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PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND ANTIMICROBIAL EFFECTS OF *ABUTILON INDICUM* (LINN.) LEAVES EXTRACTS

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Abstract

Abutilon indicum is an important shrub which is traditionally used as folk medicine on various diseases. The aim of our study was to investigate phytochemicals, antioxidant and anti-microbial potential of the shrub that will potentiate its significance in traditional medicine. Ethanol (EAI), chloroform (CAI) and aqueous (AAI) extracts of *A. indicum* leaves were taken to perform these experiments. Phytochemical screening was done qualitatively; total phenolic (TPC) and total flavonoid content (TFC) of the extracts were evaluated quantitatively by Folin-Ciocalteau regent and aluminium trichloride methods, respectively. Antioxidant effect of the extracts was evaluated by 1, 1 Diphenyl −2- picrylhydrazyl (DPPH) radical scavenging and ferric reducing power (FRP) assay methods. Anti-microbial potential was determined by Agar diffusion method. Phytochemical screening revealed the presence of major phytoconstituents. TPC and TFC were expressed as gallic acid and quercenin equivalents. Among the three extracts, EAI showed the highest TPC and TFC. The order of TPC and TFC among the extracts is EAI>CAI>AAI. In DPPH test, IC_{50} value of EAI, CAI and AAI were 25.11±2.57, 35.48±3.29 and 50.11±3.40 µg/ml, respectively. In case of FRP, EAI showed lowest EC_{50} value (28.18±2.53) followed by CAI (39.81±3.47) and AAI (70.79±4.56 µg/ml). In anti-microbial assay, both the EAI and CAI showed zone of bacterial growth inhibition of the tested organisms as concentration dependent manner whereas AAI did not show any inhibition up to 500 µg/ml concentration.

Keywords: Abutilon indicum; Antioxidant; Anti-micromial effect; Phytochemical screening; Phenolic content

Introduction

Abutilon indicum (Linn.) is a small shrub under the Malvaceae family, native to tropical and subtropical regions and sometimes cultivated as an ornamental plant [1]. It is commonly familiar as 'Country mallow' (English), 'Kanghi' (Hindi) and 'Atibala' (Sanskrit) [2,3]. It is one of the important species among 150 species of Abutilon genus [3]. It is a perennial shrub, softly tomentose and grows up to 3 m in height. The leaves are ovate, acuminate, toothed, rarely subtrilobate and 1.9-2.5 cm long. The flowers are yellow in colour, peduncle jointed above the middle. The petioles 3.8-7.5 cm long; stipules 9 mm long; pedicels often 2.5-5 mm long, axillary solitary, jointed very near the top; calyx 12.8 mm long, divided in to middle, lobes ovate, apiculate and corolla 2.5 cm diameter, yellow, opening in the evening. The fruits are capsule, densely pubescent, with conspicuous and horizontally spreading beaks. The stems are stout, branched, 1-2 m tall, pubescent. The seeds are 3-5 mm; reniform, tubercled or minutely stellate-hairy, 5-8 black or dark brown [2].

It is extensively used in folk medicine as demulcent, diuretics, anti-diabetic, anthelmintic, astringent, laxative, expectorant, antibacterial, antifungal activities [4]. It is also used as aphrodisiac, sedatives, expectorant, tonic, anti-inflammatory, anthelmintic and analgesics [5]. It is applied in snakebite, leprosy, piles, lumbago, jaundice, ulcer, toothache and liver disorders [6]. It is widely distributed in the tropical and subtropical countries of America, Africa, Asia and Australia. It needs only heat and sun and grows even in dry and poor soils. It is quite common in India on road sides and waste places, growing usually after the rains and flowering during winter. In Poland, it is also cultivated for its ornamental value [3]. It is extensively grown in Bangladesh, India, Pakistan and Srilanka [7].

