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Research Article

Bioguided extraction and Evaluation of Antioxidant studies of *Abutilon indicum* fruits

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ABSTRACT

The present study was conducted to evaluate the antioxidant activity, to determine the phytoconstituents, phenolic content and flavonoids in different fractions of *Abutilon indicum* fruits. The hydro alcoholic extract of *Abutilon indicum* fruits was fractionated with organic solvents with increasing polarity. *In vitro* antioxidant activity was assayed by diphenyl picryl hydrazyl radical scavenging assay, nitric oxide radical scavenging assay, superoxide radical scavenging assay, hydroxyl radical scavenging assay, ferric-reducing power assay. Phenolic content was determined by folin ciocalteu reagent method. Among all fractions the ethyl acetate fraction was found to possess good antioxidant activity, followed by chloroform fraction. The total phenolic (86 mg gallic acid equivalent/g), flavonoid (30 mg rutin equivalent/g) content in ethyl acetate fraction is high when compared to other fractions. These results reveal that ethyl acetate fraction of *A. indicum* fruits has strong antioxidant potential compared with all other fractions. Further studies are necessary for isolation and characterization of active constituent.

Key words: *Abutilon indicum*, fractions, folin ciocalteu reagent, antioxidant, flavonoid content.

Introduction

An antioxidant causes prevention or slowing the oxidation reactions of other molecules. An oxidation reaction produces free radicals which start chain reactions that damage cells. Antioxidants being oxidized themselves, causes termination of these chain reactions by removing free radical intermediates. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols [1]. Natural antioxidants in plants like vitamin C, vitamin E and polyphenols scavenges free radicals which can cause several diseases including diabetes, cancer, cataracts, cardiovascular diseases, arthritis, atherosclerosis and ageing [2-6]. *Abutilon indicum* (Linn) (Malvaceae), commonly known as “Atibala” is a plant of high medicinal importance. It is mainly found in the hotter parts of India with a characteristic hairy under shrub with golden yellow flowers. The different parts of the plant like leaves, roots, bark, flowers, seeds, and seed oil are used traditionally for the treatment of several ailments. Various parts of plant extracts has been reported to possess hepato-protective , hypoglycemic, immunomodulatory, analgesic, antimicrobial, anti malarial, anti fertility and wound healing properties [7,8,9]. In view of various scientific reports, it is thought to investigate the antioxidant properties of various fractions of *Abutilon indicum* fruits.

MATERIALS AND METHODS

Chemicals and instruments:

All the chemicals and solvents used were analytical grade and were obtained either from Sigma chemicals, U.S.A, S.D.fine chemicals, and Loba chemicals, Mumbai, India. The solvent was removed from all the fractions in a rotary evaporator (Superfit rotavap-PBU-6). The absorbance measurements were recorded using the double beam UV spectrophotometer (Elico, SL210).

Preparation of plant extract:

The fruits of *Abutilon indicum* were collected in December 2015 from Anajipuram (V) Penpahad

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(M) Suryapet (Dist) Telangana. The plant authentication and identification was done by Scientist & Taxonomist of the Botanical Survey of India, Hyderabad. Voucher specimen of *A. indicum* (GPRCP/AI/BR12/2015) is maintained in the department of Phytochemistry and Pharmacognosy, G. Pulla Reddy College of Pharmacy, Hyderabad, Telangana. The fruits were separated from plant, shade dried and grinded into powder. The dried fruit powder of *A. indicum* (2 kg) was extracted with 80% of aqueous ethyl alcohol by maceration process for 8 days. The solvent was removed from the extract in a rotary evaporator and dried.

Fractionation of extract:

To the concentrated aqueous ethanolic extract 500 ml of water was added and fractionated with petroleum ether (4x500 ml), chloroform (4x500 ml), ethyl acetate (4x500 ml) and n-butanol (4x500 ml).

Phytochemical screening:

The aqueous ethanolic extract and fractions of *A. indicum* were tested for detection of carbohydrates, proteins, alkaloids, glycosides, saponins, phenols, flavonoids and steroids [10, 11].

Total phenol content determination:

Phenolic content of fruits of *A. indicum* extracts were measured by Slinkard and Singleton method [12]. 5 ml of 10% Folin-Ciocalteau reagent and 4 ml of 1M sodium carbonate solution was added to 0.5 ml of test solution. At 765 nm absorbance was measured. Standard curve of gallic acid (10-100 µg/ml) was plotted by absorbance versus concentration. The total phenol content of *A. indicum* fruit fractions were determined and expressed as gallic acid equivalent per gram (mg GAE/g).

