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Research Article

A Comparison of Chemical Composition, Antioxidant and Antimicrobial Studies of *Abutilon indicum* Leaves and Seeds

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Abstract

Objective: The objective of this study was to evaluate *in vitro* antioxidant, antimicrobial activity and characterize the chemical constituents of leaves and seeds of *Abutilon indicum* by using gas chromatography-mass spectrometry (GC-MS). In addition total phenolics and flavanoids were also estimated. **Materials and Methods:** The GC-MS analysis of plant and seed extracts was performed using a GC-Brucker 436 system and interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST). Antioxidant activity was determined in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The total flavonoids assay was conducted by aluminium chloride colorimetric method and otal phenolics content was determined according to the Folin-Ciocalteu method. Antibacterial activity and minimum inhibitory concentrations were calculated by the agar diffusion method. **Results:** Twenty five chemical compounds are identified from the plant extract and 29 compounds identified from seed extract by GC-MS analysis. The leaf and seed extracts showed significant DPPH scavenging activity (IC_{50} values are 48.94±3.54 and 49.89±3.29 μg mL^{-1}) compared with the values obtained for ascorbic acid standard (IC_{50} value 39.22±5.50). The leaf and seed extracts prevented the growth of both Gram-positive and Gram-negative bacteria. The antimicrobial and antioxidant activities of the extracts were positively associated with the total phenolic and flavonoid contents of the extract. **Conclusion:** The presence of various bioactive compounds justifies the use of *Abutilon indicum* for various ailments by traditional practitioners.

Key words: *In vitro* antioxidant, antimicrobial, DPPH, GC-MS, total phenol, total flavanoid, *Abutilon indicum*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Reactive oxygen species, such as singlet oxygen, superoxide ion, hydroxyl ion and hydrogen peroxide are highly reactive, toxic molecules which are generated normally in cells during metabolism. They cause severe oxidative damage to proteins, lipids, enzymes and DNA by covalent binding and lipid per oxidation with subsequent tissue injury. Natural antioxidant agents have attracted much interest because of their ability to scavenge free radicals[1]. Free radicals have been implicated in the development of a number of disorders, including cancer, neurodegeneration and inflammation[2], giving rise to studies of antioxidants for the prevention and treatment of diseases. The presence of antioxidants such as phenolics, flavonoids, tannins and proanthocyanidins in plants may provide protection against a number of diseases for example, ingestion of natural antioxidants has been inversely associated with morbidity and mortality from degenerative disorders[3]. Medicinal plants are therefore being investigated for their antioxidant properties and the demand for natural antioxidants and food preservatives is increasing[4].

Abutilon indicum belongs to the family Malvaceae and distribute in all parts of tropical and sub tropical region of India. All parts of the plant have been recognized to have medicinal properties. The plant is commonly called as 'Thuthi' in Tamil Nadu. The traditional method of medicine, the plant used as anthelmintic, anti-inflammatory and is useful in urinary and uterine discharges, piles and lumbago[5], jaundice, ulcer and leprosy. *Abutilon indicum* leaves are used in the treatment of toothache, lumbago, piles, anti-fertility and liver disorders. Although, the plant is widely used in traditional medicine, few studies have been conducted of the pharmacological activities of the plant[6]. Phenolic and flavonoid compounds are widespread in plant kingdom where they act as antioxidants and free radical scavengers[7]. The objective of this study was to determine the total phenolic and flavonoid content and the antioxidant and antimicrobial activity of a methanol extract of leaves and seeds of *Abutilon indicum*.

MATERIALS AND METHODS

Chemicals: Quercetin, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and gallic acid were purchased from Sigma-Aldrich, USA. Folin-Ciocalteu reagent was obtained from Merck, Germany. All other chemicals and solvents used in the study were of analytical grade procured locally.

Collection and processing of plant material: The leaves and seeds of the plant *Abutilon indicum* collected from Siluvinippatti village, Sivagangai district, Tamil Nadu, India in the month of July, 2016 and authenticated by Dr. John Britto, Rapinat Herbarium, St., Joseph's College, Tiruchirappalli, Tamil Nadu, India. The leaves were cleaned and shade dried for a week and grounded into uniform powder. One gram of plant material was added to 20 mL of aqueous methanol (20%, v/v) for 18 h at room temperature. The extracts were filtered and used for the estimation of total phenols, total flavanoids contents and antioxidant activity.

