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Antioxidant activity and phytochemical screening of stem bark extracts of *Grewia optiva Drummond ex Burret*

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Abstract
Medicinal plants are traditionally used in Pakistan and in other countries for treatment of various diseases. *Grewia optiva* is a medicinal plant used in northern Pakistan for various diseases. This plant was assessed for various parameters like total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant capacity (TAC) using ABTS radical scavenging assays. Phytochemical tests are also included in the study to identify various classes of phytochemicals. Phytochemical tests resulted in the presence of secondary metabolites like alkaloids, terpenoids, tannins, saponins and flavonoid. TPC analysis showed phenolic content in extract and its fractions with maximum value in aqueous fractions 167.3 mg/g followed by n-butanol 132.5 mg/g while the lowest value was recorded in chloroform fraction 89.9 mg/g respectively with comparing to Gallic acid equivalent. Results of TFC quantitative estimation exhibited presence of flavonoids in aqueous fraction with maximum value of 84.2 mg/g followed by crude extract 81.9 mg/g while the lowest value was recorded chloroform fraction 42.6 mg/g respectively as compare to Quercetin equivalent. TAC revealed promising antioxidant potential of the crude extract and its fractions with high activity in again aqueous fraction 1.55 mg/g followed by crude extract 1.52 mg/g while the lowest value was recorded in chloroform fraction 0.82 mg/g respectively by comparing to the standard trolox. From the result is concluded that plant have various phytochemical compound and further study should be made to isolate the compounds and check for different activity.

Keywords: *Grewia optiva,* Stem, Antioxidant activity (DPPH), Phytochemistry.

1. Introduction
The genus *Grewia* is a member of family Tiliaceae that includes round about 150 species which are mostly sub scattered and climbing [1]. Linnaeus identified the said genus with 2 different varieties namely *G. occidentalis* and *G. orientalis.* These species are mainly reported from Arab, South Africa, Madagascar, Himalayan region, India, Pakistan, china, Bangladesh, Myanmar, Thailand, Malaysia, the pacific islands and northern Australia [2].
Pakistan is known to have the following 10 species: *G. micros, G. optiva, G. tenax, G. helicterifolia, G. glabra, G. damian, G. villosa, G. sapida, G. asiatica, G. elastic* are found in Pakistan [3]. All above ten species are known for their therapeutic potentials [4]. A *Grewia optiva* fruit has as an astringent & an anti-inflammatory activity and can reduce fever. Its bark has medicinal activity against diarrhea [5]. *Grewia optiva* is a medicinal plant having significant potential to be studied for its phytochemical compounds. Due to the medicinal importance of plant *Grewia optiva* the study was planned to explore its Phytochemistry and antioxidant activity.

2. Material and Methods
2.1 Collection of Plant Material
Grewia optiva plant was taken from the northern areas of Pakistan i-e lower Dir during the month of March 2011 which was identified by Dr. Inam Ullah, lecturer in the Department of Pharmacy, University of Peshawar, Peshawar and Voucher specimen No.246 was placed in the Herbarium Department of Botany, University of Peshawar, Pakistan.

2.2 Extraction through Rotary Evaporator
Stem Bark of the selected of the *Grewia optiva* was washed with running tap water and dried in shade for week. The stem bark (7 kg) of *Grewia optiva* which was dried was powdered and extracted three times with 80% methanol (3200 ml) at normal temperature and with the help of

rotary evaporator the crude sample was converted to syrup (0.75 kg) which was then fractionated with separating funnel by different organic solvents like n-hexane, Chloroform, Ethyl acetate, n-butanol and water fractions to bring about polarity.

2.3 Phytochemical Screening
Crude extracts were analyzed chemically to investigate classes of compounds in the stem bark of *Grewia optiva* using standard procedures [6] to identify the constituents. The methods for each test are given below accordingly.

2.4 Alkaloids
Test sample was taken at the rate of 0.2 gm, it was then heated with 2% H_2SO_4 for almost 2 mints. Dragendroff's reagent was added in the form of drops to filtered reaction mixture. The appearance of orange red precipitate is a positive test for alkaloids.

2.5 Tannins
Water was added to small amount of crude extract which was then heated over water bath, followed by filtration. After this ferric chloride was added in the form of drops to all filtrates. The turning of solution to dark green colour showed the attendance of tannins.

2.6 Saponins
Test sample was weighed exactly 0.2 g. 5 ml distilled. Water was added and shaken. This mixture / solution were heated till boiling point. The velvety overlook of little froth clearly resulted in the existence of saponins.

2.7 Flavonoids
Crude extract was taken 0.2 g. Dilute sodium hydroxide was added to each fraction followed by adding hydrochloric acid drops. The change in colour from yellow to colorless indicates the presence of flavonoids.