Many bioactive chemical compounds have been identified in the extracts of *Abutilon indicum*. Among these phenols, tannins, alkaloids, flavanoids glycosides, proteins, amino acids, sesquiterpenes, steroids, sterols, terpenoids, terpenes, carbohydrate are the major compounds [8]. α-tocopherol, ß–sitosterol, gallic acid, fumaric acid, p-coumaric acid, vanillic acid, caffeic acid are also found in different parts of the plant. ß-amyrin, luteolin, chrysoeriol, luteolin-7-O-beta glucopyranoside, chrysoeriol-7-O-betaglucopyranoside, quercetin-3-O-beta glucopyranoside, and p-hydroxybenzoic acid, p-ß-D-glucosyloxybenzoic acid has also been reported in it [9]. Amino acid profile of seed proteins (31%) contains threonine, glycine, serine, glutamine, lysine, methionine, isoleucine, proline, alanine, cystenine, tyrosine, phenylalanine, leucine, aspargine, histidine, valine, argininine etc [3].

Reactive oxygen species (ROS) which are composed of superoxide ($O2^-$), hydrogen peroxide (H2O2), hydroxyl radical (OH^-), and peroxynitrite ($ONOO^-$) are abundantly generated by the normal mitochondrial respiration and some environmental factors such as ultraviolet radiation, pollutants, x-rays etc. These are critical in intracellular signalling pathways. Overload of the free radicals may, however, lead to oxidative damage that causes cellular membrane injury with the subsequent alteration in metabolic processes. Moreover, these play a potential role in pathogenesis of diverse degenerative diseases like atherosclerosis, liver disorders, lung and kidney damage, aging and diabetes mellitus [10]. The intracellular concentration of ROS depends on both their production by endogenous or exogenous factors and removal by various endogenous antioxidants including both enzymatic and non-enzymatic components [11,12]. Therefore, it is very important to consume dietary or supplementary antioxidants to scavenge the abundant free radicals so that they can protect cellular DNA, proteins and lipid membranes [13].

Search for new antibacterial agents, in particular, has increased in the last decade mainly because of the increase in bacterial infections especially in countries with poor populations and more so because of bacterial resistance to current antibiotics. Bacterial infections have also been implicated in complication of chronic conditions, especially transplants, cancer, and AIDS because of weakened immunity. Studies also claimed that some plants, which are already used as traditional medicine, possess antimicrobial properties against bacteria, fungi and viruses, and preparation from such plants considered to be effective against diseases of microbial aetiology like small pox, tuberculosis, typhoid and diphtheria etc. [14]. For centuries, plants have been used throughout the world as drugs and remedies for various diseases, including infectious diseases. These drugs serve as prototypes to develop more effective and less toxic medicines. According to the WHO, medicinal plants would be the best source for obtaining a large variety of drugs. Many plants have been used as remedies for diseases and offer biologically active compounds that possess antimicrobial properties. Thousands of constituents that can be used as sources of antimicrobial agents have been reported [15].

The present study was undertaken to investigate phytochemical constituents, antioxidant and anti-microbial effect of ethanol, chloroform and aqueous extracts of *A. indicum* that may unveil the rationality of use of the plant as traditional medicines and potentiality of it in the herbal medicine.

Methods

Plant materials

For the investigation fresh leaves of *Abutilon indicum* were collected from Dinajpur, Bangladesh in May, 2012 and identified by experts of the Bangladesh National Herbarium, Dhaka, where a voucher specimen has also been retained with accession no. 39430. The collected plant parts were cleaned, dried for one week and pulverized into a coarse powder using a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark, and dry place until further analysis.

Extract preparation

Approximately 500 g of powdered material was placed in a clean, flat-bottomed glass container and soaked in ethanol. Similarly 300g of the powder was soaked in distilled water and in chloroform separately. All the containers with its contents were sealed and kept for 5 days. Then extraction was carried out using ultrasonic sound bath accompanied by sonication (40 minutes). The entire mixture then underwent a coarse filtration by a piece of clean, white cotton material. The extract then was filtered through Whatman filter paper (Bibby RE200, Sterilin Ltd., UK) and dried by electric oven at 45^0C temperature and continued up to obtain ethanol (15.25g), chloroform (5g) and aqueous (13g) extracts. The gummy extracts were stored in an air tight container.