Total phenols determination: The amounts of total phenolic contents of plant were determined by the spectrophotometric method of Kim *et al.*[8] with slight modification. A diluted plant sample (1 mL) was added to a 25 mL volumetric flask, containing 9 mL of distilled water. One milliliter of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 mL of 7% Na_2CO_3 solution was mixed in to the test sample solution was diluted to 25 mL distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at 23°C, after which the absorbance was read at 750 nm. Total phenol content was determined from extrapolation of calibration curve which was made by preparing gallic acid solution (20-100 μg mL^{-1}). The estimation of the phenolic compounds was carried out in triplicate. The total phenolic content was expressed as milligrams of gallic acid (GAE) equivalents per gram dried sample.

Total flavonoids determination: The total flavonoids assay was conducted according to Damodar *et al.*[9]. Total flavonoids content was determined by using aluminium chloride colorimetric method. Plant sample (0.5 mL) was mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. It retained at room temperature for 30 min. The absorbance of the reaction mixture was measured at 510 nm using UV-visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at concentrations 20-100 μg mL^{-1} in methanol. The total flavonoids content was expressed as milligrams of quercetin equivalents per gram of dried sample.

GC-MS analysis

Preparation of extract for GC-MS analysis: Leaves and seeds of *Abutilon indicum* were shade dried. Twenty grams of the powdered samples were soaked in 95% ethanol for 12 h. The extracts were then filtered through Whatmann filter paper

No. 41 along with 2 g of anhydrous sodium sulphate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with anhydrous sodium sulphate was wetted with 95% ethanol. The filtrate was then concentrated by bubbling nitrogen gas into the solution. The extract contained both polar and non-polar phytocomponents of the plant material used. Two microliter of these solutions were employed for GC-MS analysis.

GC-MS specification

GC programme: Column BR-5MS (5% diphenyl/95% dimethyl poly siloxane), 30 m×0.25 mm, ID×0.25 µm df, equipment scion 436-GC bruker, carrier gas 1 mL min^{-1}, split 10:1, detector TQ quadrupole mass spectrometer, software MS work station 8, sample injected 2 µL, oven temperature programme: 110°C hold for 3.50 min, upto 200°C at the rate of 10°C min^{-1}, no hold, upto 280°C at the rate of 5°C min^{-1}, 12 min hold, injector temperature 280°C, total GC running time: 40.50 min MS programme: Library used NIST version-11, inlet line temperature 290°C, source temperature 250°C, electron energy 70 eV, mass scan (m/z) 50-500 amu. Solvent delay 0-3.5 min, total MS running time: 40.50 min.

Identification of components: Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

***In vitro* antioxidant activity**

DPPH radical-scavenging activity: Different concentrations of leaf and seed extracts (20, 40, 60 and 80 µg mL^{-1}) were chosen for *in vitro* antioxidant activity. The L-ascorbic acid (20, 40, 60 and 80 µg mL^{-1}) was used as the reference standard. The DPPH radical-scavenging activity was determined by the method of Shimada *et al.*[10]. Briefly, a 2 mL aliquot of DPPH methanol solution (25 µg mL^{-1}) was added to 0.5 mL sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity:

$$\text{Radical scavenging activity (\%)} = 100 - \left[\frac{A_c - A_s}{A_c}\right] \times 100$$

where, A_C is control of the absorbance and A_S is sample of the absorbance of reaction mixture (in the presence of sample).

Assay of antimicrobial activity: Twenty five grams of plant powder was weighed and macerated in methanol, individually in the ratio of 1:4 (w/v). They were kept at the room temperature for 24 h. Each mixture was stirred every hours using sterile glass rod. Then it was filtered through the Whatmann No. 1 filter paper. The extracting procedure was done further twice for complete extraction of the active compounds. The obtained filtrate was combined together and concentrated using Soxhlet apparatus. The dried residue of respective solvent extract was used for evaluating the antibacterial activity. They were kept in refrigerator until use. Sterile empty antibiotic discs (6 mm diameter) were purchased from Hi-media Company, Mumbai. Twenty milligrams of dried crude extract was dissolved in 1 mL of 20% DMSO (Dimethyl sulphoxide). From this stock solution 10 µL of respective solvent extracts were added to the disc (0.2 mg $disc^{-1}$) individually and aseptically. Each disc contained 0.2 mg of extract. Then the discs were allowed for drying at room temperature. After drying they were used for screening the antibacterial activity.

Bacteria causing infectious diseases both in animals and humans were used in the present study. They were both Gram positive and Gram negative. Such as *Aspergillus niger*, *Bacillus subtilis* and *E. coli* were used. They were collected from the Microbial Type Culture Collection (MTCC) at Chandigarh, India. Pure cultures of bacterial pathogens in nutrient agar slant was transferred to tryptone broth and incubated at 37°C for 24 h.

