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FREE RADICAL SCAVENGING ACTIVITY OF *ABUTILON INDICUM (LINN)* SWEET STEM EXTRACTS

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ABSTRACT: Currently, there has been an increased interest globally to identify antioxidant compounds that are pharmacologically potent and have low or no side effects for use in preventive medicine. Antioxidant compounds in food play an important role as a health-protecting factor and it neutralizes the free radicals, which are unstable molecules and are linked with the development of a number of degenerative diseases and conditions including hepatic disease, immune dysfunction, cataracts and macular degeneration. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases and conditions.
Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide; hydro-peroxide or lipid peroxyl which are thereby involved in reducing the risk of diseases associated with oxidative stress. Thus in the present study an attempt was made to determination the Total Phenolic Content (TPC), quantified by Folin–Ciocalteu method and free radical scavenging (antioxidant) activity by DPPH (2, 2-diphenyl-1 picryl hydrazyl) in methanolic (AIM) , Hydro-alcoholic (AIHA) and aqueous (AIA) extracts of the *Abutilon indicum* Stem. The TPC and percentage inhibition of DPPH radical were calculated and respectively. Thus it could be concluded that the AIA showed a potent total phenolic content and possessed a significant scavenging activity.
Key words: *Abutilon indicum*, Total Phenolic Content (TPC), Antioxidant activity, Oxidative stress, DPPH scavenging activity.

INTRODUCTION

Abutilon indicum (Linn) family (Malvaceae) commonly known as 'Thuthi /Atibala' is distributed throughout the hotter parts of India[1] and used in our Traditional System of Medicine for healing various diseases. Almost all parts of Atibala are of medicinal importance and are used traditionally for the treatment of various ailments. The roots of the plant are considered as demulcent, diuretic, used in chest infection and in urethritis. The infusion of root is described in fevers as cooling medicines and is useful in stangury, haematuria and in leprosy. The leaves are effective in ulcer, for the treatment of diabetes, diuretic infection and gingivitis. Fomentation of plant materials are used to relief body pain. The decoction of the leaf is used in toothache, tender gums and internally for inflammation of bladder. In some places, juice from the leaves in combination with the liquid extract of *Allium cepa* is used to treat jaundice, and in cases of hepatic disorders[2, 3]. The bark is used as febrifuge, anthelmintics, alexeteric, astringent and diuretics. The seed are used in piles, laxative, aphrodisiac, expectorant, in chronic cystitis, gleets and gonorrhea[4, 5, 6, 7]. The leaves and seeds are crushed with water to form paste which is applied to penis to cure syphilis.[8, 9, 10]. In Siddha system of medicine, it used as a remedy for jaundice, piles, ulcer and leprosy [11]. It also exhibits marked hepatoprotective action, which has been related to their antioxidant properties. As plants produce significant amount of antioxidants to prevent the oxidative stress caused by photons and oxygen

radicals, they represent a potential source of new compounds with antioxidant activity. Antioxidants help organisms deal with oxidative stress, caused by free radical damage. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability[12]. Extensive review on the effect of free radicals and antioxidants in normal physiological functions and human were studied[13]. It is possible to reduce the risks of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants[14].

Alkaloids, flavonoids, steroids, terpenoids and saponins have been isolated and characterized from genus *Abutilon* [15, 16]. Previous phytochemical investigations of *Abutilon indicum* showed the presence of seven flavonoids, two sesquiterpene lactones, [17] gallic acid, β-sitosterol, geraniol and caryophylline[16]. The analysis of phenolic compounds in plants is of considerable commercial importance, since it is known that they contribute to the flavor and antioxidant property [18]. However, no attention has been paid to its comparative Total Phenolic Content (TPC) and antioxidant status of stem extracts. Thus a common spectrophotometric method for total polyphenol content according to Follin-Ciocalteu has been widely used in the area of oncology and viticulture[19] and in-vitro antioxidant property by common DPPH Scavenging method. In the present investigation these *in-vitro* studies were carried out for the first time to study the comparative properties of total polyphenol content (TPC) and its antioxidant properties on stem extracts.