Drugs and chemicals

DPPH, quercetin, gallic acid, ascorbic acid were purchased from Sigma Aldrich, USA. Nutrient ager was parched from Mark India. Other chemicals and solvents were of highest analytical grade commercially available. All the tested organisms which were used to conduct the study were used from stored sample of Southeast university laboratory.

Phytochemical screening

Qualitative tests of the EAI, CAI and AAI for the presence of alkaloids, tannins, resins, saponins, flavonoids, steroids and terpenoids were carried out.

Test for alkaloids (Mayer's and Dragendroff's test)

0.4 g of EAI, CAI and AAI were stirred with 8 ml of 1% HCl in three separate test tubes. The mixtures were warmed and filtered. 2 ml of filtrate were treated separately with (a) few drops of potassium mercuriciodide (Mayer's reagent) and (b) potassium bismuth (Dragendroff's reagent). Turbidity or precipitation with either of these reagents was taken as evidence for existence of alkaloids [16].

Test for saponins (Froth test)

The ability of saponins to produce emulsion with oil was used for the screening test. 20 mg of the extracts (EAI, CAI and AAI) were boiled in 20 ml of distilled water in a water bath for five min and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for froth formation. 3 drops of olive oil were mixed with froth, shaken vigorously and observed for emulsion development [16].

Test for terpenoids (Salkowski test)

Presence of terpenoids in the extracts was carried out by taking 5 ml (1 mg/ml) of EAI, CAI and AAI in test tubes. Then 2 ml of chloroform, followed by 3 ml of concentrated H_2SO_4 were added in the test tubes. A reddish brown coloration of the interface confirmed the presence of terpenoids [16].

Test for tannins (Braymer's test)

50 mg of EAI, CAI and AAI were boiled in 20 ml of distilled water then filtered. A few drops of 0.1% $FeCl_3$ was added in filtrate and observed for color change. Appearance of brownish green or a blue-black coloration indicated the presence of tannins [17].

Test for steroids (Liebermann-Burchard's test)

One ml of the extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids [16].

Test for flavonoids (NaOH Tests)

To 2-3 ml of extract, few drops of sodium hydroxide solution were added in a test tube. Formation of intense yellow colour that became colourless on addition of few drops of dilute HCl indicated the presence of flavonoids [18].

Test for reducing sugars (Benedict's test)

All the extracts (EAI, CAI and AAI) were dissolved

individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates. Filtrates were treated with Benedict's reagent and heated on a water bath. Formation of an orange red precipitate indicates the presence of reducing sugars [18].

Anthraquinone (Borntrager's test)

About 0.5 g of the extract was taken into a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate shaken with equal volume of 10% ammonia solution. A pink violet or red color in the ammoniacal layer (lower layer) indicates the presence of anthraquinone [18].

Determination of phytoconstituents

Determination of total phenol content (TPC)

Total phenol content was determined using Folin-Ciocalteu reagent method as described by Yang et al. [19] with slight modifications. Total phenolic assay was conducted by mixing 2.7 mL of demonized water, 0.01 ml (50, 100, 200µg/ml) of extracts (EAI, CAI and AAI), 0.3 ml 20% Na_2CO_3 and 0.10 ml Folin-Ciocalteu reagent. Absorbance of mixture was measured at 725 nm. A standard curve was prepared with gallic acid (r^2= 0.936) and final results were given as mg/g gallic acid equivalent.

Determination of total flavonoid content (TFC)

1 ml of plant extract (EAI, CAI and AAI) in methanol (50, 100, 200µg/ml) was mixed with 1 ml aluminium trichloride in ethanol (20 mg/ ml) and a drop of acetic acid, and then diluted with ethanol to 25 ml. The absorption at 415nm was read after 40 min. Blank samples were prepared using all the reagents with equal volume used in the sample except extract. The total flavonoid content was determined using a standard curve (r^2= 0.922) of quercetin (12.5-200 µg/ml) where quercetin was used as standard sample. Total flavonoid content was expressed as mg/g of quercetin equivalent [20].

