EVALUATION OF PHYTOCHEMICALS AND IN VITRO ANTIOXIDANT ACTIVITY OF DIFFERENT SOLVENT EXTRACTS FROM GREWIA *TILIAEFOLIA* (Vahl) BARK

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Abstract

Medicinal plant remedy for human health is based on the empirical findings of hundreds and thousands of years. They are the source of many important phytochemicals. The present study was carried out to investigate various extracts of *Grewia tiliaefolia* Vahl for its qualitative phytochemical screening and free radical scavenging activity by DPPH assay. Phytochemical analysis showed the presence of alkaloids, saponins, Phenolic compounds, tannins, phenols and flavanoids. Maximum percent inhibition of DPPH free radical was exhibited by ethyl acetate extract with less IC50 value 53.13µg/ml. Ascorbic acid was used as standard antioxidant.

Key Words: *Grewia tiliaefolia*, phytochemicals analysis, antioxidant activity, free radicals.

Introduction

Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies (Gurib-Fakim, 2006). This is only possible due to their different assortment of organic compounds that can be producing a definite physiological action on human body. Most important of such compounds are secondary metabolites produced by the plants (Briskin, 2000). Now a day there is increase interest in the field of natural product chemistry is only due to the vast effect of phytochemical or secondary metabolites produce by the plants. This may also due to several factors, including therapeutic needs, the remarkable diversity of both chemical structure and biological activities of naturally occurring secondary metabolites (Dar *et al*., 2017).

Antioxidants are substance that delays or inhibits oxidative damage. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus restrain the oxidative mechanisms that lead to degenerative diseases (Mahdi-Pour *et al*., 2012). Therefore, diet enrich with antioxidants has become need these days due to this the plants with antioxidant properties are becoming more and more popular all over the world (Boligon *et al*., 2014).

In present study various extracts of *Grewia tilifolia* bark were evaluated for secondary metabolites and antioxidant property. *G. tilifolia* belonging to the family Tiliaceae has high medicinal values. The bark is acrid with sharp sweetish test, digestive, aphrodisiac, used in hypertension, ulcer and diarrhea (Goyal, 2012).

Materials and Methods

Collection of plant material

The fresh sample of bark of *G. tiliaefolia* were collected from various parts of Buldhana, Maharashtra and identified with the help of floras (Cooke, 1903, Dhore, 1986, Kirtikar and Basu, 1995). Collected material was air dried under shade. After drying, the plant material was ground well using mechanical blender into fine powder and transferred into airtight containers with proper labeling.

Preparation of the plant extracts

Crude plant extracts were prepared by Soxhlet extraction method. About 50 gm of dried powdered material of bark was uniformly packed into a thimble and extracted with 500 ml of different solvents separately. Solvents used were ethanol, ethyl acetate, acetone, chloroform and distilled water. The process of extraction continues for 24 hours or till the solvent in siphon tube of an extractor become colorless. All

the extracts were evaporated to dry and dried extracts were kept in refrigerator at 4ºC for their future use in phytochemical analysis.

Phytochemical analysis

The solvent free extracts were subjected to preliminary phytochemical screening for identification of various plants constituents using standard methods (Harbone,1973, Kokate,1994).

Antioxidant activity with DPPH assay

The ability of the bark extracts to scavenge DPPH free radical was assessed by standard method (Blois,1958). Five concentrations (25, 50, 75, 100ug/ml) of each sample were prepared. 0.1 mM solution of DPPH in methanol was prepared and 180 µl of this solution was added to 20µl of different plant extracts in 96 well plates and incubated. After 30 min incubation in dark at room temperature the absorbance was recorded at 490 nm. Ascorbic acid was used as a positive control. Percentage inhibition was calculated using following equation, while IC50 values were estimated from the % inhibition versus concentration plot. The effective concentration of sample required to scavenge DPPH radical by 50% (IC50 value) was obtained by linear regression analysis of dose response curve plotting between % inhibition and concentrations. The absorbance was recorded and % inhibition was calculated using formula given below, (Badami and Gupta, 2005).