Total flavonoid content determination:

Flavonoid content of *A. indicum* fruit fractions were assayed by aluminum chloride method. To 0.5 ml of test sample, 3 ml of 95 % ethanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water were added. After 30 min absorbance was measured at 415 nm[13].

In vitro antioxidant studies:

DPPH radical scavenging assay:

DPPH radical scavenging assay was determined by Aquino *et al* method [14]. To 1ml of test solution 2 ml of 90 µM of DPPH solution was added. After 60 min incubation in dark condition 1 ml of ethanol was added to reaction mixture. Absorbance was measured 517 nm. Ascorbic acid and curcumin were used as reference materials. All the tests were performed in triplicate; radical scavenging capacity is calculated by comparing with control. IC_{50} was determined by plotting graph between concentrations versus %RSC.

$$\%RSC = \frac{\text{Absorbance of blank - Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

Nitric oxide radical scavenging assay:

Nitric oxide radical scavenging assay was determined by the Griess reaction. To 0.5 ml of sodium nitroprusside, 1 ml of various concentrations of *A. indicum* extracts and reference compounds were added. After 17 hrs incubation at 25° C, 1.5 ml of incubated mixture is diluted with 1.5 ml of Griess reagent. Absorbance was measured at 540 nm against a blank sample. IC_{50} was determined by %RSC values. Curcumin and ascorbic acid were used as reference compounds [15].

Super oxide radical scavenging assay:

Super oxide radical scavenging assay was determied by Nshimiki *et al* [16] method. To 1 ml of 156 mM of nitro blue tetrazolium solution, 1ml of 468 µM of nicotinamide adenine dinucleotide, 1ml of *A. indicum* extracts and reference samples, 100 µl of 60 mM phenazine metho sulphate (PMS) in phosphate buffer (100 mM, PH - 7.4) was added. After incubation at 25° C for 5 min, absorbance was read at 560 nm against control. Gallic acid and rutin were used as reference samples. The %RSC and IC_{50} values were determined.

Hydroxyl radical scavenging assay:

To 0.1 ml of EDTA (1mM) add 0.01 ml of ferric chloride (10mM), 0.1 ml of hydrogen peroxide (10 mM), 0.36 ml of deoxyribose (10 mM), 1 ml of different concentrations of *A. indicum* extracts and reference compounds, 0.33 ml of phosphate buffer (50 mM, pH -7.4) and 0.1ml of ascorbic acid (0.1 mM) in sequence. After incubation at 37°C for

60 min, 1 ml of the above solution was added to 1 ml of 0.5% TBA, 1 ml 10% TCA and the mixture was heated at 100°C for 20 min to get pink color. Read the absorbance at 532 nm. BHT and mannitol were used as standards. The %RSC and IC_{50} values were determined [17].

Assay of Reducing power:

A. indicum fruit reducing power was measured according to Oyaizu method. 1 ml of extracts and reference compounds with different concentrations were mixed with 2.5 ml of buffer (0.2M; pH 6.6) and 2.5 ml of 1% potassium ferricyanide and incubated for 20min at 50°C. To the above solution 2.5 ml of 10% TCA was added and centrifuged for 20 min at 3000 rpm. To 2.5 ml of supernatant, 2.5 ml of water, 0.5 ml of 0.1% $FeCl_3$ was mixed and absorbance was read at 700 nm. Curcumin and BHT were positive controles [18].

Statistical analysis:

All the data were expressed as the mean values±SD and were obtained from experiments repeated three times. Statistical analysis was performed by one-way ANOVA and Dunnett's test. P-values less than 0.05 were considered significant.

RESULTS AND DISCUSSIONS

Extraction and Fractionation:

The dried *A. indicum* fruits were extracted with 80% aqueous ethyl alcohol by maceration at room temperature, the yield of aqueous ethanolic extract (AIFM) is 130g (6.5 %w/w). The percentage yields of petroleum ether (AIFP), chloroform (AIFC), ethyl acetate (AIFE), n-butanol (AIFB), and remaining aqueous extract (AIFA) of AIFM were 3.6, 1.4, 1.2, 3.7 and 74%.

Preliminary phytochemical screening:

Phytochemical tests revealed that *A. indicum* fruit extracts containing flavonoids, steroids and/or their glycosides, carbohydrates, phenols and proteins (Table 1).