MATERIALS AND METHODS

PLANT MATERIAL

The fresh stems of the plant *A. indicum* were collected from the wild sources of the Dhulia district of Maharashtra, in the month of June-July and was identified and authenticated by Prof. S.R. Kshirsagar, of Shri. Shivaji Vidya Prasarak Sanstha's, Late Karmveer Dr. P.P. Ghogrey Science College, Dhule (M.S.) Maharashtra, 425 405.A voucher Specimen is kept in the P.G. Department of Pharmacognosy, NMiMS, SPTM, Shirpur, Dhulia, for further references. The fresh dried fine powder was used for the preparation of extracts.

PREPARATION OF EXTRACTS

The freshly collected stems were washed; shade dried and was treated with a mechanical pulverizer for size reduction. The fine powder was collected and was used for preparation of extracts. The powder was successively extracted with methanol (< 60°C), methanol-water (1:1) and double distilled water using soxhlet apparatus for 72 h. The extracts were concentrated under reduced pressure using a rotary vacuum evaporator and dried at room temperature. A greenish brown, brown and dark-brown residue was obtained respectively. The percentage yields of extracts were 4.3% w/w (AIM), 1.5%w/w (AIHA) and 2.4% w/w (AIA). The extracts were kept in desiccators for further use.

CHEMICALS AND INSTRUMENTS

REAGENTS: 2, 2-diphenyl-1-picryl hydrazyl (DPPH), Ascorbic acid, Folin Ciocatteu's (FC) phenol reagent, Gallic acid, anhydrous sodium carbonate, all other reagents used were of analytical grade. UV spectra were recorded in Perkin-Elmer model Lambda-25, UV-Visible spectrophotometer.

DETERMINATION OF TOTAL PHENOLIC CONTENT (Folin and Ciocatteu's, 1927)

The content of Total Phenolic in stem of Atibala plant (AIM, AIHA & AIA) extracts were determined spectrophotometrically using Folin–Ciocalteu reagent by the method of Macdonald et. al.[20] with modifications. Calibration curve was prepared by mixing ethanolic solution of gallic acid (1 ml; 0.010–0.400 mg/ml) with 5ml Folin-Ciocalteu reagent (diluted tenfold) and Sodium carbonate solution in Distilled Water (4ml, 0.7 M). The absorption was measured at 765 nm using a UV-Vis Perkin-Elmer spectrophotometer model Lambda-25. One millimeter of plant extracts (10g/l= 10mg/ml) was mixed instead of 1ml gallic acid with the same reagents as described above in three different Test tubes and after 1 hour the absorption was measured to determination the total phenolic contents. The absorbance was measured against a reagent blank, which was composed of the same reagents except test extract. The gallic acid standard calibration curve was established by plotting concentration (mg/ml) versus absorbance (nm) ($y = 0.005x - 0.101$, $R^2 = 0.986$), where y is absorbance and x is concentration. (Graph. 1) Total content of phenolic in the plant extracts were expressed as gallic acid equivalents (mg of GAE/g sample) and were calculated by the formula:

$$T = \frac{C \times V}{M}$$

Where, T=total content of phenolic compounds, milligram per gram plant extract, in GAE; C=the concentration of gallic acid established from the calibration curve, milligram per milliliter; V=the volume of extract, milliliter; M=the weight of ethanolic plant extract, gram.