I% = (Ac – As) / Ac x 100 …… (1)

Where, Ac – absorbance of the control

As – absorbance of the sample

Results and discussion

Table-1: Phytochemical analysis of various extracts of *Grewia tiliaefolia* (Bark)

Phytochemical Components	Bark				
	Ethanol	**Ethyl acetate**	**Acetone**	**Chloroform**	**Water**
Alkaloids Mayer's Hager's Wagner's	+ + +	- - -	- + +	- - -	- + +
Saponins Foam Test	+	+	+	+	+
Phenolic compounds Lead acetate	+	+	+	+	+
Tannin Ferric chloride	+	+	+	+	+
Protein	-	-	-	-	-
Amino acids	-	-	-	-	-
Reducing sugar	-	+	-	+	-
Glycosides	+	-	+	-	+
Flavonoids	+	+	-	+	-
Phenols	+	+	-	-	+
Coumarins	-	-	-	-	-
Resins	-	-	-	-	-
Steroids/ Terpenoids	+	+	+	+	+

Key: (+) = indicate present, (-) = indicate absent

The phytochemical characteristic of *G. tiliaefolia* bark were summarized Table-1. The qualitative analysis showed the presence of medicinally active compounds in ethanol, ethyl acetate, acetone, chloroform and water extracts *G. tiliaefolia*. Screening of the bark showed the presence of various secondary metabolites like alkaloids, saponins, Phenolic compounds, tannins, glycosides, flavanoids, phenols and coumarins. Goyal, (2012) reported triterpenoids, fatty component, flavonoids, steroids, saponins and tannins from *Grewia.* The presence of Phenolic compounds (flavanoids, tannins and total phenols) are responsible for free radical scavenging effect observed by Kumar and Venkatachalam (2016) and Rani *et al*., (2011).

Table-2: Evaluation of DPPH free radical scavenging activity of *Grewia tiliaefolia* (Bark)

Extracts	Concentration (µg/ml)	Absorbance (Mean± SD) n=2	% Inhibition (Mean± SD) n=2	IC50 (µg/ml)
Ethanol	25	0.238± 0.001	40.64±0.35	74.85
	50	0.233± 0.006	41.77±1.58	
	75	0.189± 0.009	52.86±2.46	
	100	0.182 ±0.003	54.48±0.88	
Ethyl acetate	25	0.218 ±0.009	45.51±2.29	53.13
	50	0.21±0.002	47.63±0.70	
	75	0.177±0.002	55.86±0.70	
	100	0.168±0.003	57.98±0.80	
Acetone	25	0.293± 0.012	26.80±2.99	106
	50	0.288± 0.009	28.05±2.29	
	75	0.255 ±0.014	36.28±3.70	
	100	0.194± 0.011	51.62±2.82	
Chloroform	25	0.270 0.007	32.54±1.93	80.87
	50	0.250 ±0.020	37.53±5.11	
	75	0.192± 0.009	52.11±2.46	
	100	0.183± 0.005	54.36±1.41	
Aqueous	25	0.307±0.007	23.44±1.76	102.93
	50	0.294±0.007	26.55±1.93	
	75	0.245±0.027	38.77±6.87	
	100	0.198±0.002	50.62±0.70	
Ascorbic acid	25	0.189 ±0.015	52.86±3.87	9.39
	50	0.144 ±0.010	63.96±2.64	
	75	0.103± 0.007	74.31±1.76	
	100	0.098± 0.011	75.56±2.82	