2.8 Terpenoids
Extract was weighed 0.2 g exactly. Chloroform was added to the test sample in the amount of 2 ml. This mixture was then added with 3ml concentrated sulfuric acid due to which a layer was formed. The appearance of red color in the solution indicates the presence of terpenoids.

2.9 Determination of Total Phenolic Content (TPC)
Total phenolic content (TPC) of the test samples were resolved using Folin- Ciocalteu method [7] employing gallicacid as standard. 100 µl of the crude sample was taken. After which 900 µl of water was added to the crude sample. When mixture was prepared then it was provided with 500 µl of Folin-Ciocalteu reagent. Then 20% Na_2CO_3 solution was prepared from which 1.5 ml was taken and added to the above mixture which increased the volume upto 5 ml. finally this total volume was warmed for two hours. Systronics 2202UV–Vis Spectrophotometer was used for the absorbance at the wavelength of 765 nm. Totals phenols were estimated using gallic acid equivalents (GAE) as standard.

2.10 Determination of Total Flavonoid Content (TFC)
The method given by [8] was studied for determining the total flavonoid content (TFC) employing Quercetin as astandard. Total flavonoid content of the test sample was determined using colorimetric method. To 1 ml of 100 µg/ml test fraction, 4 ml of double distilled water was added followed by addition of 300 µl of $NaNO_2$ and 300 µl of $AlCl_3$, which was incubated for 5 minutes. To this 2 ml sodium hydroxide was poured and the volume reached upto 10 ml. Systronics 2202 UV–Vis Spectrophotometer was used for absorbing the wavelength set at 510 nm and total flavones were then expressed as Quercetin equivalents (QE) in mg/g of dry sample.

2.11 Total Antioxidant Activity
This activity was estimated by using superior ABTS.+ radical cation test by comparing it with standard trolox [9]. First of all ABTS solution was made which was then treated with K-persulfate. On account of getting the ABTS.+ radical cation the solution was kept for the whole night. After that 10 µl from our test sample was taken and treated with 1 ml of ABTS.+solution. 734 nm frequency was used for the absorbance. We did our experiment 6 times for accuracy. The Trolox equivalent antioxidant capacity (TEAC) was measured by comparing it with standard.

2.12 Statistical Analysis
The experiments were performed in triplicates and the data was analyzed by using SPSS 16.1 software. The data were arranged as Mean±SD (Standard Deviation).

3.1 Results and Discussion
Grewia optiva plant has been recognized for the folkloric use throughout the world. This plant was collected from its natural habitat in the month of March 2011. This plant was analyzed for the phytochemical in-vitro studies as well as different activities like total phenolics, total flavonoids and antioxidant potential to check its drug potential.

3.2 Phytochemical Screening of Grewia optiva
Screening of *G. optiva* phytochemically resulted in the existence of secondary metabolites such as alkaloids, terpenoids, flavonoids, saponins and tannins.

Table 1: Phytochemical screening of crude extract of *Grewia Optiva*

S. No.	Chemical components	Status
1	Alkaloids	+
2	Terpenoids	+
3	Flavonoids	+
4	Tannins	+
5	Saponins	+

Key: – = absent, + = present

3.3 Total Phenolic Content
This test was performed on the plant extracts to check its absorbing capacity using Folin-Ciocalteu reagent. The results were evaluated with standard gallic acid equivalent [10]. Different grades were analysed using colorimetry instrument in Table 2 and Fig.1. Phenols were maximum in aqueous fraction (167.3 mgGAE/g), followed by n-butanol (132.5 mgGAE/g), crude extract (129.3 mgGAE/g), and Ethyl acetate (129.2 mg GAE/g). Choroform fraction had the lowest total phenolic content (89.9 mgGAE/g). Different studies are made on Grewia genus and total phenolic content has been found which support our findings.

Table 2: TP content of various extracts from *Grewia optiva*

Stem Extract	Total phenolic (mg GAE per g extract)
Crude extract	129.3 ±3.16[c]
Chloroform	89.9±2.15[d]
Ethyl acetate	129.2 ±2.41[c]
n-Butanol	132.5 ±2.37[b]
Aqueous extract	167.3 ±4.32[a]

*Values with different letters in the same column are significantly different (P<0.05).