EVALUATION OF ANTIOXIDANT ACTIVITY

This method was given by Brand-Williams, Cuvelier, and Berset (1995)[21] and later was modified by Sanchez-Moreno, Larrauri, and Saura-Calixto (1998)[22]. It is one of the most extensively used antioxidant assay for plant samples. This assay is based on the measurement of the scavenging ability of antioxidant test substances towards the stable radical. The free radical scavenging activity [23] of the extracts (AIM, AIHA & AIA) were examined *in vitro* using DPPH radical. The radical scavenging activities of the plant extracts against DPPH radical (Sigma Aldrich) were determined by UV Perkin Elmer UV-Vis model Lambda 25 spectrophotometer at 517 nm. Radical scavenging activity was measured by a slightly modified method previously described by various scientists[23, 24.] Hydrogen atom or electron-donating ability of the stem extracts was measured from the bleaching of the purple-colored methanol solution of DPPH. This spectrophotometric assay uses stable DPPH radical as a reagent [25]. One ml of various concentrations of the (AIM 0.05 - 5 mg/ml, AIHA1.0 – 8.0 mg/ml samples in methanol and AIA 1.0- 8.0 mg/ml sample re dissolved in 5% ethanol) extracts were added to 3ml of methanol followed by 0.5 ml of 1mM methanolic solution of DPPH. After incubation period at room temperature, the absorbance was read against a blank (A blank solution was prepared containing the same amount of methanol and DPPH except the test compound). Ascorbic acid (Vitamins C) were used as the antioxidant standard at concentrations of 0.005 - 0.06 mg/ml. The radical scavenging activity (Inhibition of DPPH free radical in percent) was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{[Ab - Aa]}{Ab} \times 100$$

Where, Ab- is the absorption of the blank sample (containing all reagents except the test compound) and Aa- is the absorption of the test compound. Sample concentration providing 50% inhibition (IC50) was calculated from the graph plotting inhibition percentage against extract concentration.

RESULTS

TOTAL PHENOLIC CONTENT

The total phenolic content of the *A. indicum* stem extracts (AIM, AIHA & AIA) measured by Folin Cicalteu reagents in terms of gallic acid equivalent (GAE) were 20.94, 27.77 and 35.45mg/g. respectively showed in Table 1.

ANTIOXIDANT ACTIVITY (DPPH ASSAY)

Radical Scavenging Increased with Antioxidant. The results of scavenging effect of tested plant extracts on DPPH radical are given in Table 1. These results indicated that the extracts (AIA, AIHA & AIM) of *A. indicum* have notably reduced the stable free radical of DPPH (Graph 2, 3, 4 and 5) to the yellow-colored Diphenyl picryl hydrazyl with an IC_{50} values (the concentration that Inhibits 50% of the DPPH radical) 1343.89, 2487.14 and 1154.20µg/ml respectively showed in (Table 1) comparison with Ascorbic acid (Vitamin-C) (IC50 = 20.73 µg/ml).

DISCUSSIONS

The TPC of the *A. indicum* stem were found more in AIM than AIHA and AIA extracts. The free radical scavenging activity of the extracts was evaluated based on the ability to scavenge the synthetic DPPH. This assay provided useful information on the reactivity of the compounds with stable free radicals, because of the odd number of electrons. DPPH shows a strong absorption band at 517 nm in visible spectrum (deep violet colour). As the electron became paired off in the presence of free radical scavenging, the absorption vanishes and the resulting discoloration stoichiometrically coincides with respect to the number of electrons taken up. The bleaching of DPPH absorption is representative of the capacity of the test drugs to scavenge free radicals independently. Hydroxyl radical is the principal contributor for tissue injury of liver. The (AIM, AIHA & AIA) extracts of the *Abutilon indicum* stem showed promising free radical scavenging effect of DPPH in a concentration dependant manner. The results of scavenging effect of different extracts of *A. indicum* on DPPH radical are given in Graph 1, 2 and 3 respectively. It indicated that the AIA extract showed more reduction with the stable free radical DPPH to the yellow-colored Diphenyl-picryl-hydrazyl than AIM and AIHA extracts. The reference standard ascorbic acid (Vitamin-C) also demonstrated a significant radical scavenging potential in the concentration of 1µg / ml. The extracts of *A. indicum* plants were considered to play important roles in the prevention of number of degenerative diseases like Hepatic disorders, immune dysfunction, cataracts and macular degeneration by inhibiting the ROS production which may lower the risk and allows for proper functioning of the organs.