Antioxidant ability assays

DPPH• radical scavenging activity

The DPPH free radical scavenging activity of the extracts (EAI, CAI and AAI) was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH [21]. Briefly, 0.004% w/v of DPPH radical solution was prepared in methanol and then 900 µl of this solution was mixed with 100 µl of extract solution (12.5–200 µg/ml) and kept in a dark place for thirty minutes. Then absorbance was measured at 517 nm where methanol (98%), DPPH solution and ascorbic acid were used as blank, control and standard antioxidant respectively. Scavenging capacity of DPPH radicals (% Inhibition) was measured by the following formula and finally 50% inhibition concentration (IC_{50}) was calculated using Microsoft Excel software.

$$\text{Inhibition (\%)} = (A_0\text{-}A_s)/A_0 \times 100$$

Where A_0=Absorbance of control group, A_s= Absorbance of sample

Reducing power assay (RPA)

The Fe^{3+}reducing power of the extracts (EAI, CAI and AAI) were determined by the method of Oyaizu [22] with slight modifications. Different concentrations of the extracts and standard ascorbic acid (12.5, 25, 50, 100, 200 µg/ml) were prepared. 1ml of both the extracts and standard ascorbic acid of all concentrations were taken in separate test tubes and were mixed with 2.5 ml of phosphate buffer solution (0.2 M, pH 6.6). 2.5 ml of potassium ferricyanide (1%) was added in each test tube, and incubated at 50°C for 30 min. Then, 2.5 ml of trichloroacetic acid (10%) was added to the mixture which was then centrifuged at 4000 rpm for 10 min. Finally, 2.5 ml of supernatant was mixed with 2.5 ml of distilled water and 0.1 mL of $FeCl_3$ (0.1%) solution followed by incubation at 35^0C for 10 minutes. The absorbance was measured at 700 nm and the reducing powers of the extracts were compared with the standard ascorbic acid. A higher absorbance indicates a higher reducing power. EC_{50} (µg/ml) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from linear regression analysis. Ascorbic acid was used as control.

Antibacterial test

Microbial strains tested

The following strains of Gram positive and negative bacteria were used. Gram positive bacteria: *Sarcina luteae* ATCC# 9341, *Bacillus cereus ATCC*# 14579, *Bacillus megaterium ATCC*# 10778, *Staphylococcus aureus* ATCC# 25923. Gram negative bacteria: *Salmonella typhi* ATCC# 19430, *Escherichia coli* ATCC# 1053, *Shigella dysenteriae* ATCC# 11835, *Vibrio parahemolyticus* ATCC# 17802, *Pseudomonas aeruginosa* ATCC# 27853. Tested microorganisms were obtained from the culture collection of Southeast University, Department of pharmacy, Faculty of Science, Dhaka, Bangladesh. The microorganisms were grown overnight at 37°C in 2% Nutrient Agar solution (Merk Germany) at pH 7.

Their sensitivity to the reference antibiotics was checked. Kanamycin (Sigma, USA) was used for this purpose.

Preparation of inocula

The inocula were prepared by inoculating a loop of each bacterial strain from a 24 hours old culture into a sterile nutrient broth aseptically. The culture was allowed to grow for 24 hours in a shaking incubator at 37°C. The overnight culture is taken and checked until the visible
turbidity is equal or greater than that of 0.5 McFarland standards (Pro-Lab Diagnostics) at 560 nm using UV-Visible spectrophotometer (IRMECO UV–VIS U2020, Germany). Sterilized nutrient broth is used as blank. If the absorbance is higher, then the culture is diluted with sterilized nutrient broth and absorbance is noted again. The standardized cultures were used for further analysis.