Table 1: Phytochemical analysis of various fractions of *A. indicum*

Test	AIFM	AIFP	AIFC	AIFE	AIFB	AIFA
Carbohydrates	++	++	++	+	++	++
Proteins	++	-	+	+	++	-
Alkaloids	-	-	-	-	-	-
Glycosides	-	-	-	-	-	-
Phenols	++	+	++	++	+	+
Flavonoids	++	+	+	++	+	+
Saponins	+	-	-	+	+	+
Steroids	++	++	++	+	+	-

+ Present, - absent.

AIFM - Mother extract of *A. indicum* fruit; AIFP - Petroleum ether fraction of *A. indicum* fruit.

AIFC - Chloroform fraction of *A. indicum* fruit; AIFE - Ethyl acetate fraction of *A. indicum* fruit.

AIFB - Butanol fraction of *A. indicum* fruit; AIFA - Aqueous fraction of *A. indicum* fruit.

Total phenol content determination:

Phenols are the major plant compounds with antioxidant activity [19]. Among all fractions ethyl acetate fraction (86 mg GAE/g) showed highest phenolic content followed by chloroform fraction (56 mg GAE/g). Table 2 represents the results of total phenolic content.

Total flavonoid content determination:

Total flavonoid content of mother extract and all the fractions were assayed by aluminium chloride using rutin as a standard. The total flavonoid content was found to be higher in ethyl acetate (30 mg RE/g) and chloroform (20 mg RE/g) fractions (Table 2).

Table 2: Total phenolic and flavonoid content of various fractions of *A. indicum*

Fraction	Phenol content (mg GAE/g)	Flavonoid content (mg RE/g)
AIFM	18± 1.2	15 ± 0.4
AIFP	14 ± 1.4	11 ± 0.6
AIFC	56 ± 0.7	20 ± 0.2
AIFE	86 ± 1.3	30 ± 0.5
AIFB	32 ± 1.1	18 ± 0.8
AIFA	16 ± 0.8	11 ± 0.3

Values are mean of three replicate ± SD

AIFM - Mother extract of *A. indicum* fruit; AIFP - Petroleum ether fraction of *A. indicum* fruit.
AIFC - Chloroform fraction of *A. indicum* fruit; AIFE - Ethyl acetate fraction of *A. indicum* fruit.
AIFB - Butanol fraction of *A. indicum* fruit; AIFA - Aqueous fraction of *A. indicum* fruit.

In vitro antioxidant studies:

DPPH radical scavenging assay:

DPPH in its radical form has purple color which disappears by hydrogen donor. Different extracts of *A. indicum* exhibited considerable DPPH radical scavenging activity as shown in figure.1, 2, and 3. AIFE (25 µg/ml), AIFC (73 µg/ml) DPPH radical scavenging capacity is higher than all other fractions. The decreasing order of radical scavenging capacity is ascorbic acid (1.3 µg/ml) > curcumin (6 µg/ml) >AIFE (25 µg/ml) >AIFC (73 µg/ml) >AIFB (250 µg/ml) >AIFP (330 µg/ml) >AIFA (690 µg/ml) >AIFM (920 µg/ml).