Antibacterial assay was carried out by agar diffusion method[11]. The sterile Muller-Hinton agar plates were prepared. The test organisms were spread over the Muller-Hinton agar plates by using separate sterile cotton swabs. The prepared sterile disc was placed on the surface of the medium at equidistance. Then the plates were incubated at 37°C for 24 h to determine the antibacterial activity of the respective solvent extraction. Gentamicin antibiotic discs (30 mg $disc^{-1}$) were used as positive control and disc with respective solvents (10 µL) were used as negative control. Each extract was treated in triplicate for calculation of mean value.

Statistical analysis: The results were presented as Mean±SD. Data was statistically analyzed using student "t" test. For the calculation of IC_{50}, linear regression analysis was done using GraphPad prism statistical software.

RESULTS AND DISCUSSION

Phenolic and flavonoid contents: The total phenolic content of the methanolic leaf and seed extract, calculated from the calibration curve (R^2 = 0.997) were 24.60±1.68 and 30.20±2.10 mg gallic acid equivalents g^{-1} and the total flavonoid content (R^2 = 0.997) were 148.10±10.30 and 80.9±5.60 mg quercetin equivalents g^{-1} (Table 1). Phenolic compounds have redox properties, which allow them to act as antioxidants[12]. As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity. Flavonoids, including flavones, flavanols and condensed tannins are plant secondary metabolites, the antioxidant activity of which depends on the presence of free OH groups, especially 3-OH. Plant flavonoids have antioxidant activity *in vitro* and also act as antioxidants *in vivo*[13].

Chemical composition by GC-MS analysis: Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. In the GC-MS analysis, 25 bio-active phytochemical compounds were identified in the ethanol extract of leaves (Fig. 1, Table 2) and 29 bio-active compounds were identified in the ethanol extract of seeds (Fig. 2, Table 3). Biological activity of some identified components listed in Table 4.

Table 1: Quantitative analysis of total phenolic and flavonoids content of leaf and seeds

Name of samples	Flavonoids (Expressed as milligrams of quercetin equivalents per gram of dried sample)	Phenol (Expressed as milligrams of gallic acid (GAE) equivalents per gram dried sample)
Leaf	148.10±10.30	24.60±1.68
Seed	80.90±5.60	30.20±2.10

