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Investigation of Preliminary Phytochemical Screening, Antioxidant Property, Total Phenolic and Total Flavonoid Contents of Ethanolic Extract of Stem Bark of *Crataeva nurvala.*

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Abstract : The present study was undertaken to screen the phytochemicals, to evaluate the total flavonoid and total phenolic contents as well as antioxidant activity of ethanolic extract of bark of *Crataeva nurvala*. Various studies have been done to identify antioxidants from plant sources and efforts have been taken to incorporate them in conventional therapy. In our present study, ethanolic extract of Bark of *Crataeva nurvala* showed antioxidative potential, total phenolic and flavonoid contents along with the presence of various phytoconstituents such as alkaloids, terpenoids, glycosides, steroids, triterpenoids, flavonoids, carbohydrates and tannins. Our current results emerged that *Crataeva nurvala* act as an antioxidant agent due to its free radical scavenging activity. So, the plant may be further pursued to find out for its pharmacological active natural products.

Keywords: *Crataeva nurvala*, Antioxidants, Reactive oxygen species, total flavonoid content, total phenol content.

Introduction

There is growing interest on up keeping and development of medicinal plants in all parts of the world. Medicinal plants have long been employed in traditional medicine and global ethnomedicine. The World Health Organization estimated that 80% of the populations of developing countries rely on traditional medicines, mostly plant derived drugs, for their prime health care needs. Also, modern pharmacopoeias still have 25% plant derived drugs and synthetic ones are also prototype compounds of plant origin. Use of medicinal plants for treatment of various ailments before prehistoric period is evident from Ancient Unani manuscripts, Egyptian papyrus and Chinese writings. Medicinal herbs are the most chief source of folk medicines and majority of the world's population depends on them (Samejo *et al.,* 2013). Traditional medicines are widely practiced on many accounts mainly due to Population increase, derisory supply of drugs, exorbitant cost of treatments, adverse effects of synthetic ones and development of confrontation to synthetic. The golden fact is that, use of herbal treatments is independent of any age groups and the sexes. Among prehistoric civilizations, India is well known to have a rich diversity of medicinal plants (Gangola *et al*., 2017). The forest in India are considered as treasure trove because of having rich assortment of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. Medicinal plants encloses various ingredients such as essential oils, alkaloids, flavonoids, tannins, saponins, generally produced by plants for self defense have been recommended for their therapeutic values either pharmacopoeial, non- pharmacopoeial or synthetic drugs (Ghribia *et al*., 2014).

Crataeva nurvala (Varuna) is distributed in sub-Himalayan tracts and is indigenous to Tamil Nadu, Kerala and Karnataka. It is found in abundance, in Kerala, Madhya Pradesh, Bengal and Assam. Varuna is cultivated throughout India, especially along the streams and riverbanks.

Crataeva nurvala is small sized tree. The mature bark is typically 6-15 cm long and 3-10 cm wide with a thickness varying from 5-15 mm. The external surface of the bark is grey to greyish-brown in colour and rough in texture, due to the presence of several small and rounded lenticels. The inner surface is smooth and whitish-brown to buff coloured. Leaves are trifoliate. Varuna is one of the most excellent litholytic herbs and has been used all the way through the ages for the treatment of urolithiasis and crystalluria. Varuna is mentioned in vedic literature, its therapeutic use being known to ancient Ayurvedic physicians, especially as a blood purifier, to maintain homeostasis. Bark juice of this plant is given orally to prevent childhood diseases among the inhabitants of the Kanyakumari district, India (Kiruba *et al.,* 2011).

The plant has various synonyms in Ayurvedic scriptures outlines its peculiarities *viz.* triparna-trifoliate, bilvapatra-leaves resemble to those of bilva (Aegle marmelos). Vrttaphala – fruits, ovoid berries, asmari-ghna- litholytic, tikta- bitter etc. Maharsi Susruta has mentioned varuna as a litholytic agent in treating kapha and vata varieties of asmari (Wagner and Farnsworth, 1994). An amount of pharmacological activities have been reported from various parts of the *Crataeva nurvala* such as antibacterial (Malini *et al*., 1995; Parvin *et al*., 2012), anti-diabetic (Sikarwar and Patil, 2010), antimalarial activity (Khalid *et al*., 1986), anti-fertility (Bhaskar *et al.,* 2009), analgesic (Khatun *et al.,* 2012) and wound-healing (Dinesh *et al*., 2010).

However, literature survey revealed a scanty study about *in vitro* antioxidant activity of ethanolic extract of Bark of *Crataeva nurvala*. Therefore, this present study was

designed to evaluate antioxidant property, total Phenolic and total Flavonoid contents of ethanolic extract of Bark of *Crataeva nurvala* for the first time.