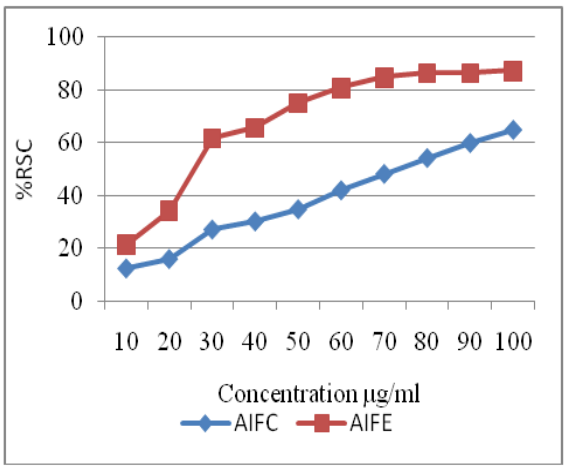

Figure 1: DPPH radical scavenging activity of AIFE, AIFC

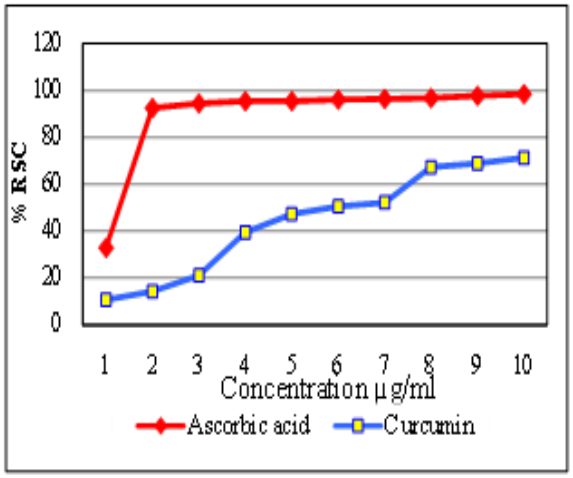

Figure 2: DPPH radical scavenging activity of ascorbic acid, curcimine

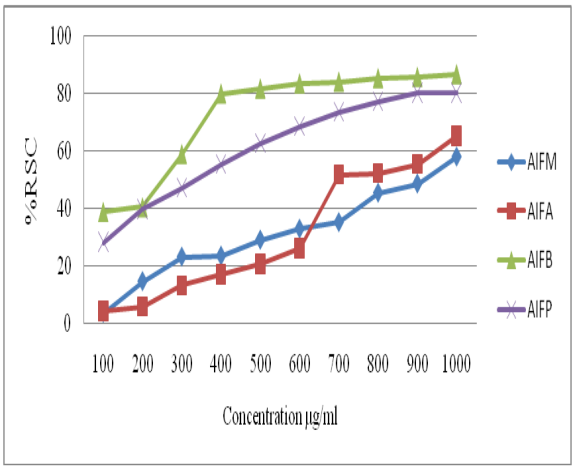

Figure 3: DPPH radical scavenging activity of AIFM, AIFA, AIFB, AIFP

Nitric oxide radical scavenging assay:

Among all fractions AIFE only showed good results in concentration range of 100-1000 µg/ml, like standard ascorbic acid. Whereas Standard curcumin showed good inhibition in concentration range of 10-100 µg/ml and decreasing order of IC_{50} values were curcumine (57 µg/ml) > ascorbic acid(310 µg/ml) > AIFE(380 µg/ml). The other extracts are not having the activity. Figure 4, 5 represents the radical scavenging capacity of AIFE, ascorbic acid and curcumin.

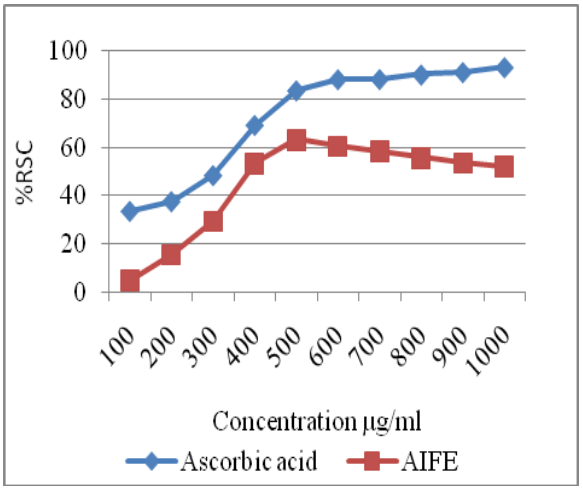

Figure 4: Nitric oxide radical scavenging activity of AIFE, ascorbic acid

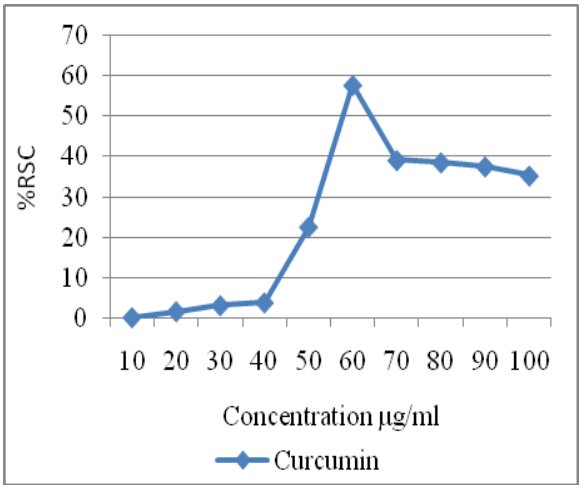

Figure 5: Nitric oxide radical scavenging activity of curcumine

Super oxide radical scavenging assay:

Among all extracts of *A. indicum,* AIFE exhibited superoxide radical scavenging activity in concentration range of 100-1000 µg/ml like standard rutin. Whereas gallic acid inhibited the superoxide radicals in concentration range of 10-100 µg/ml. The decreasing order was gallic acid (37 µg/ml) > rutin (930 µg/ml) > AIFE(1000 µg/ml). Figure 6, 7 represents the results of test and standard compounds.