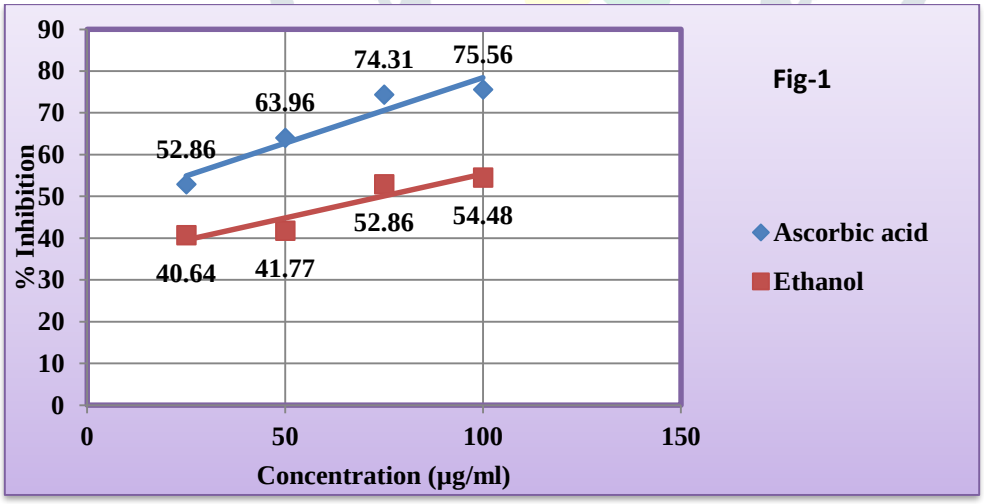


Fig-1

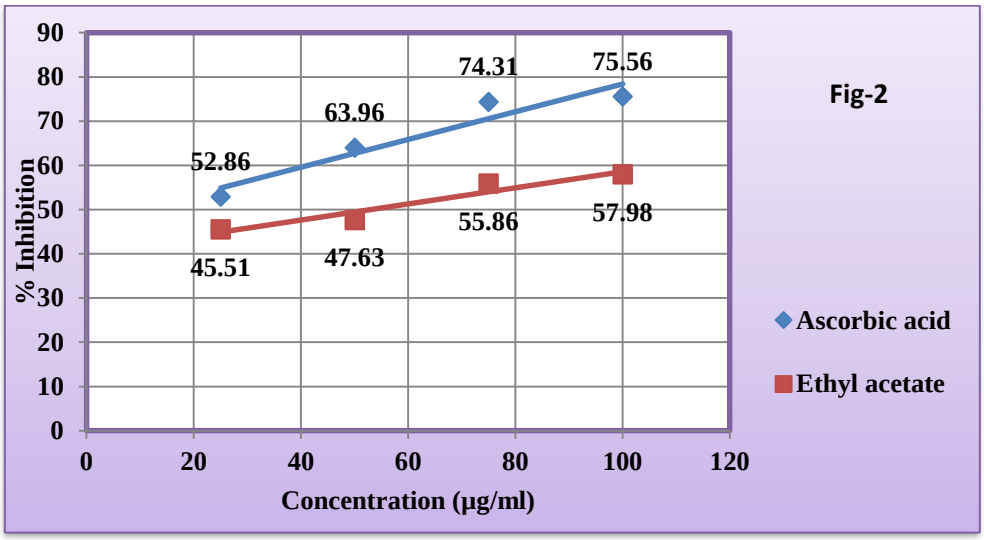
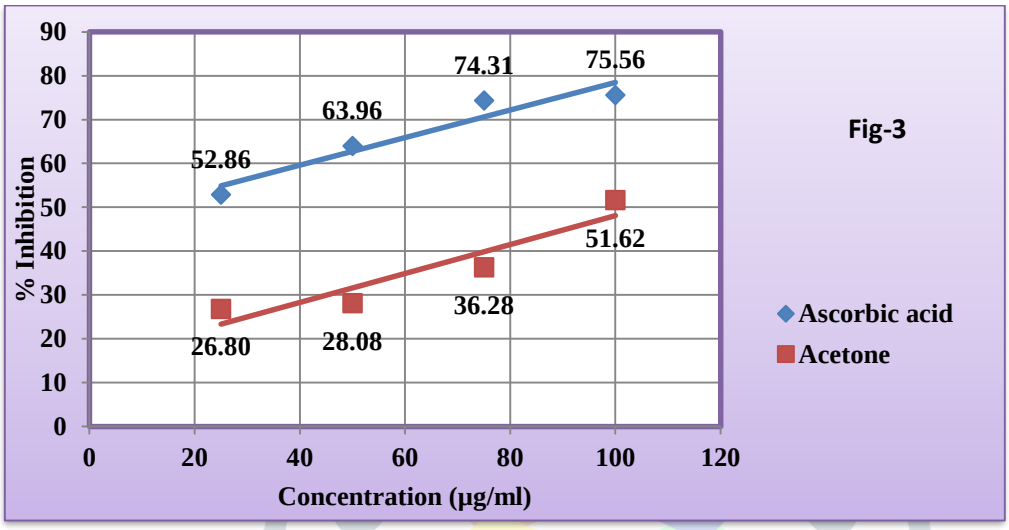