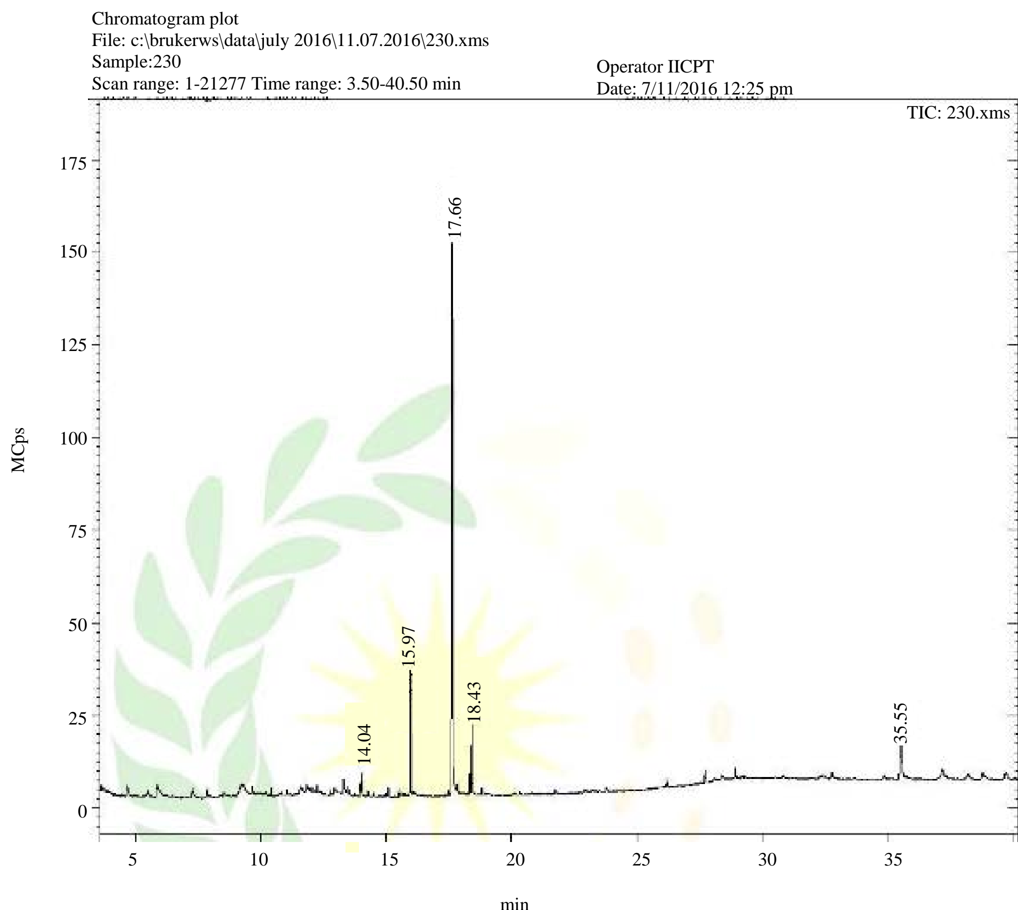


Fig. 1: GC-MS chromatogram of leaves extract

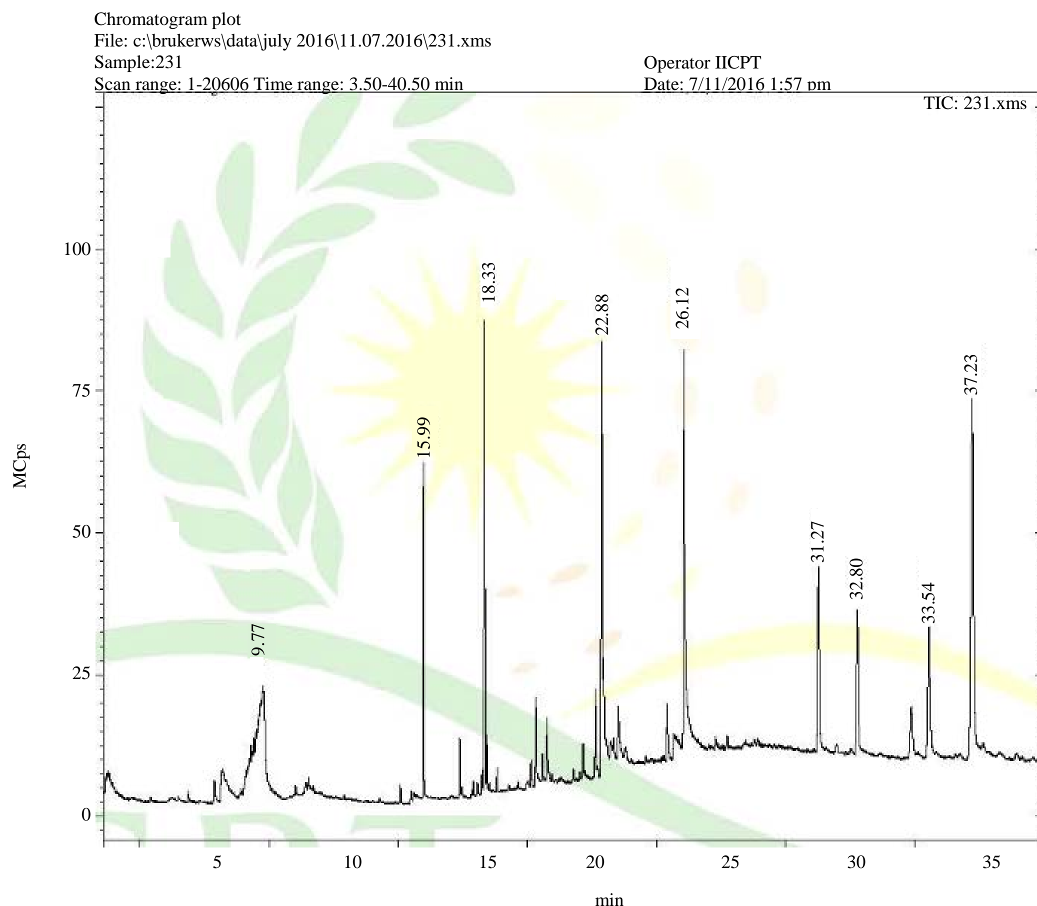


Fig. 2: GC-MS chromatogram of seeds extract

Table 2: Compounds identified in the leaves of *Abutilon indicum*

RT	Name of the compounds	Molecular formula	Molecular weight	Peak area (%)
4.67	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6- methyl-	$C_6H_8O_4$	144	1.90
5.50	11-Oxa-dispiro[4.0.4.1]undecan-1-ol	$C_{10}H_{16}O_2$	168	1.36
5.87	Benzofuran, 2,3-dihydro-	C_8H_8O	120	5.78
7.29	Phenol, 3-methyl-5-(1-methylethyl)-, methylcarbamate	$C_{12}H_{17}NO_2$	207	2.65
7.90	Bicyclo[3.1.1]hept-3-ene-spiro-2,4'-(1',3'-dioxane), 7,7-dimethyl-	$C_{12}H_{18}O_2$	194	0.75
9.26	α-D-Glucopyranose, 4-O-β-D-galactopyranosyl-	$C_{12}H_{22}O_{11}$	342	3.04
9.64	4-(2,4,4-Trimethyl-cyclohexa-1,5-dienyl)-but-3-en-2-one	$C_{13}H_{18}O$	190	0.50
10.20	2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	$C_{14}H_{22}O$	206	0.45
10.42	Menthol, 1'-(butyn-3-one-1-yl)-, (1R,2S,5R)-	$C_{14}H_{22}O_2$	222	0.81
11.61	Spiro-6-(bicyclo[3.2.1]octane)-2'-(oxirane), 7,8- di(hydroxymethyl)-5-methyl-2-isopropyl-	$C_{15}H_{26}O_3$	254	0.35
13.31	2,5,5,8a-Tetramethyl-4-methylene-6,7,8,8a- tetrahydro-4H,5H-chromen-4a-yl hydroperoxide	$C_{14}H_{22}O_3$	238	0.84
14.04	Benz[e]azulene-3,8-dione, 5-[(acetyloxy)methyl]-3a,4,6a,7,9,10,10a,10b-octahydro-3a, 10a-dihydroxy-2,10-dimethyl-,(3aα,6aα,10β,10aβ,10bβ)-(+)-	$C_{19}H_{24}O_6$	348	1.22
15.08	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1.19
15.97	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	12.21
17.66	Phytol	$C_{20}H_{40}O$	296	53.32
18.33	Butyl 9,12-octadecadienoate	$C_{22}H_{40}O_2$	336	1.75
18.43	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	5.61
26.18	Strychane, 1-acetyl-20α-hydroxy-16-methylene-	$C_{21}H_{26}N_2O_2$	338	0.35
27.74	9,12,15-Octadecatrienoic acid, 2,3- bis[(trimethyl)oxy]propyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4$	496	0.73
28.42	1-Monolinoleoylglycerol trimethyl ether	$C_{27}H_{54}O_4$	498	1.07
28.93	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	326	0.93
32.76	Vitamin E	$C_{29}H_{50}O_2$	430	0.85
35.55	Stigmasterol	$C_{29}H_{48}O$	412	0.50
37.20	Androstane-11,17-dione, 3-[(trimethyl)oxy]-, 17-[O-(phenylmethyl)oxime], (3α, 5α)-	$C_{29}H_{43}NO_3$	481	0.65