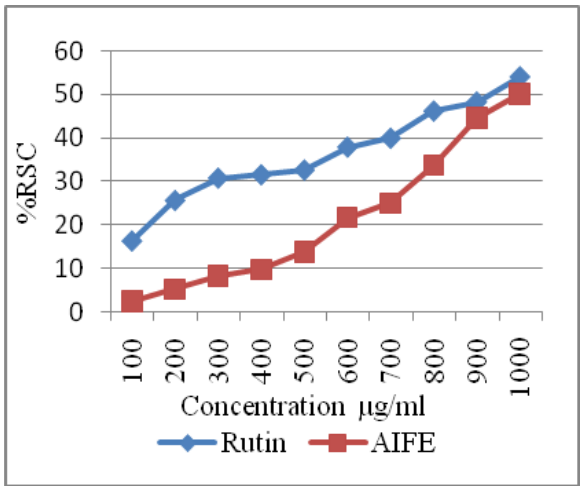

Figure 6: Super oxide radical scavenging activity of AIFE, rutin

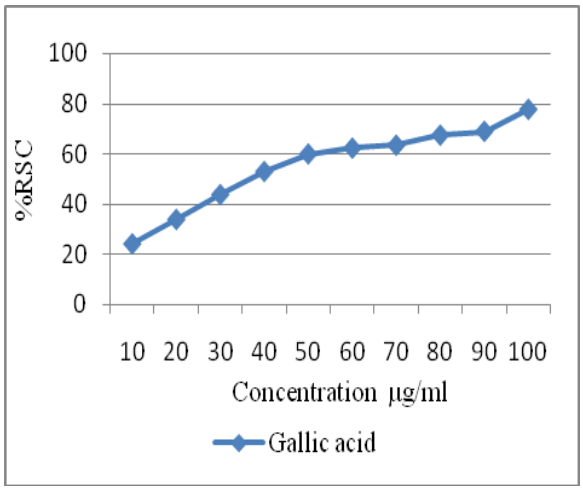

Figure 7: Super oxide radical scavenging activity of gallic acid

Hydroxyl radical scavenging assay:

A. indicum ethanol extract and all fractions failed to produce hydroxyl radical scavenging property even at the concentrations up to 1000 µg/ml. Standard mannitol has shown good hydroxyl radical scavenging activity in concentration range of 10- 100 µg/ml with IC_{50} value 45 µg/ml. Whereas standard BHT has shown good hydroxyl radical scavenging activity in concentration range of 100- 1000 µg/ml with IC_{50} value 150 µg/ml. Scavenging effects of mannitol and BHT were shown in Figure 8, 9.