Determination of antimicrobial activity

The antibacterial tests were performed using agar well diffusion method [23]. Agar plates were prepared by using sterile Mueller-Hinton (MH) agar (Bio Lab). Bacterial cultures of standardized cultures were prepared by adding the seed culture in the autoclaved agar medium followed by pouring into petri plates. The wells were made with 8 mm sterile cork borer. 50 µl of each extract (125, 250, 500 µg/ml) was added in the pre labeled wells together with ethanol, chloroform and water as negative control and kanamycin as positive control. The reference antibiotic was used in the concentration of 50µg/ml. The diffusion of extracts was allowed for 1 hr at room temperature on a sterile bench. The plates were then sealed with Parafilm X and incubated for 24 hrs at 37°C. However, in case of *S. cerevisieae* the plates were incubated for 48 hr at 30°C. The plates were observed for the presence of inhibition of bacterial growth and that was indicated by clear zone of inhibition of bacterial growth around the wells. The size of zone of inhibition was measured in millimeters (mm).

Statistical analysis

All samples were analyzed in triplicate. Data are presented as mean ± standard error mean (SEM). Differences were evaluated by one-way analysis of variance (ANOVA) test, Folled by Dunnett ´s Test, completed by SPSS version 15.0. Differences were considered significant at $p < 0.01$. The IC_{50} and EC_{50} values were calculated by regression equation using Microsoft Excel 2007. The dose response curve of DPPH was obtained by plotting the percentage inhibition versus concentration.

Results

Phytochemical screening

The preliminary phytochemical screening of the extracts (EAI, CAI and AAI) revealed the presence of secondary metabolites such as alkaloids, saponins, terpenoids, flavonoids, tannins, steroids and reducing sugars. (Table 1).

Total phenol content (TPC)

Phenolics are important classes of phytochemicals that poses significant antioxidant and subsequently biological functions. Quantitatively, phenolics have measured in terms of total phenolic contents by using Folin-ciocalto reagent test and expressed as gallic acid equivalent. EAI, among the three extracts have shown highest (212.36±8.23 mg/g gallic acid) TPC. CAI and AAI exhibited 190.40±9.47 and 90.23±5.78 mg/g gallic acid of TPC respectively at 200µg/ml concentration. TPC of the extracts varied significantly ($p < 0.01$) with each other (Table 2).

Total flavonoid content (TFC)

Total flavonoid content (TFC) of the three extracts was measured as quercetin equivalents. EAI presented the highest TFC (85.69±8.12) followed by CAI (72.37±5.89) and AAI (55.10±5.08) at 200µg/ml concentration. The flavonoid content of the three extracts was significantly different from each other ($p < 0.01$) (Table 2).

DPPH radical scavenging assay

Antioxidant potential of these phytochemicals is evaluated by some established methods. Among these, DPPH radical scavenging is the most popular and authentic method. As DPPH is a stable free radical, scavenging potential of the phytochemicals are by means of donating a hydrogen atom to it (DPPH radical). In this assay ascorbic acid (AA) is used as a standard antioxidant. Here, all the three extracts of *A. indicum* have shown inhibition of the DPPH radicals as concentration dependent manner. Scavenging activity is also expressed as median inhibition concentration (IC_{50}) value. Here, less IC_{50} value of the extracts indicates more antioxidant power. Among the three extracts of *A. indicum* the crude ethanol extract (EAI) showed maximum inhibition (47.12±3.69 to 92.08±6.23%) in respect to dose ranging from 12.5 to 200µg/ml) and minimum IC_{50} value (25.11±2.57 µg/ml). CAI showed moderate inhibition (24.36±2.17 t0 84.20±6.12%) in the same dose range of EAI with the IC_{50} value of 35.48±3.29 µg/ml. On the other hand, AAI showed least

Inhibition (21.45±2.45 to 83.20±6.78%) in the same dose with the highest IC_{50} value of 50.11±3.40 µg/ml. The standard AA showed least value of IC_{50} (12.58±2.13 µg/ml) indicating the highest antioxidant potential. So, the order of DPPH radical scavenging activity of the standard and the three extracts is AA>EAI>CAI> AAI (Table 3).