Materials and Methods

Collection of plant material

The bark of *Crataeva nurvala* was collected from local area of Bhopal India, in the months of February-March, washed with double distilled water and shade dried for 3 weeks at room temperature and was identified and authenticated by Dr. Zia Ul Hassan (H.O.D. Botany, Safia Science College, Bhopal Madhya Pradesh India). The voucher specimen bearing numbers 508/Bot/Safia/14 was submitted in the said department for future reference.

Chemicals

All chemicals, solvents used were of analytical grade obtained from Merck, Mumbai and HiMedia, Mumbai.

Preparation of extract

The fully dried Bark of *Crataeva nurvala* was powered with the help of grinder. The powder was then extracted in 90 % ethanol by using the Soxhlet extractor apparatus for seven days at 40-50°C temperature. The percentage yield of the extract was determined by using the formula given below. The extract was then dried under vacuum evaporator and the semi solid material thus obtained was stored in glass vials which were kept at -4^0 C for further use. Yield was calculated by formula; % yield = weight of extract / Weight of plant material used X 100. Prepared extract was observed for colour, odour, and texture, and was stored in labeled bottles till any further proceedings.

Phytochemical investigations of crude extract of *Crataeva nurvala*

Prilimnary Phytochemical screening of the extracts was carried out according to the standard procedures (Trease and Evans, 1989 and Kokate *et al.,* 2006). The ethanolic extract *Crataeva nurvala* (EECN) was subjected to preliminary phytochemical screening to identify the various phytoconstituents present in them like alkaloids, terpenoids, glycosides, steroids, triterpenoids, flavonoids, carbohydrates, saponins, tannins, mino acids and proteins.

***In vitro* Antioxidant Assay:**

DPPH radical scavenging activity (Gulcin *et al*., 2006; Jain and Jain 2011**)**

0.1 mM DPPH solution (4 mg/100 ml) was prepared in methanol. Different concentrations of test sample with methanol were prepared. To the 2 ml of test sample was added 1ml of DPPH solution. The mixture was incubated at room temperature for 10 minutes. The absorbance was taken at 515 nm against blank (methanol). Percentage Inhibition was calculated by using following formula :

% Inhibition = [(AC 515 nm- AS 515 nm/ AC 515 nm) x 100].

A curve for % Inhibition and concentration was plotted and IC50 was estimated by using line of regression.

Reducing Power Assay (Jain and Jain, 2011)

Compounds with reducing power indicate that they are electron donors and associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity (Oktay *et al*., 2003). The higher the absorbance of the reaction mixture, the higher would be the reducing power. Different concentrations of test sample were prepared. Added 0.5 ml of different concentrations of sample to 0.5 ml of phosphate buffer (0.2 M, pH 6.6) and 0.5 ml of potassium ferricyanide (0.5 ml, 1%W/V). Reaction mixture was incubated at 50° C for 20 min. After cooling, 1.5 ml of trichloroacetic acid solution (10% W/V) was added to terminate the reaction. 0.5 ml ferric chloride (0.1% W/V) was added and absorbance was measured at 700 nm. The curve between absorbance and concentration was plotted. Increased absorbance of the reaction mixture indicated increase in reducing power.

Estimation of Total Phenolic Content (Ainsworth and Gillespie, 2007)

Different concentrations of Gallic acid (10 to 100 µg/ml) in methanol were prepared. The test sample was prepared in methanol (100 µg/ml). 0.5 ml of different concentrations of Gallic acid/ test sample with 2 ml Folin-Ciocalteu reagent (1:10 in deionized water) was added. Also, 4 ml of sodium carbonate solution was added to the resulting solution. The testing mixture was incubated at room temperature for 30 minutes with intermittent shaking. The absorbance was taken at 765 nm (due to developed blue colour) using methanol as blank. A standard curve of different concentrations of gallic acid was prepared and line of regression was found. The absorbance of test sample was put in line of regression of standard curve of gallic acid. Total phenolic content was calculated and expressed as mg/gm or µg/mg galic acid equivalent.

Estimation of Total Flavonoid Content (Alhakmani *et al*., 2013)

Different concentrations of rutin (10 to 100 µg/ml) in methanol were prepared. The test sample was prepared in methanol (100 µg/ml). 0.5 ml aliquots of appropriately diluted sample solution with 2 ml of distilled water and subsequently with 0.15 ml of 5% $NaNO_2$ solution were mixed. After 6 minutes, 0.15 mL of a 10% $AlCl_3$ solution was added and allowed to stand for 6 minutes, then 2 ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml and then mixed the mixture thoroughly and allowed to stand for another 15 minutes. Absorbance of the mixture at 510 nm was taken using water as the blank. The standard curve for different concentration of Rutin was prepared and line of regression was drawn. Total flavonoid content was calculated and expressed as mg/gm or, µg/mg rutin equivalent.