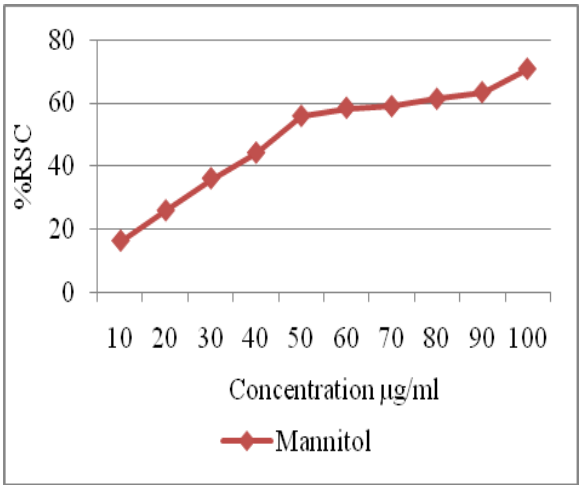

Figure 8: Hydroxyl radical scavenging activity of Mannitol

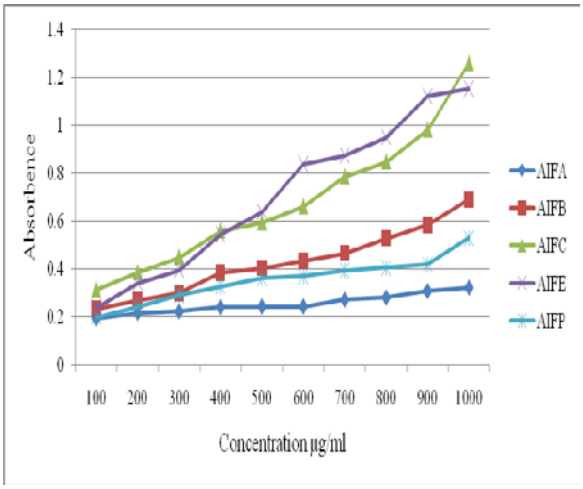

Figure 10: Reducing power of AIFA, AIFB, AIFC, AIFE, AIFP

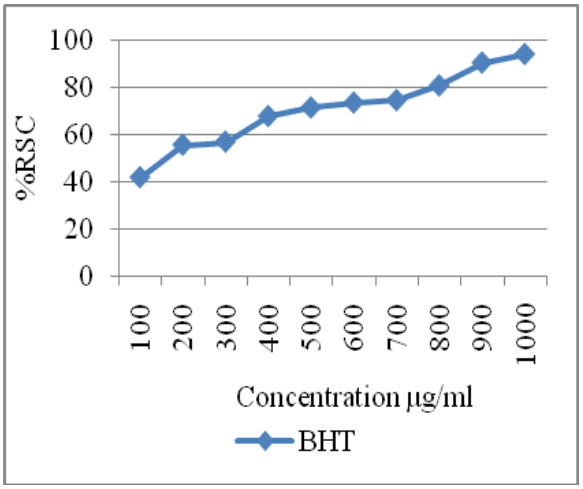

Figure 9: Hydroxyl radical scavenging activity of BHT

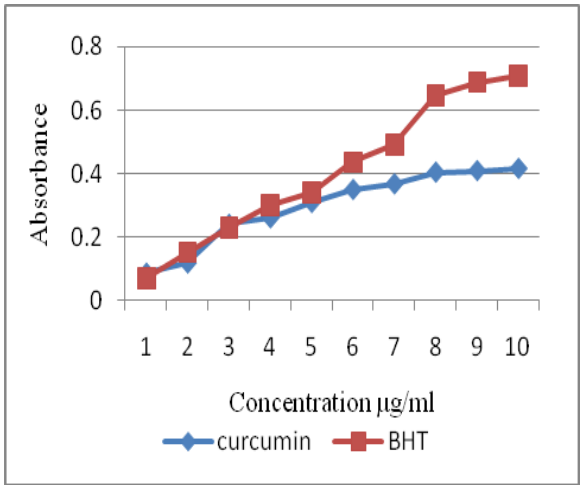

Figure 11: Reducing power of Curcumine, BHT

Assay of reducing power:

The measurement of reductive ability is a significant indicator for determination of potential antioxidant activity [20]. Increasing of absorbance values indicates increased reducing power activity. Standard curcumin, BHT has shown good reductive capabilities in concentration range of 1-10 µg/ml. AIFM has shown good reductive capabilities in concentration range of 10-100 µg/ml. AIFA, AIFB, AIFC, AIFE and AIFP has shown good reductive capabilities in concentration range of 100-1000 µg/ml. The decreasing order of reductive capabilities were BHT > curcumin>AIFM> AIFC > AIFE > AIFB> AIFP> AIFA. Reducing power of test and standard drugs were shown in Figure 10, 11, 12.

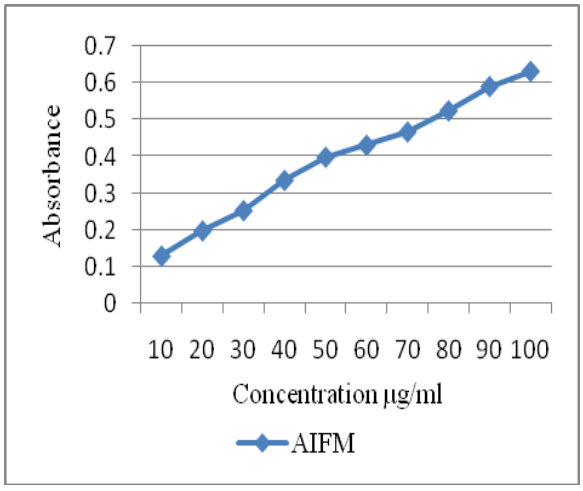

Figure 12: Reducing power of AIFM

Conclusion:

Ethyl acetate fraction of *Abutilon indicum* has produced highly significant antioxidant activity followed by chloroform extract. The both extracts are rich in total phenol and flavanoid content. The

observed antioxidant potential may be attributed to these constituents. The ethyl acetate extract can be considered as new source of natural antioxidant. These finding may provide basis for developing a valuable food additive to enhance human immune system.

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