Reducing power assay (RPA)

Reducing power, another important method of antioxidant evaluation, determines electron donating capacity of extracts. Here AA has used as standard antioxidant. Reducing power was evaluated according to absorbance value of the samples. More absorbance value indicates more reducing power. As, AA had highest absorbance value, it had highest reducing power. All the three extracts have shown reducing power as concentration dependant fashion in significantly different extents. Among the extracts, EAI had highest reducing power. In contrast, AAI had lowest reducing power. EC_{50} value of the three extracts have been calculated which is another way of reducing power determination. If EC_{50} value of a sample is less than the reducing power of it is more. The EC_{50} value of the standard AA, and extracts EAI, CAI, AAI were 19.95±2.34, 28.18±2.53, and 39.81±3.47 and 70.79±4.56 µg/ml, respectively. So, the order of reducing power of the standard and the extracts is AA> EAI> CAI> AAI (Table 3).

Correlations between antioxidant activities and the total phenolic and flavonoids content

Table-4 shows the correlations (linear regression coefficients, r^2) between antioxidant assays results (DPPH and RPA) and the total phenolic content (TPC) as well as total flavonoids content (TFC) of the three extracts. The DPPH method showed a good correlation with the TPC (R^2= 0.935) and a very good correlation (R^2= 0.99) with the TFC, indicating that most phenolic compounds of EAI, CAI and AAI were likely to contribute to the radical scavenging activity in this method. The RPA results, also, were well correlated with the TPC (R^2 = 0.99) and the TFC (R^2 = 0.96). So, it may be hypothesized that the chemicals of the extracts, having antioxidant properties, are able to donate hydrogen or electron to a free radical, and are capable to reduce oxidants properly (Table 4).

Antimicrobial effect

The antimicrobial activity of each extract was monitored in three different concentrations such as 125, 250 and 500 µg/ml. The activity of each extract was then compared against control group (only solvent), and the standard drug kanamycin at 50 µg/ml dose. The samples were run in triplicate to get a complete picture of effectiveness of extracts against each experimented microorganism. The effectiveness of the extracts was measured in the form of zone of growth inhibition. The extracts were tested against Gram positive and Gram negative strains of bacteria. Table 5 represents the antimicrobial activity (zone of inhibition) of the three extracts and the standard kanamycin against respective microbial strain. Kanamycin showed maximum inhibition against the organisms. Among the three extracts of *A. indicum* EAI showed maximum zone of inhibition followed by CAI. On the other hand, AAI had no potential to inhibit growth of any of the organisms up to 500µg/ml dose (Table 5).

Discussion

In recent years, there has been an increasing interest in finding natural antioxidants from medicinal plants [24]. Antioxidants are the prominent bioactive compounds that can effectively protect cells of different organs from damage by opposing the activities of the free radicals [25]. The coordinate action of antioxidant system is very critical for the detoxification of free radicals. SOD reduces the concentration of highly reactive superoxide radical by converting it to H_2O_2 whereas CAT and GSH-Px decomposes H_2O_2 and protect the tissues from highly reactive hydroxyl radicals [26, 27]. Recently, interest has considerably increased in finding naturally occurring antioxidant for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their side effects such as carcinogenesis. Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods. Hence, studies on natural antioxidants have gained increasingly greater importance [28].

The antimicrobial effects of plant materials are believed due to secondary products present in the plant, although it is usually not attributed to a single compound, but to a combination of metabolites [29]. The exact mechanism of bacterial growth inhibition of the plant materials is yet to know. One of the most important mechanisms is the hydrophobic activity of the bioactive compounds which enables them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable. Extensive leakage from the bacterial cells or the exit of critical molecules and ions will consequences death of the