39.72	Glycine, N-[(3α, 5β)-24-oxo-3-[(trimethyl)oxy]cholan-24-yl]-, methyl ester	$C_{30}H_{53}NO_4$	519	1.19

Table 3: Compounds identified in the seeds of *Abutilon indicum*

RT	Name of the compounds	Molecular formula	Molecular weight	Peak area (%)
3.73	Guanethidine	$C_{10}H_{22}N_4$	198	0.32
7.88	Trans-isoeugenol	$C_{10}H_{12}O$	164	0.52
8.19	5-methoxymethoxyhex-3-yn-2-ol	$C_8H_{14}O_3$	158	0.97
9.77	β-D-Glucopyranose, 4-O-β-D-galactopyranosyl-	$C_{12}H_{22}O_{11}$	342	18.25
11.00	α-D-Glucopyranoside, methyl 2-(acetylamino)-2- deoxy-3-O-(trimethyl)-, cyclic methylboronate	$C_{13}H_{26}BNO_6$	331	0.15
11.52	β-D-Galactopyranoside, methyl 2,6-bis-O-(trimethyl)- , cyclic methylboronate	$C_{14}H_{31}BO_6$	362	0.17
15.09	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.32
15.99	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	5.58
17.37	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	1.19
17.89	Octadecanoic acid, 9,10-epoxy-18-(trimethyl)-, methyl ester, cis-	$C_{22}H_{44}O_4$	400	0.34
18.09	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	0.19
18.33	Butyl 9,12-octadecadienoate	$C_{22}H_{40}O_2$	336	9.31
18.43	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	1.13
18.81	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	0.48
20.15	Fumaric acid, 2-dimethylaminoethyl tetradecyl ester	$C_{22}H_{41}NO_4$	383	0.29
20.33	Palmitic anhydride	$C_{32}H_{62}O_3$	494	2.01
20.58	2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280	0.57
20.75	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	1.41
22.16	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{38}O_4$	354	0.85
22.63	2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)-one, 9-[2-(dimethylamino)ethyl]amino]methyl]octahydro-2,5a-dimethyl-	$C_{19}H_{32}N_2O_3$	336	2.06
22.88	Butyl 9,12,15-octadecatrienoate	$C_{22}H_{38}O_2$	334	11.31
23.53	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl Ester	$C_{35}H_{68}O_5$	568	0.32
25.42	cis-5,8,11-Eicosatrienoic acid, trimethyl ester	$C_{23}H_{42}O_2$	378	1.39
26.12	Butyl 9,12-octadecadienoate	$C_{22}H_{40}O_2$	336	0.79
31.27	γ-tocopherol	$C_{28}H_{48}O_2$	416	6.39
32.80	Vitamin E	$C_{29}H_{50}O_2$	430	5.77
34.87	Campesterol	$C_{28}H_{48}O$	400	3.21
35.54	Stigmasterol	$C_{29}H_{48}O$	412	3.39
37.23	β-sitosterol	$C_{29}H_{50}O$	414	21.33