Results

Loss on drying and yield

The percentage loss of weight on drying was evaluated and it was found that there was 62.05% loss in weight of bark of *Crataeva nurvala estimated to* 54.50 % and 5.6% yield.

Organoleptic evaluation

The extract thus obtained was subjected to organoleptic evaluations and it was found that the ethanolic extract of bark of Crataeva *nurvala* was brown in colour, bitter in

taste and having non sticky nature.

Solubility test

The solubility of the extract was checked in various solvents and it was found that ethanolic extract of bark of *Crataeva nurvala* was soluble in water, methanol, petroleum ether, chloroform, ethanol, acetone, ethyl acetate and DMSO.

Observations of phytochemical investigation

The preliminary phytochemical screening of ethanolic extracts of bark of *Crataeva nurvala* was done by using the standard procedures which revealed the presence of alkaloids, terpenoids, flavonoids, carbohydrates, glycosides, tannins, phenolic compounds, saponins, amino acids and proteins (Table:1).

Table 1: Showing the presence of different phytochemicals in bark of *Crataeva nurvala*.

Phytochemicals	Tests	*Crataeva nurvala*
Alkaloids	Mayer's Test	+
	Wagner's Test	-
	Hager's Test	+
	Dragendroff's Test	+
Terpenoids	Salkowski Test	+
	Libermann Burchards Test	+
Flavonoids	Lead Acetate Test	+
	Alkaline Reagent Test	-
	Shinoda Test	+
Carbohydrates	Molish's Test	-
	Fehling's Test:	+
	Benedict's Test:	+
	Barfoed's Test	-
Glycosides	Killer Killians Test	+
	Borntrager's Test	-
	Legal's Test	+
Tannins and Phenolic compounds	$FeCl_3$ Test	+
	Dilute Iodine Solution Test	-
	Lead Acetate Test	-
	Gelatin Test	+
Saponins	Froth Test	+
Amino acids Proteins	Biuret's Test	+
	Millon's Test	+
	Ninhydrin Test	-

+ = Presence, - = Absence

Antioxidant activity of ethanolic extract of *Crataeva nurvala*

The *in vitro* antioxidant effect of the extracts was evaluated by using DPPH assay. The DPPH radicals reacts with suitable reducing agents losing colour stoichometrically with the number of electrons consumed, which is measured spectrophotometrically at 517 nm. The scavenging activity of bark extracts of *Crataeva nurvala* was IC_{50} 0.85 mg/ml. The scavenging effect was compared to that of the standard ascorbic acid. The results, thus obtained suggest that ethanolic extracts of the selected medicinal plant bark has the proton donating ability and can serve as free radical inhibitors or scavenger or exhibit significant DPPH radical inhibition (Table: 2 and Fig: 1).

Table 2: Showing % inhibition of DPPH by ethanol extract of bark of *Crataeva nurvala.*

S. No.	Conc. (mg/ml)	% Inhibition	IC_{50} (mg/ml)
1	0.4	28.17	0.85 mg/ml
2	0.6	33.13	
3	0.8	52.7	
4	1	62.33	
5	1.2	67.15	
6	1.4	71.67	
7	1.6	84.08	

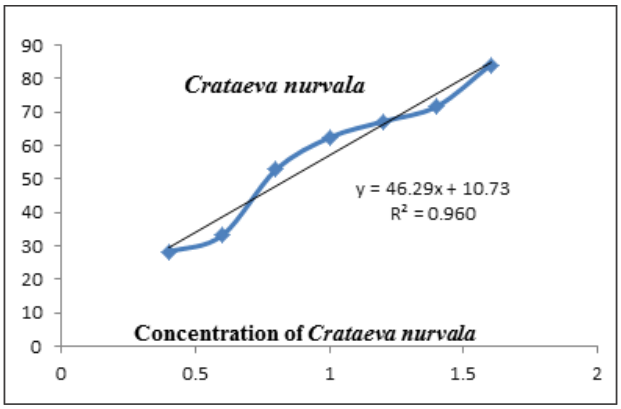

Fig: 1 - Represents percentage inhibition of DPPH by ethanolic extract of bark of *Crataeva nurvala.*

Reducing Power Assay

The antioxidant activity of bark of *Crataeva nurvala* was also determined by reducing power assay. In case of reducing power assay the higher the absorbance of the reaction mixture, the higher would be the reducing power. Table: 3 represents the absorbance of ethanol extracts of bark of *Crataeva nurvala* at different concentrations which shows the clear increase in the absorbance with increase in concentration.