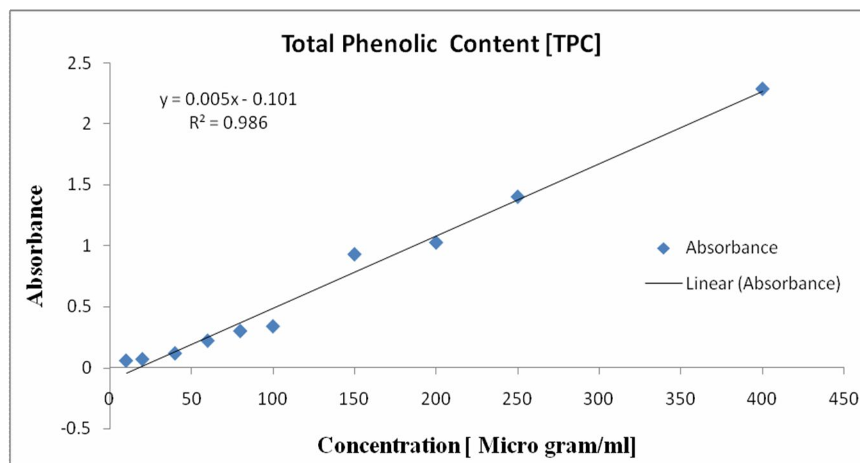
CONCLUSION

Thus from the present study it can be concluded that the different extracts of the *A. indicum* stem exhibited a significant phenolic content with promising free radical scavenging effect of DPPH in a concentration dependant manner. The AIA showed more total phenolic content, hence more scavenging activity than the AIM and AIHA extracts which help to show a synergetic effect on hepatoprotective activity.

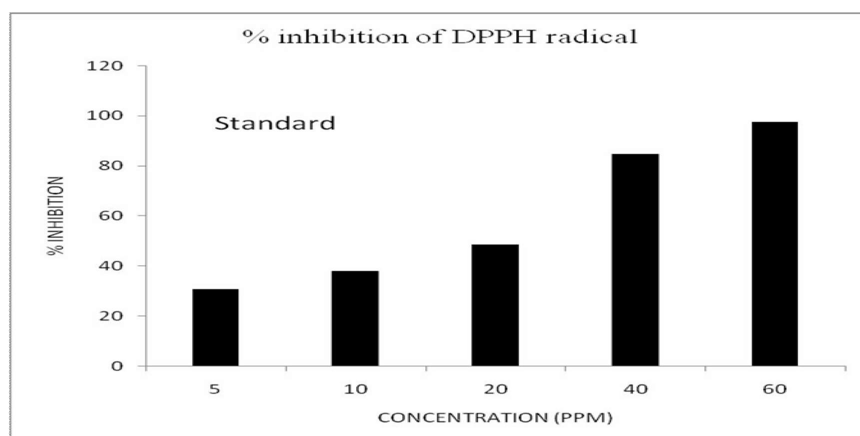
Table1.TPC and Inhibition of DPPH free radical by different (AIM, AIHA and AIA) extracts of *A. indicum*.

Name, Plant Part, Family	Extracts	Yield of extracts (% w/w)	TPC value (GAE mg/g)	IC_{50} Values (µg/ml)
Abutilon indicum Stem (Malvaceae)	Methanolic	2.4	20.94	1343.89
	Hydro-alcoholic	1.5	27.77	2387.14
	Aqueous	4.3	35.45	1154.20
Ascorbic acid (Vitamin-C) (IC50 = 20.73 µg/ml).				