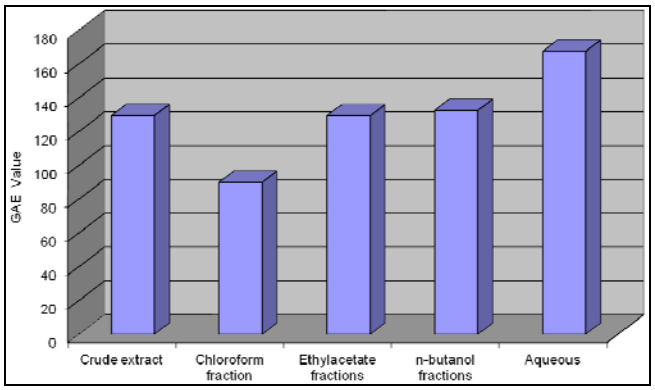

Fig 1: TP content of various extracts from *Grewia optiva*

3.4 Total Flavonoid Content

The total flavonoid contents were usually higher in aqueous fraction 84.2 mg quercetin equivalent/g of extract with reference to standard, followed by crude extract 81.9 mg quercetin equivalent/g, n-butanol 72.2 mg quercetin equivalent/g, ethyl acetate 51.2 mg quercetin equivalent/g. The lowest total flavonoid content 42.6 mg quercetin equivalent/g was observed in chloroform fraction (Table 3 & Fig. 2).

Table 3: TF content of various extracts from *Grewia optiva*

Stem Extract	Total flavonoids (mg QE per g extract)
Crude extract	81.9±3.22[b]
Chloroform	42.6±1.18[e]
Ethyl acetate	51.2±1.63[d]
n-Butanol	72.2±3.37[c]
Aqueous Extract	84.2±2.70[a]

*Values with different letters in the same column are significantly different (P<0.05).

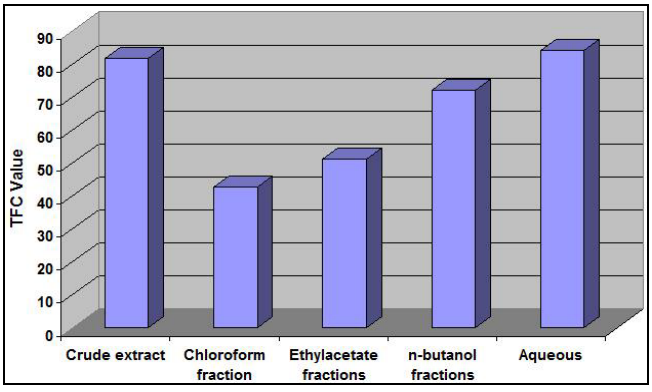

Fig 2: TF content of various extracts from *Grewia optiva*

3.5 Antioxidant Activity

Results were expressed as Trolox equivalent antioxidant capacity (TEAC). Aqueous fraction demonstrated the highest free radical scavenging activity, followed by, crude extract, n-butanol, Ethyl acetate and Chloroform fraction all of which were higher than gallic acid (Table 4 & Fig.3). They were also superior to chlorogenic acid and quercetin, whereas the extracts from aqueous fraction and crude extract were comparable to Trolox. Additionally, their total phenolic contents were significantly correlated with antioxidant activity: r = 0.721 ($P < 0.01$) (Table 4).

Table 4: Antioxidant Activity of *Grewia optiva* in various extracts.

Stem Extract	Antioxidant activity (TEAC)
Crude extract	1.52±0.13[b]
Chloroform	0.82±0.13[e]
Ethyl acetate	1.22±0.15[d]
n-Butanol	1.26±0.25[c]
Aqueous extract	1.55±0.12[a]

*Values with different letters in the same column are significantly different (P<0.05).

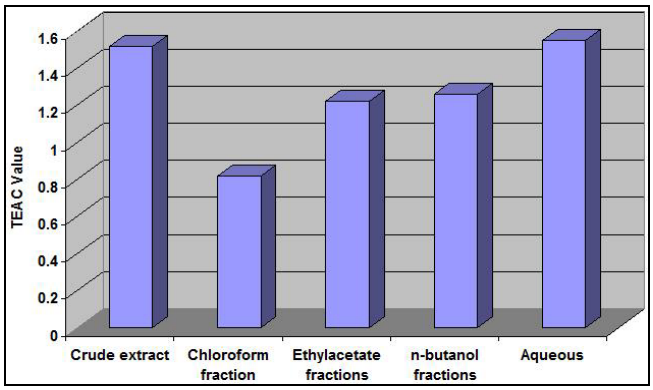

Fig 3: Antioxidant Activity of *Grewia optiva* in various extracts.

4. Conclusion

From the result it has been concluded that this plant possess phenolic content as well as flavonoid content in addition to antioxidant capacity. Various classes of Phytochemicals were also found. Further phytochemical studies are required to isolate and identify compounds from natural products. Discovery of new antioxidants for better health is a challenging field in nutraceuticals. Keeping the significant antioxidant potential of *G. optiva* in view, extensive bioactivity-guided studies are necessary to identify new and effective antioxidant compounds.

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