Table: 3 – Showing the Reducing Power of ethanol extract of bark of *Crataeva nurvala.*

S. No	Concentration µg/ml	Absorbance
1	100	0.299
2	200	0.425
3	300	0.503
4	400	0.599
5	500	0.701

Total phenolic content

The total phenol content of the extracts was calculated and it was found that the ethanolic bark extract of *Crataeva nurvala* had the value of TPC as 8.996±1.563 µg/100 µg Gallic acid equivalent (Table: 4).

Total Flavonoid content

The total flavonoid content was estimated in bark of *Crataeva nurvala* which contained 14±3.0 µg/100 µg rutin equivalent (Table: 5).

Table 4: Showing Total Phenol Content (in µg/100 µg Gallic acid equivalent) in ethanol extract of bark of *Crataeva nurvala* bark.

S. No.	Concentration	Absorbance	Total Phenolic content
1	100 µg/ml	0.170	7.5
2	100 µg/ml	0.195	10.62
3	100 µg/ml	0.181	8.87
Mean	-	0.182	**8.996**
S. D.	-	0.01253	**1.563**

Table: 5 - Showing Total Flavonoid content (in µg/100 µg rutin equivalent) in ethanolic extract of bark of *Crataeva nurvala* bark.

S. No.	Concentration	Absorbance	Total Flavonoid content
1	100 µg/ml	0.023	14
2	100 µg/ml	0.026	17
3	100 µg/ml	0.025	11
MEAN±SD			**14 ± 3.0**

Discussion

The current investigation was intended to evaluate phytochemicals, *in vitro* antioxidant activity by measuring total phenol and flavonoid contents of ethanolic extract of Bark of *Crataeva nurvala,* which has not been much reported. Results of our investigation indicated that the bark of *Crataeva nurvala* richly contains alkaloids, terpenoids, flavonoids, carbohydrates, glycosides, tannins, phenolic compounds, saponins, amino acids and proteins. These phytochemicals are the non-nutritional plant compounds that are mainly related to self defense to protect them from pest, microbes, and environmental stress factors. Since ages, a variety of phytochemical compounds from plants origin have been used for the synthesis of new drugs (Kumar *et al.,* 2013**).** Our results of a phytochemical study of stem bark extract of the *C. nurvala* are in concurrence with the previous studies (Bhattacharjee *et al.,* 2012).

Presence of steroid and terpenoids as well as alkaloids, phenolics, flavanoids, tannin and saponin was reported in stem bark of *C. nurvala* (Hade *et al.,* 2016). These compounds are known to be pharmacologically active and, therefore, aids to the various biological activities of *C. nurvala.*

Present study showed that the ethanolic bark extract of *Crataeva nurvala* have significant antioxidant activity as compared to that of the standard (ascorbic acid). The presence of total phenol in the tested extract might be responsible for the free radical quenching activity. The hydroxyl groups of the phenolic compound(s) confer scavenging activity (Saeed *et al.*, 2012). The antioxidant activities of natural antioxidants have been proved responsible for various mechanisms, such as impediment of chain initiation, binding with transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reducing capacity and radical scavenging ability (Liu *et al.,* 2013). *C. nurvala* stem bark showed good antioxidant property, and it possesses a higher amount of terpenoids. This implies that the antioxidant property may be due to terpenoids (Hade *et al.,* 2016).

Our results on bark extract of *Crataeva nurvala* indicate presence of flavanoid content in appreciable manner. The antioxidant activity of flavonoids is coupled with several features of chemical structures. Usually flavonols with free hydroxyl group at the C-3 position and double bond between C-2 and C-3 show maximum antiradical activity (burda and Oleszek, 2001).

Earlier studies find that the bark of this plant possesses cadabicine, cadabicine diacetate, phragmalin triacetate, lupeol, lupenone, succinic acid, mannitol, lactic acid, betulinic acid, β-sitosterol and stigmasterol (Haque *et al.,* 2008; Slipi *et al*., 2011; Parvin *et al*., 2011; *Rao et al.*, 2011). One of the constituents of *C. nurvala* bark, lupeol, has antioxidant property (Saleem *et al.,* 2001).

Therfore, it could be suggested that phenols and flavonols present in the ethanolic bark extract might have greater role to the antioxidant activity.

Conclusion

It was concluded that ethanolic extract of bark of *Crataeva nurvala* (EECN) can be used as a potent source of innate antioxidants with consequential health benefits. For this reason, further research is needed to isolate and recognize the novel antioxidants present in *Crataeva nurvala***.**

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