EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *ABUTILON INDICUM*

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Summary

The present study was designed to evaluate the antioxidant and antimicrobial activities of chloroform fraction of alcoholic extract of whole plant of *Abutilon indicum* belonging to Malvaceae family. The extract was screened for antioxidant and free radical scavenging effects at various concentrations (100, 300 and 500 µg/ml) by reducing power assay and DPPH free radical scavenging method. All these antioxidant activities were concentration dependent which were compared with standard antioxidants such as BHA and ascorbic acid. The antimicrobial activity was studied using the agar well diffusion assay. Extract of *Abutilon indicum* was found to be most effective against *Staphylococcus aureus* followed by *Bacillus sublitis* whereas in case of Gram negative bacteria, extract was found to be most effective against *Escherichia coli* showing the maximum zone of inhibition followed by *Pseudomonas aeruginosa* .The activity of the extract against *S. aureus* and *B. subtilis* was comparable to that of standard drug ciprofloxacin. Interestingly, the ethanolic extract showed high activity against *C. albicans* than that of standard drug amphotericin B.

Keywords: *Abutilon indicum*, Antioxidant activity, Antimicrobial activity, chloroform fraction.

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Introduction

Abutilon indicum (Linn.) Sweet (Malvaceae) is a shrub distributed throughout India, Sri Lanka, topical regions of America and Malaysia [1]. The various parts of the plant (leaves, roots, seeds and seed oil) are widely used by various tribal communities and forest dwellers for the treatment of variety of ailments. A scrutiny of literature revealed some notable pharmacological activities of the plant such as antibacterial [2], analgesic [3], antimalarial [4], antifertility [5], hepatoprotective [6], and wound healing [7]. The plants contain saponins, flavonoids, alkaloids, hexoses, n-alkane mixtures (C_{22-34}), alkanols, β-sitosterol, vanillic, p-coumaric, caffeic acid, fumaric acid, sesquiterpene lactones (Alantolactone and isoalantolactone) and amino acids. The plant *A.indicum* contains 0.15% of essential oil which mainly consists of α-pinene, caryophyllene, caryophyllene oxide, endesmol, farnesol, borenol, geraniol, geranyl acetate, elements and 1:8-cineole along with number of other minor constituents [8-11], [12], [13]. The present work was therefore undertaken to study the antioxidant activity and antimicrobial activity of the organic extractives of the plant *Abutilon indicum*.

Materials and methods

Plant material

Abutilon indicum (Malvaceae) was collected from surrounding local areas and identified by Department of Botany, Kurukshetra University, Kurukshetra, Haryana, India. A voucher specimen (Sr.No. KUK/IPS/2008/AI-106) was deposited in the herbarium of the Botany Department, Kurukshetra University, Kurukshetra, Haryana, India.

Preparation of Extract

The dried and coarsely powdered plant material was extracted with petroleum ether (60-80°) by hot percolation in soxhlet apparatus until it become colorless. The defatted plant material was then extracted with alcohol until it become colorless. The extract was concentrated under reduced pressure to yield a crude semi-solid mass. This material was stirred with 6% acetic acid and kept overnight, and then extracted with chloroform.

Drugs

Chemicals used in this study were 2, 2-diphenyl-1-picrylhydrazyl (DPPH), potassium ferricyanide, trichloroacetic acid, butylated hydroxy anisole (BHA), ascorbic acid, phosphoric acid, nitro blue tetrazolium, phenazine methosulfate. All reagents used for the study were of analytical grade. All the standard drugs (Ciprofloxacin, Amphotericin) were obtained from various chemical units – E.Merck India Ltd. And S.D.Fine Chem. Ltd. (India).

Test microorganisms

A total of five microbial strains were selected on the basis of their clinical importance in causing diseases in humans. Two Gram positive bacteria, *Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (MTCC 121) and two Gram negative bacteria, *Escherichia coli* (MTCC 1652) and *Pseudomonas aeruginosa* (MTCC 741) and one yeast, Candida albicans (MTCC 227) were chosen for evaluation of antimicrobial activity of the extract of *A. indicum.* All the strains used for these studies were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India. All the test microorganisms were maintained on Nutrient Agar at 37^0C.

Determination of antimicrobial activity

Various concentrations (100mg/ml, 75mg/ml, 50mg/ml and 25mg/ml) of extract of *A.indicum* were evaluated for antimicrobial activity by agar well diffusion method.[14] All the microbial strains were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately 1.5×10^8 cfu/ml.[15]

20ml of specific agar media was poured into each petri plate and plates were swabbed with 100 µl inocula of each test bacterial strain and kept for 15 min for adsorption. Wells of 8mm diameter were punched into seeded agar plates and loaded with a 100 µl volume with different concentrations of leaf extract of *A.indicum,* reconstituted in the dimethylsulphoxide (DMSO). All the plates were incubated at 37°C for 24 hrs. Antimicrobial activity was evaluated by measuring the diameter of inhibition zone with zone reader (Hi Antibiotic zone scale). DMSO served as the negative control and cipropfoxacin (for bacteria) and amphotericin-B (for fungi) served as the positive control. The experiment was carried out in triplicate and mean of the diameter of inhibition zones was calculated.