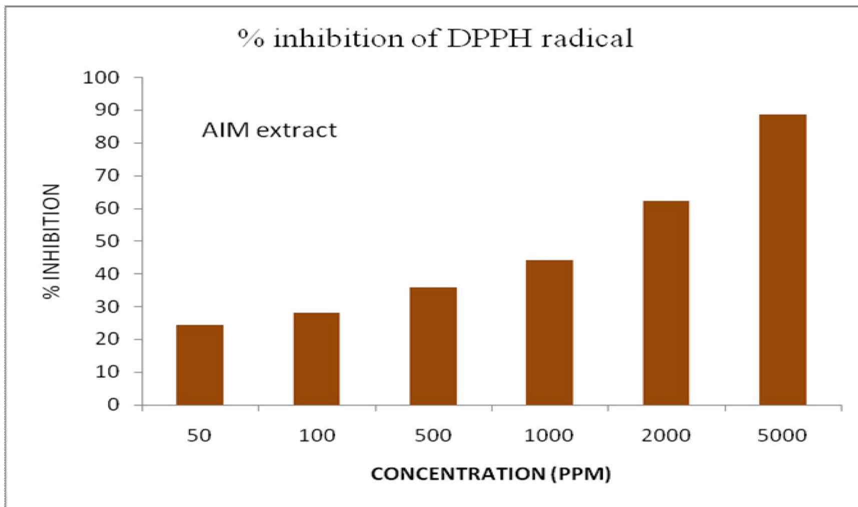
Graph1. Standard Plot of Total Phenolic Content



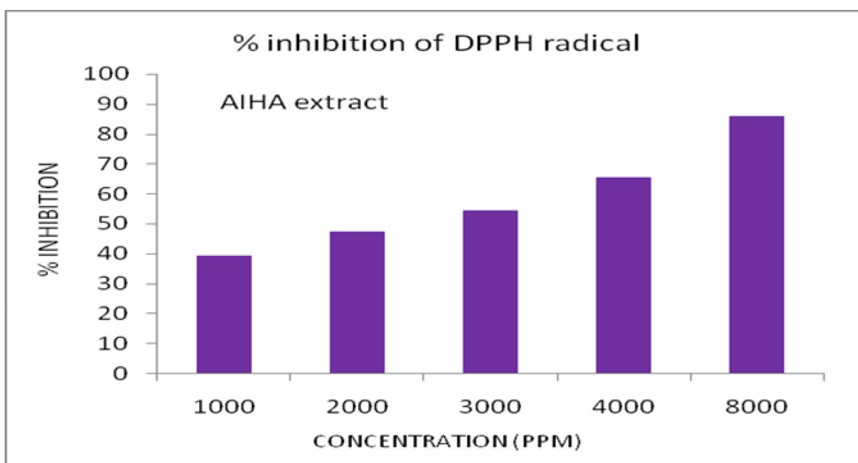
Graph 2. Percentage inhibition of DPPH by standard Ascorbic acid (Vitamins-C).



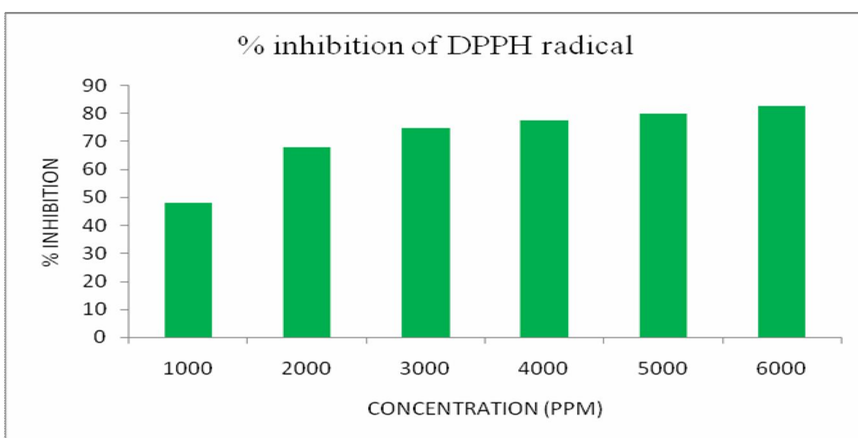
Graph3. Percentage inhibitions of DPPH by Methanolic extract (AIM).



Graph4. Percentage inhibitions of DPPH by Hydro-alcoholic extract (AIHA).



Graph5. Percentage inhibitions of DPPH by Aqueous (AIA) extract.



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