Fig-2



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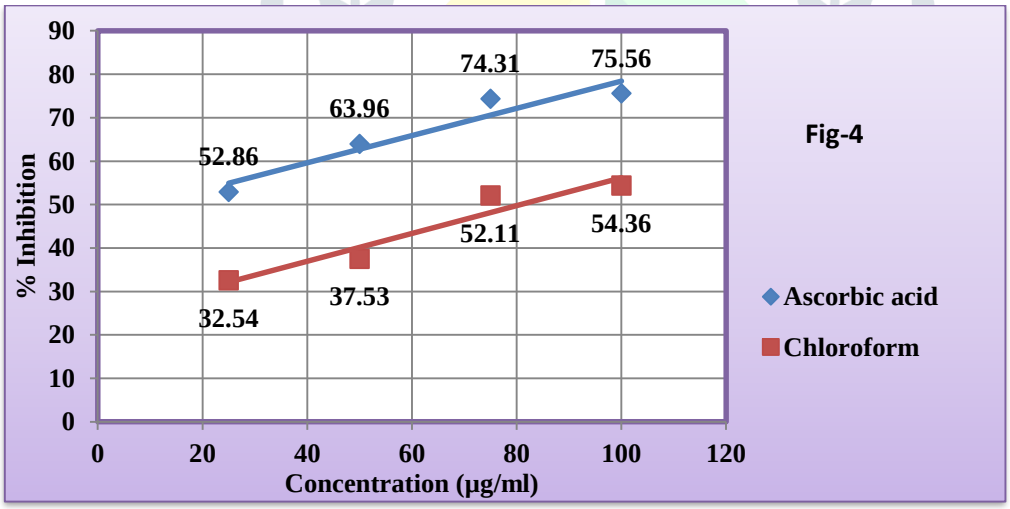

