [14]. Extract solution (400 μg/ml) of plant extract was added to 4 ml of distilled water. Sodium nitrite solution (0.3 ml, 5%) was then added to the mixture followed by incubation for 5 min after which 0.3 ml of 10% aluminium chloride was added. The mixture was allowed to stand for 6 min at room temperature before 2 ml of 1 M sodium hydroxide was finally added and the mixture diluted to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm with a UV/Vis spectrophotometer immediately. Quercetin was used as the standard for the calibration curve. Total flavonoids were expressed as mg quercetin equivalent (mg QE/g dry weight) (table 3).

**Determination of tannin content**

The tannin content was determined by Folin-Denis reagent method [14]. 0.1 ml of the sample extract was added with 7.5 ml of distilled water and 0.5 ml of Folin -Denis reagent, 1 ml of 35% sodium carbonate solution and diluted to 1 ml of distilled water. The mixture was shaken well, kept at room temperature for 30 min and further incubated in boiling water bath for 1 min and the absorbance was measured at 700 nm. Blank was prepared with water instead of the sample. Tannic acid was used as a standard. The results of tannins are expressed in terms of tannic acid equivalent (mg TAE/g of dry extract) (table 4).

**In vitro antioxidant activity**

DPPH radical scavenging activity

DPPH radical scavenging activity was measured according to the method of Chu et al. [16] and Barku et al. [17]. An aliquot of 2 ml of 0.004% DPPH solution in methanol and 1 ml of plant extract in methanol at various concentrations (100, 200, 300, 400 and 500 μg/ml) were mixed and incubated at 25 °C for 30 min and the absorbance of the test mixture was read at 517 nm using a spectrophotometer against a DPPH control containing only 1 ml of methanol in place of the extract. Ascorbic acid was used as a standard. Percent inhibition was calculated using the following expression:

\[ \text{% Inhibition} = \frac{\text{A}_{0} - \text{A}_{1}}{\text{A}_{0}} \times 100 \]

Where \( A_0 \) and \( A_1 \) stand for absorbance of the blank and absorbance of tested extract solution respectively.

**Results**

**Phytochemical Screening**

The present study was carried out in leaf extracts of Grewia heterotricha mast. Each extract showed the presence of different bioactive components. Terpenoids, flavonoids, saponins, steroids, glycosides, coumarins and carbohydrates were present in all extracts whereas phenols, tannins and proteins were present in methanol and aqueous extracts. Alkaloids were present only in a pet. ether and aqueous extracts. The results are presented in table 1.

| Phytochemicals          | Petroleum ether leaf extract | Chloroform leaf extract | Methanol leaf extract | Aqueous leaf extract |
|-------------------------|------------------------------|-------------------------|-----------------------|---------------------|
| Alkaloids               | +                            | +                       | +                     | +                   |
| Flavonoids              | +                            | -                       | +                     | +                   |
| Di-terpenoids           | +                            | +                       | -                     | +                   |
| Tri-terpenoids          | -                            | +                       | +                     | +                   |
| Saponins                | -                            | +                       | -                     | +                   |
| Tannins                 | -                            | +                       | +                     | +                   |
| Glycosides/Stereoids    | -                            | -                       | -                     | -                   |
| Phenols                 | -                            | +                       | +                     | +                   |
| Anthocyanins            | +                            | -                       | +                     | -                   |
| Coumarins               | +                            | +                       | +                     | +                   |
| Carbohydrates           | +                            | +                       | +                     | +                   |
| Proteins                | -                            | +                       | +                     | +                   |

+ represents compound present, represents compound absent.

**Determinations of total phenolic content**

The total phenolic content of the extracts was measured by using Folin-Ciocalteu reagent and results were expressed in terms of catechol equivalent (the standard curve equation \( y = 0.02x + 0.002, R^2 = 0.9978, \) fig. 1) shown in table 2. Methanolic extract of Grewia heterotricha leaves showed good phenolic content (87.58±2.52 mg CE/g) than aqueous extract (78.46±5.36 mg CE/g).