Table 4: Biological activity of some identified components

RT	Name of the compounds	Biological activity
4.67	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6- methyl-	Antimicrobial, anti-inflammatory, antioxidant
5.87	Benzofuran, 2,3-dihydro-	Neuropathic pain, analgesic and anti-inflammatory
7.29	Phenol, 3-methyl-5-(1-methylethyl)-, Methylcarbamate	Antifungal, anti cancer
9.26	α-D-Glucopyranose, 4-O-β-D-galactopyranosyl-	Preservative
15.97	Hexadecanoic acid, ethyl ester	Antioxidant, hypocholesterolemic nematicide, pesticide, anti-sandrogenic flavor, hemolytic, 5-alpha reductase inhibitor
17.66	Phytol	Antimicrobial, anticancer, anti-inflammatory, diuretic
27.74	9,12,15-Octadecatrienoic acid, 2,3- bis[(trimethyl)oxy]propyl ester, (Z,Z,Z)-	Anti-inflammatory and CNS depressant activity
32.76	Vitamin E	Antiageing, analgesic, anti-diabatic anti-inflammatory, antioxidant, anti-dermatitic, anti-leukemic, anti-tumor, anti-cancer, hepatoprotective, hypocholesterolemic, anti-ulcerogenic, vasodilator, anti-spasmodic, anti-bronchitic, anticoronary
3.73	Guanethidine	Antihypertensive drug
7.88	trans-Isoeugenol	Antioxidant, anticancer
31.27	γ-Tocopherol	Antioxidant, anticancer
34.87	Campesterol	Anti-inflammatory
35.54	Stigmasterol	Anti-inflammatory , anti-osteoarthritic
37.23	β-Sitosterol	Anti-inflammatory, anti-pyretic, anti-arthritic, anti-ulcer,

*Source: Dr. Duke's phytochemical and ethnobotanical databases [Online database]

Corresponding to the peak at RT 17.66 and peak area 53.32% is phytol. The molecular formula and molecular weight of this compound is $C_{20}H_{40}O$ and 296, respectively. Phytol is known to be antimicrobial, anti-cancer, anti-inflammatory, hepatoprotective and anti-androgenic[14]. Guanethidine (Peak area 0.32%) is an antihypertensive drug that reduces the release of catecholamines, such as norepinephrine. Guanethidine is transported across the sympathetic nerve

Table 5: DPPH radical scavenging activity of leaf and seed extract at different concentrations

Parameters	20 (μg mL^{-1})	40 (μg mL^{-1})	60 (μg mL^{-1})	80 (μg mL^{-1})	IC_{50} (μg mL^{-1})
Leaf extract	21.37±1.47	34.55±2.38	58.64±4.03	90.46±6.3	48.94±3.54
Seed extract	19.10±1.33	30.00±2.10	54.10±3.78	85.46±5.95	49.89±3.29
Standard (ascorbic acid)	25.60±2.04	51.26±4.90	78.98±7.11	95.34±7.94	39.22±5.50

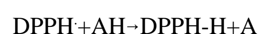
Values were expressed as Mean±SD for triplicate

Table 6: Assay of antibacterial activity

	Zone of inhibition (mm in diameter)			
Bacteria	Control	Standard	Leaves	Seed
Aspergillus niger	-	12	10	-
Bacillus subtilis	-	28	21	19
Escherichia coli	-	26	-	22