bacteria [30]. Useful antimicrobial phytochemicals can be divided into several categories, such as, phenolics; flavones, flavonoids, and flavonols; quinones, tannins, coumarins, terpenoids, and essential oils; alkaloids,
lectins, and polypeptides etc. [31]. The mechanisms thought to be responsible for phenolic toxicity to microorganisms including enzyme inhibition by the oxidized compounds, possibly through reaction with the sulfhydryl groups or through more nonspecific interactions with the proteins [32]. Quinones probably target the surface-exposed adhesions, cell-wall polypeptides, and membrane-bound enzymes in the microbial cell. Quinones may also render substrates unavailable to the microorganisms. The anti-microbial activity of flavones and flavonoids may probably be due to their ability to react with extracellular and soluble proteins and to form complex materials with bacterial cell walls [33]. Tannins are a group of polymeric phenolic substances and they are found in almost every plant part. They have an astringent effect on the cell membrane of microorganisms that is responsible for formation of a protein complex through the so-called nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation [34]. The protein complex is the main substance that renders the overall biochemical process of bacteria leading to death [35]. Hydroxycinnamic acids related to coumarins seem to be inhibitory to gram-positive bacteria. Terpenoids and essential oils are active against growth of microorganisms such as bacteria, fungi, viruses, and protozoa where the speculated mechanism of action is to involve membrane disruption by the lipophilic compounds [36].
It has been unveiled that *Abutilon indicum* contains many biologically active compounds such as phenols, tannins, alkaloids, flavanoids glycosides, proteins, amino acids, sesquiterpenes, steroids, sterols, terpenoids, terpenes, carbohydrate, β–sitosterol, gallic acid, p-coumaric acid, quercetin-3-O-beta glucopyranoside etc. [3]. So, the anti-microbial effect of the extracts is due to any of these compound(s). However, further analysis is necessary to separate these compounds and to find out the most active compound having antimicrobial effect as well as to clarify the actual mode of microbial growth inhibition of that compound.

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Conflict of interest

Authors have declared that they have no conflict of interest.

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Table 1. Results of preliminary phytochemical screening of three solvent extracts of *A. indicum* leaves. + = Present, - = absent

Phytochemicals	Name of test	Extracts		
		EAI	CAI	AAI
Alkaloids	Mayer's, Dragendroff's test	+	+	+
Saponins	Froth test	+	-	+
Terpenoids	Salkowski test	+	-	+
Flavonoids	NaOH Test	+	+	+
Tannins	Braymer's test	+	-	+
Steroids	Liebermann-Burchard's test	+	-	+
Reducing sugars	Benedict's test	+	+	+
Anthroquinone	Borntrager's test	-	-	-

Table 2. Total phenol content, total flavonoid content and extraction yield of EAI, CAI and AAI. Each value is represented as mean ± SEM (n = 3). Values in the three column followed by a different letter (a-c) are significantly different with each other ($P < 0.01$).

Phytoconstituents	Concentration (µg/ml)	Extracts		
		EAI	CAI	AAI
Total phenolic contents (mg gallic acid equivalent/g)	50	121.23±6.25^{c}	87.21±7.26^{c}	48.78±3.27^{c}
	100	175.69±7.12^{c}	115.26±8.25^{c}	75.36±4.36^{c}
	200	212.36±8.23^{c}	190.40±9.47^{c}	90.23±5.78^{c}
Total flavonoid contents (mg quercetin equivalent/g)	50	32.45±3.47^{a}	24.36±2.78^{a}	17.35±1.27^{a}
	100	62.72±5.23^{a}	50.10±3.45^{a}	43.58±3.56^{a}
	200	85.69±8.12^{a}	72.37±5.89^{a}	55.10±5.08^{a}
Extraction yield (%)	--	3.05±0.14	1.66±0.25	4.33±0.10

Table 3. Antioxidant capacity of EAI, CAI and AAI of *A. indicum* leaves.