Antioxidant assay

DPPH free radical scavenging activity

The free-radical scavenging activity of extract was measured as decrease in the absorbance of methanol solution of DPPH [16]. A stock solution of DPPH (33 mg in 1 L) was prepared in methanol, which gave initial absorbance of 0.493, and 5 ml of this stock solution was added to 1 ml of extract solution at different concentrations (100, 300 and 500 µg/ml). After 30 min, absorbance was measured at 517 nm and compared with BHA and ascorbic acid taken as standards. Scavenging activity was expressed as the percentage inhibition calculated using the following formula:

$$\%\ Inhibition = \frac{Control\ absorbance - Sample\ absirbance}{Control\ absorbance}\ X\ 100$$

Reducing power assay

The reducing power of extracts was determined as per the reported method [17]. Different concentrations of extract (100, 300 and 500 µg/ml) in 1 ml of methanol were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferrocyanide (2.5 ml, 1%). The mixture was incubated at 50oC for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1%). The absorbance was measured at 700 nm and compared with standards. Increased absorbance of the reaction mixture indicated increased reducing power.

Statistical analysis

All data were represented as mean ± S.E.M. and as percentage. Results were statistically evaluated using Dunnett's *t*- test. $P<0.01$ was considered significant.

Results

DPPH free radical scavenging activity

DPPH radical was used as a substrate to evaluate free radical scavenging activities of extract. It involves reaction of specific antioxidant with a stable free radical 2, 2-diphenyl-1- picryl-hydrazyl (DPPH). As a result, there is reduction of DPPH concentration by antioxidant, which decreases the optical absorbance of DPPH; this is detected by spectrophotometer at 517 nm. BHA and ascorbic acid were used as standards. The scavenging effect of MP extract on the DPPH radical was 87.6%, (Fig.1) at a concentration of 500 µg/ml. These results indicated that extract has a noticeable effect on scavenging the free radicals.

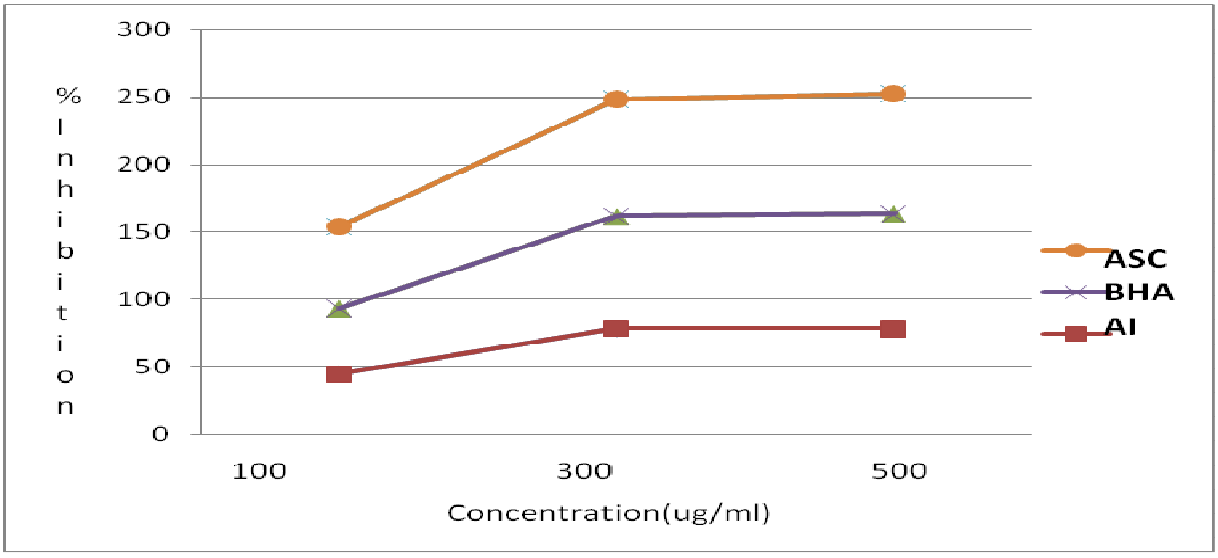

Fig.1. Anti-radical activity of AI extract, Ascorbic acid and BHA against 1,1-diphenyl-2-picrylhydrazyl free radicals

Reducing power assay

For the measurements of the reducing ability, 'Fe3+- Fe2+ transformation' in the presence of extract was found. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reductive capabilities of extract were compared with BHA and ascorbic acid. The reducing power of extract was found to increase with increasing concentrations (Fig. 2).