Fig-4

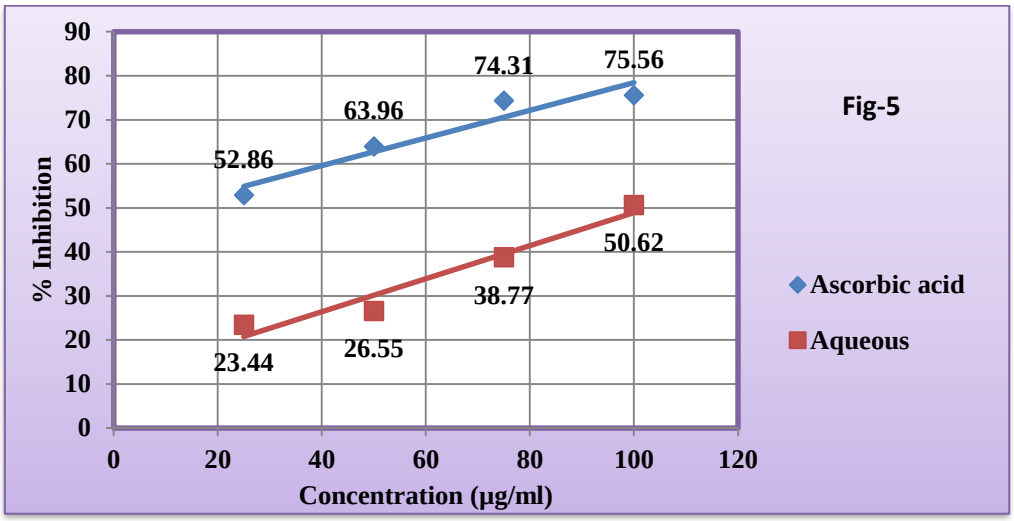

Figure-1-5: Percentage inhibition of DPPH free radical by various extracts of *Grewia tiliaefolia* (Bark) and ascorbic acid.

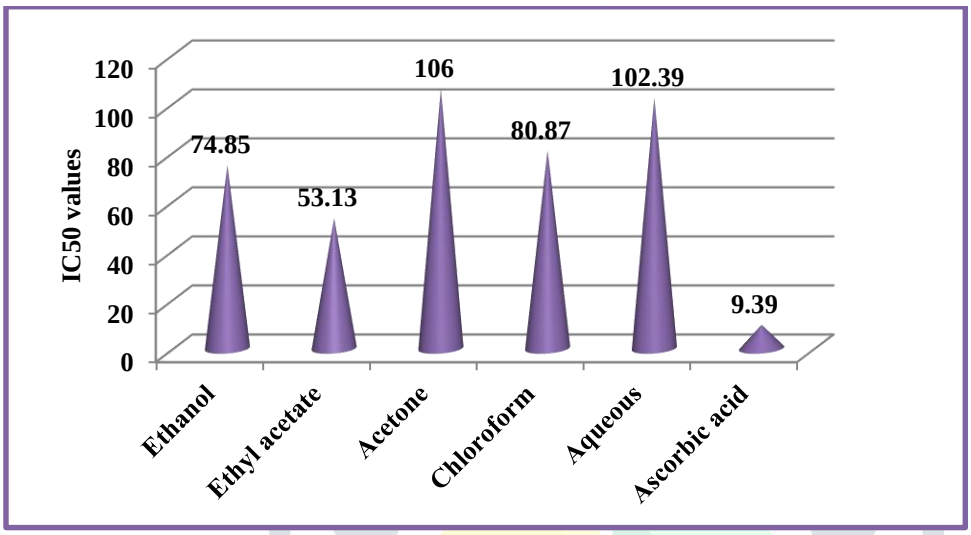

Figure-6: IC50 values of various extracts of *Grewia tiliaefolia* (Bark) compared with ascorbic acid.

The antioxidant activity of prepared extracts was determined by DPPH radical scavenging assay. Table-2 and Figure 1–5 shows the results of the free radical (DPPH) scavenging activity in % inhibition. The ethyl acetate extract showed highest DPPH radical scavenging activity with 57.98% inhibition of DPPH radical at (100µg/ml) concentration. IC50 values for DPPH radical scavenging assay showed significant variation from 53.13µg/ml to 106µg/ml (Fig.-6). The ethyl acetate extract showed lowest IC50 i.e. 53.13µg/ml, followed by ethanol 74.85µg/ml, chloroform 80.87µg/ml, aqueous 102.93µg/ml and acetone 106µg/ml. Selvam *et al*. (2010) also proved the potential free radical scavenging activity of *G. tiliaefolia* bark against DPPH. Thamizh *et al*. (2010), Karuppusamy *et al*. (2011), Sharma *et al*. (2016) and Badami *et al*. (2005) had earlier claimed the antioxidant activity of *Grewia* species. This is in agreement as observed in this study that *G. tiliaefolia* bark displayed potent antioxidant property.

Conclusion

In the present study phytochemical screening and antioxidant activity *G. tiliaefolia* bark extracts were evaluated. The ethyl acetate extract showed maximum antioxidant activity. The antioxidant property as reported in this study could be a result of phytochemicals such as phenols, flavanoids and tannins present in this plant.

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