**Determinations of total flavonoid content**

The total flavonoid content of the extracts in terms of quercetin equivalent (the standard curve equation \( y = 0.0009x + 0.003, R^2 = 0.9988, \) fig. 2) were shown in table 3. The results revealed that the chloroform extract of Grewia heterotricha showed higher total flavonoid content (31.49±25.06 mg QE/g) followed by aqueous (242.98±32.42 mg QE/g) and methanolic extract (217.0±18.32 mg QE/g). Pet Ether fraction had the lowest total flavonoid content (188.86±23.35 mg QE/g).

**Table 1: Phytoconstituents in different leaf extracts of Grewia heterotricha mast**

| Extracts                | mg Catechol/g dry extract |
|-------------------------|---------------------------|
| Methanol                | 87.58±2.52                |
| Aqueous                 | 78.46±5.36                |

Each value represents mean±SD (n=3).
Fig. 1: Standard calibration curve for total phenolic content

Table 3: The total flavonoid content present in the leaf extracts of *Grewia heterotricha* mast

| Extracts     | mg quercetin/g dry extract |
|--------------|----------------------------|
| Pet. ether   | 188.86±23.35               |
| Chloroform   | 314.9±25.06                |
| Methanol     | 217.0±18.32                |
| Aqueous      | 242.98±32.42               |

Each value represents mean±SD (n = 3).

Fig. 2: Standard calibration curve for total flavonoid content

**Determination of tannin content**

The tannin content of the extracts was expressed as tannic acid equivalent/g of the dry extract (the standard curve equation y=0.006x+0.004, R²=0.995, fig. 3) were shown in table 4. The results revealed that the aqueous extract of *Grewia heterotricha* have higher tannin content (148.0±8.96 mg TAE/g) than methanolic extract (130.7±8.05 mg TAE/g).

Table 4: The tannin content present in the leaf extracts of *Grewia heterotricha*

| Extracts     | mg tannic acid/g dry extract |
|--------------|------------------------------|
| Methanol     | 130.7±8.05                   |
| Aqueous      | 148.0±8.96                   |

Each value represents mean±SD (n = 3).

**In vitro antioxidant activity**

**DPPH radical scavenging activity**

The antioxidant activity of different leaf extracts of the plant was investigated by DPPH radical scavenging assay using ascorbic acid as a standard. The results were summarized in table 5. The methanolic extract showed maximum free radical scavenging activity (IC₅₀ 98.95 µg/ml) which was significantly comparable with free radical scavenging activity of ascorbic acid (IC₅₀ 13.44 µg/ml).

Table 5: DPPH scavenging activity of the extracts

| Concentration(µg/ml) | Extracts | % inhibition |
|----------------------|----------|--------------|
| 100                  | Pet. ether | 9.3          |
| 200                  | Pet. ether | 18.61        |
| 300                  | Pet. ether | 25.97        |
| 400                  | Pet. ether | 32.46        |
| 500                  | Pet. ether | 40.25        |
| IC₅₀ (µg/ml)         | Pet. ether | 631.5        |

| Concentration(µg/ml) | Chloroform | 10.8          |
|----------------------|------------|---------------|
| 100                  | Chloroform | 30.05         |
| 200                  | Chloroform | 39.48         |
| 300                  | Chloroform | 41.6          |
| 400                  | Chloroform | 43.41         |
| 500                  | Chloroform | 525.9         |
| IC₅₀ (µg/ml)         | Chloroform | 131.8         |

| Concentration(µg/ml) | Methanol | 52.6          |
|----------------------|----------|---------------|
| 100                  | Methanol | 71.74         |
| 200                  | Methanol | 85.39         |
| 300                  | Methanol | 93.80         |
| 400                  | Methanol | 94.60         |
| 500                  | Methanol | 98.95         |
| IC₅₀ (µg/ml)         | Methanol | 69.66         |

| Concentration(µg/ml) | Aqueous | 19.2          |
|----------------------|---------|---------------|
| 100                  | Aqueous | 31.11         |
| 200                  | Aqueous | 38.50         |
| 300                  | Aqueous | 43.55         |
| 400                  | Aqueous | 49.8          |
| 500                  | Aqueous | 50.83         |
| IC₅₀ (µg/ml)         | Aqueous | 508.3         |

Values are mean±SD (n = 3).

**Reducing power assay**

The results of reducing power assay are provided in table 6. Absorbance is increased with increasing the concentration of the extracts. All the extracts showed potent reducing power ability. Among all the extracts tested, methanolic extract showed highest reducing ability (131.8±11.67).