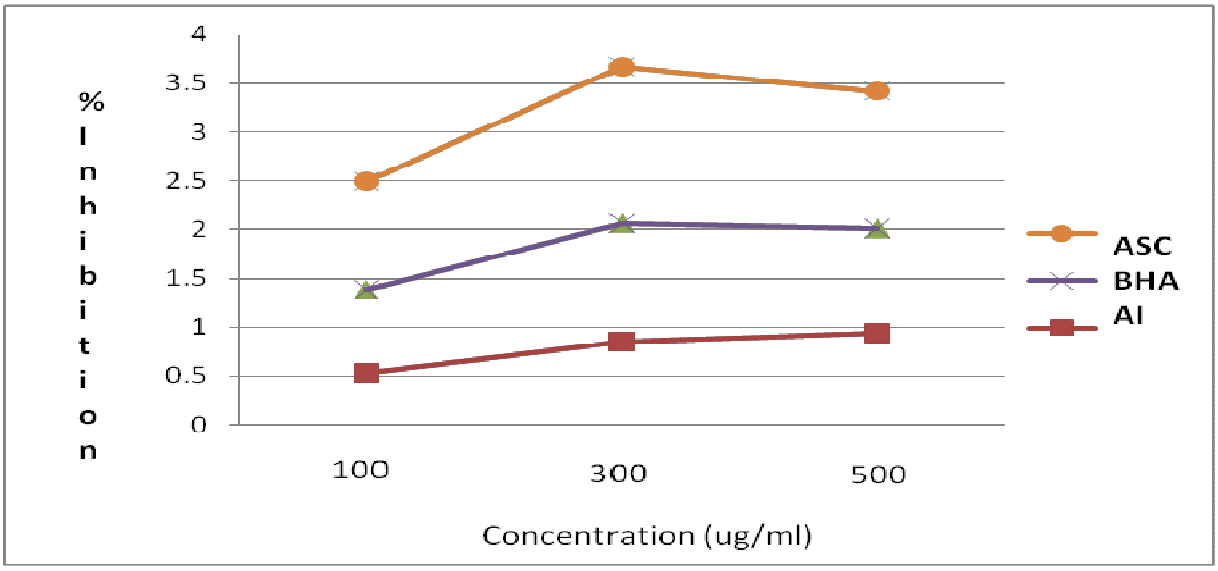

Fig. 2 Reducing power assay of AI extract, Ascorbic acid and BHA

***In vitro* antimicrobial activity**

The extract of *Abutilon indicum* inhibited all the tested bacterial and fungi strains as shown in Table 1. The results showed that the increase in concentration of extract increased the zone of inhibition against all the tested microbial strains. From the results, extract of *Abutilon indicum* was found to inhibit the Gram positive bacteria at all the tested concentrations (100mg, 75mg, 50mg and 25mg) whereas Gram negative bacteria did not showed any activity at an concentration of 25mg/ml. The extract of *Abutilon indicum* showed good activity against the tested microorganisms except for *P. aeruginosa*, which showed low activity as compare to other tested microorganisms. Extract of *Abutilon indicum* was found to be most effective against *S. aureus* showing the maximum zone of inhibition (31.6mm) followed by *B. sublitis* (24.3mm) whereas incase of Gram negative bacteria, ethanolic extract was found to be most effective against *E. coli* showing the maximum zone of inhibition (24.6mm) followed by *P. aeruginosa* (15.6mm). The activity of the extract against *S. aureus* and *B. subtilis* was comparable to that of standard drug ciprofloxacin. Interestingly, the extract showed high activity against *C. albicans* with a zone of inhibition (18.6mm) than that of standard drug amphotericin B (13.6mm).

Table-1. Antimicrobial activity of *A. indicum* extract

Micro-organisms	Diameter of growth of inhibition zone (mm)[a]						
	Extract concentration (mg/ml)				Control		
	100	75	50	25	Ciprofloxacin (20 µg/ml)	Amphotericin B (100 µg /ml)	DMSO
Staphylococcus aureus	31.6	25.3	23.6	15.6	26.3	ns	-
Bacillus subtilis	24.3	20.6	20.6	18.3	25.6	ns	-
Escherichia coli	24.6	22.3	20.3	-	25	ns	-
Pseudomonas aeruginosa	15.6	14	13.6	-	23.3	ns	-
Candida albicans	18.6	15.6	15.3	-	ns	13.6	-

- No activity, ns –not studied

[a] Values, including diameter of the well (8mm), are means of three replicates

Discussion

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. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [18]. Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and treatment of diseases associated with oxidants or free radicals [19]. Extract of *Abutilon indicum* showed variable activity against all the tested microbial strains. It was observed that the zone of inhibition varies from one organism to another at different concentrations. According to Prescott [20], the activity of antimicrobial agent is concentration dependent. Among the Gram positive and negative bacteria tested, Gram positive bacteria were more susceptible to the extracts. These results are in accordance with the earlier reports indicating that plant extract are most active against Gram positive bacteria than that of Gram negative bacteria [21]. The activity of extracts was comparable to those of standard antibiotics in case of Gram positive bacteria and found to be more active, in case of yeast (*C. albicans*).Thus, extract of *Abutilon indicum*, showed broad spectrum activity against the tested bacteria and in case of *C. albicans,* it showed activity greater than the standard drug, so it can also be used for the treatment of candidiasis.

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