Sample	DPPH radical scavenging test			Ferric reducing power (FRP) assay		
	Concentration (µg/ml)	% Inhibition	IC_{50}	Concentration	Absorbance	EC_{50}
AA	12.5	43.25±3.10	12.58±2.13^{a}	12.5	0.37±0.03	19.95±2.34^{a}
	25	66.75±4.12		25	0.51±0.07	
	50	78.23±3.20		50	0.89±0.08	
	100	86.12±4.26		100	1.58±0.13	
	200	94.23±5.12		200	2.26±0.19	
EAI	12.5	35.35±2.45	25.11±2.57^{b}	12.5	0.25±0.02	28.18±2.53^{b}
	25	47.12±3.69		25	0.46±0.05	
	50	68.47±4.12		50	0.68±0.08	
	100	84.10±6.58		100	0.98±0.10	
	200	92.08±6.23		200	1.15±0.16	
CAI	12.5	24.36±2.17	35.48±3.29^{c}	12.5	0.16±0.02	39.81±3.47^{c}
	25	37.20±3.47		25	0.37±0.03	
	50	59.28±3.10		50	0.56±0.08	
	100	75.12±3.72		100	0.83±0.12	
	200	84.20±6.12		200	1.02±0.17	
AAI	12.5	21.45±2.45	50.11±3.40^{c}	12.5	0.12±0.01	70.79±4.56^{c}
	25	32.34±3.45		25	0.28±0.03	
	50	51.07±4.56		50	0.41±0.06	
	100	76.41±5.78		100	0.52±0.08	
	200	83.20±6.78		200	0.75±0.19	

Table 4:. Correlations between the IC_{50} and EC_{50} values of antioxidant activities and total phenol and flavonoid content of *A. indicum.*

	Correlation r^2	
Assays	**TPC**	**TFC**
IC_{50} of DPPH	0.935	0.99
EC_{50} of RPA	0.99	0.96

Table 5:. Antimicrobial activities of three *A. indicum* leaves extracts against gram positive and gram negative bacteria.

Sample	Concentration (µg/ml)	Zone of inhibition (mm)				
		EAI	CAI	AAI	Control (Solvent)	Kanamycin 50 µg/ml
***Sarcina luteae* (ATCC#9341,+ve)**	125	10.20±0.25	8.23±0.12	-	-	30±2.15
	250	14.50±0.30	12.58±0.37	-	-	
	500	20.23±0.15	16.42±0.45	-	-	
***Bacillus cereus* (*ATCC#*14579, +ve)**	125	11.42±0.52	9.50±0.24	-	-	32±3.10
	250	16.33±0.62	14.40±0.36	-	-	
	500	23.25±0.45	19.36±0.60	-	-	
***Bacillus megaterium* (*ATCC*#10778,+ve)**	125	8.55±0.20	6.78±0.27	-	-	25±2.40
	250	10.20±0.35	8.56±0.21	-	-	
	500	15.56±0.45	13.25±0.22	-	-	
***Staphylococcus aureus* (ATCC#25923, +ve)**	125	11.07±0.64	10.39±0.69	-	-	28±3.55
	250	15.12±1.32	13.20±0.78	-	-	
	500	22.21±1.56	20.58±1.04	-	-	
***Salmonella paratyphi* (ATCC#19430, -ve)**	125	9.15±0.56	7.25±0.37	-	-	26±3.10
	250	14.32±0.15	12.36±0.12	-	-	
	500	20.05±1.26	18.25±0.39	-	-	
***Escherichia coli* (ATCC#1053, -ve)**	125	12.70±1.03	10.65±0.28	-	-	32±2.08
	250	17.64±0.62	14.23±0.69	-	-	
	500	23.36±1.62	19.30±1.15	-	-	
***Shigella dysenteriae* (ATCC#11835, -ve)**	125	9.14±0.78	7.65±0.57	-	-	29±3.10
	250	14.15±0.65	9.45±0.39	-	-	
	500	18.25±0.73	15.74±0.48	-	-	
***Vibrio parahemolyticus* (ATCC#17802, -ve)**	125	12.15±0.15	8.45±0.23	-	-	31±2.65
	250	17.23±0.71	13.10±0.47	-	-	
	500	22.20±0.64	20.22±0.37	-	-	
***Pseudomonas aeruginosa* (ATCC#27853, -ve)**	125	12.09±0.32	10.45±0.78	-	-	33±3.45
	250	16.78±1.10	15.54±1.07	-	-	
	500	24.34±1.46	21.23±1.49	-	-	

Each value in the table is represented as mean ± SEM (n = 3). - = No zone of inhibition