Table 6: Reducing power assay of the leaf extracts of *Grewia heterotricha* mast

| Leaf extracts | mg ascorbic acid/g dry extract |
|---------------|-------------------------------|
| Aqueous       | 73.0±4.47                     |
| Methanol      | 131.8±11.67                   |
| Chloroform    | 69.66±11.56                   |
| Pet. ether    | 51.7±5.19                     |

Values are mean±SD (n = 3).
Hydrogen peroxide scavenging activity
Scavenging of hydrogen peroxide by various extracts of the leaf was found to be concentration dependent. Maximum inhibition was shown by methanolic extract (IC_{50}=11.01 µg/ml) followed by chloroform (IC_{50}=403.8 µg/ml) and aqueous (IC_{50}=685.6 µg/ml) extracts (table 7 and fig. 6). Least scavenging activity was observed in a pet. ether extract (IC_{50}=403.8 µg/ml).

### Table 7: Hydrogen peroxide scavenging activity of the leaf extracts Grewia heterotricha mast

| Concentration(µg/ml) | Pet. ether | Chloroform | Methanol | Aqueous | Ascorbic acid |
|----------------------|------------|------------|----------|---------|--------------|
| 100                  | 5.26       | 20.61      | 48.38    | 7.14    | 66.21        |
| 200                  | 6.57       | 29.89      | 64.51    | 10.71   | 69.59        |
| 300                  | 11.84      | 36.08      | 72.04    | 17.85   | 79.72        |
| 400                  | 14.47      | 49.48      | 77.41    | 23.21   | 87.83        |
| 500                  | 17.10      | 62.08      | 83.87    | 39.28   | 95.94        |
| IC_{50} (µg/ml)      | 1531       | 403.8      | 110.1    | 685.6   | 57.96        |

Values are mean±SD (n = 3).

### DISCUSSION
Secondary metabolites derived from plants are responsible for diverse pharmacological properties [20]. The antioxidant property of many plants is due to the presence of secondary metabolites such as phenolic compounds, flavonoids, tannins. Antioxidants play an important role in scavenging free radicals and provide protection against degenerative diseases [21, 24]. It has been reported that the phenolic compounds present in plants possess antioxidant activity due to the presence of hydroxyl groups and they can act as potent hydrogen donors [22, 25]. Flavonoids are the most diverse and widespread secondary metabolites involved in thrombosis, atherogenesis and carcinogenesis. It has been reported that pharmacological effects of flavonoids are correlating with their antioxidant activity. Flavonoids contain phenol groups and these are potent antioxidants. Tannins are abundant in plants and are used as astringents and also important in cancer treatment [23, 26-28]. These natural bioactive components are useful therapeutic agents in various degenerative diseases.

In the present study, preliminary phytochemical screening of different leaf extracts of grewia heterotricha mast showed the presence of steroids, alkaloids, tannins, diterpenoids, triterpenoids, saponins and phenolic compounds. All the four-leaf extracts of Grewia hetertricha were analyzed for total phenolic content, flavonoids and tannin content as well as for antioxidant activity by DPPH assay, reducing power assay and H2O2 scavenging activity. Results obtained for the above studies indicated maximum antioxidant activity in the methanolic extract of Grewia hetertricha which was significantly comparable with that of standard ascorbic acid. The methanol extract also exhibited the highest total phenolic content which can be positively correlated with its DPPH free radical scavenging activity.

The results suggested that all the four extracts of Grewia heterotricha have reducing property. Higher phenolic content in methanol extract might be responsible for maximum reducing ability. In the present study, the methanol and chloroform extracts exhibited highest H2O2 scavenging activity suggests that polyphenols, as well as flavonoids, may be responsible for antioxidant activity.

### CONCLUSION
In conclusion, the present study revealed the presence of various bioactive components in the leaf extracts of Grewia heterotricha mast. The plant leaves possess good phenolic content, total flavonoids and tannin content, in addition to other phytochemical components. The data obtained in the present study show that the leaf extracts have powerful antioxidant activity. The presence of phenolics, tannins and flavonoids may be responsible for this activity. Hence may be used for wound healing and in oxidative stress related diseases. Further studies are necessary to find the exact bioactive component involved in antioxidant activity.

### CONFLICT OF INTERESTS
Declare none

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