membrane by the same mechanism that transports norepinephrine itself (NET, uptake 1) and uptake is essential for the drug's action. Once guanethidine has entered the nerve, it is concentrated in transmitter vesicles, where it replaces norepinephrine. It may also inhibit the release of granules by decreasing norepinephrine and was often used safely during pregnancy. Eugenol (4-allyl-2-methoxyphenol), one of these phytochemicals is a biologically active phenolic component of syzigium aromaticum (cloves). Eugenol has been used traditionally in Asian countries, mainly as a medicinal antiseptic, analgesic and antibacterial agent. Eugenol has been used as a flavoring agent in cosmetics and food products and also plays a role in dentistry as cavity filling cement. Eugenol is said to possess various biological properties like antiviral, antioxidant, anti-inflammatory, etc. At low concentrations it usually acts as an antioxidant and anti-inflammatory agent, whereas at higher concentration act as a pro-oxidant causing increased generation of tissue-damaging free radicals. It has been reported to possess anti-genotoxic activity[15]. The β-vitamin E (RT 32.76) has many biological functions, the antioxidant function being the best known. Vitamin E has also found use as a commercial antioxidant in ultra high molecular weight polyethylene (UHMWPE) used in hip and knee implants to replace faulty joints, to help resist oxidation. Vitamin E also plays a role in neurological functions and inhibition of platelet coagulation. Vitamin E also protects lipids and prevents the oxidation of polyunsaturated fatty acids[16]. Sitosterol (RT 37.23) reduce carcinogen-induced cancer of the colon. It shows anti-inflammatory, anti-pyretic, anti-arthritic, anti-ulcer, insulin releasing and oestrogenic effects and inhibition of spermatogenesis stigmasterol (RT 35.54) is a plant sterol able to bind to chondrocyte membrane and possesses potential anti-inflammatory, anti-osteoarthritic and anti-catabolicproperties. It is thought to have anti-inflammatory effects. It was demonstrated that it inhibits several pro-inflammatory and matrix degradation mediators typically involved in osteoarthritis-induced cartilage degradation. The β-sitosterol is mainly known and used for its anti-inflammatory cholesterol lowering property, easing symptoms of benign prostatic enlargement, reducing risk of cancer and prevention of oxidative damage through its antioxidant activity[17]. Results reveals that most of the predominate compounds were identified in leaf and seed extracts are biologically active molecules. Among the identified phytochemicals, 2,3-dihydro benzofuran and guanethidine were identified first time in this plant. These identified active compounds are considered to be plant defence systems. Some of the chemical compounds were isolated and identified in different crude extracts from *Abutilon indicum* samples those active compounds are currently used as natural antioxidant, antimicrobial agents and in the formulation of different medicines.

Antioxidant activity: The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen-donating ability. The DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule[18]. Extracts of plants are allowed to react with the stable radical, DPPH in methanol solution. The reduction capability of DPPH radicals is determined by the decrease in its absorbance at 517 nm, induced by an antioxidant (AH) after 30 min, as follows:



$$DPPH^{\cdot}+AH \rightarrow DPPH\text{-}H+A$$

The concentration of antioxidant needed to decrease the initial DPPH concentration by 50% (IC_{50}) is a parameter widely used to measure the antioxidant activity[19]. A lower IC_{50} value corresponds with a higher antioxidant power. The leaf and seed extracts showed significant DPPH scavenging activity (IC_{50} values are 48.94±3.54 and 49.89±3.29 μg mL^{-1}) compared with the values obtained for ascorbic acid standard (IC_{50} value 39.22±5.50). All results are shown in Fig. 3 and Table 5.

Antimicrobial activity: The antibacterial properties of methanolic extract of the leaves and seeds of *Abutilon indicum* are presented in Table 6. The extracts had antibacterial activity against both Gram-positive and Gram-negative bacteria with MICs of 10-22 mm in diameter. The leaves extract had the greatest activity against *Bacillus*

subtilis and the least against *Aspergillus niger* (Fig. 4). The seeds extract had the greatest activity against *E. coli* and the least against *Bacillus subtilis.* These results suggest that *Abutilon indicum* is a potential source of broad-spectrum antimicrobial agents. The antimicrobial activity of the extract may be attributed to the high content of flavonoids, which have been reported to be involved in inhibition of nucleic acid biosynthesis and other metabolic processes[20].

The objective of this study is to identify good number of organic chemical compounds in leaf and seed extracts of *Abutilon indicum* might have some ecological and medicinal benefits. This study shows that the identified chemical might be used as a vital source of natural antioxidant for food and pharmaceutical industry. Results suggest that *Abutilon indicum* is a potential source of antioxidant and antimicrobial agents and could be used as a natural antioxidant and preservative in food and non-food systems. Further phtyochemical analysis is required to isolate the elements of the plant that show a broad spectrum of pharmacological activity.

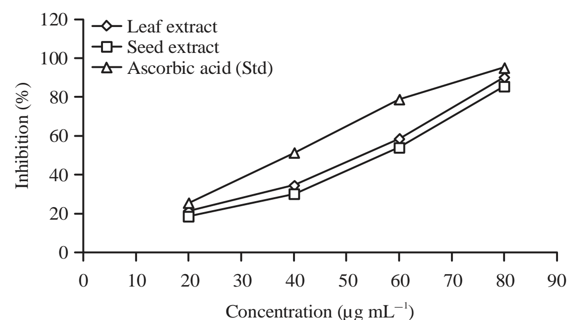


Fig. 3: DPPH radical scavenging activity of leaf and seed extracts at different concentrations

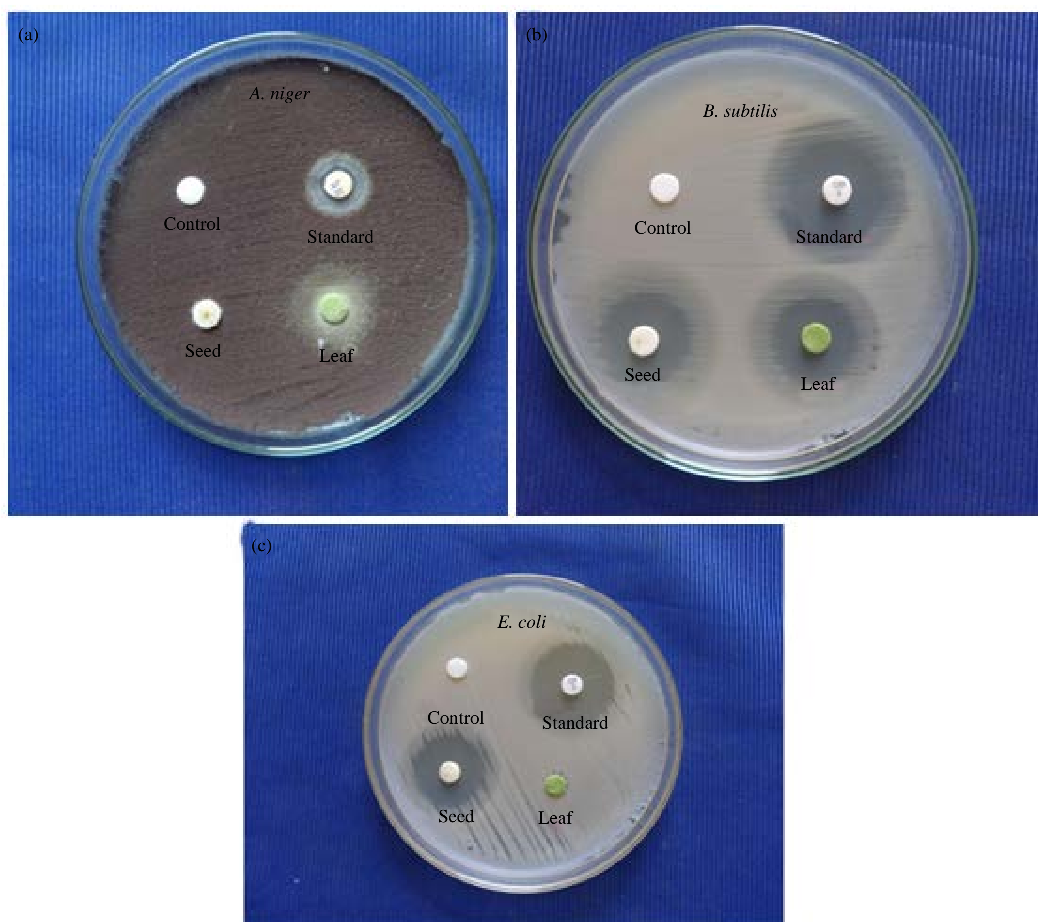


Fig. 4(a-c): Antimicrobial activity of *Abutilon indicum* leaf and seed extracts on pathogenic microorganisms, (a) *A. niger*, (b) *B. subtilis* and (c) *E. coli*

CONCLUSION

The present study provided the potential antimicrobial and antioxidant properties of *Abutilon indicum* leaf and seed. The radical scavenging activity and other complementary assays are also in good correlation. In the GC-MS analysis, 25 phytochemicals were identified in the leaves extract and 29 phytochemicals were identified in the seeds extract. Moreover, the potency of the constituents such as trans isoeugenol and sterols could provide a chemical basis for some of the health benefits claimed for *Abutilon indicum*. The present results of this study do not reveal that which chemical compound is responsible for different activity. To the best of our knowledge this is the first report to investigate *in vitro* antioxidant activity as well as the chemical composition of leaf and seed extracts of *Abutilon indicum* by GC-MS. Hence, the whole plant could be of use as a good source of antioxidant. Further studies are needed for the isolation and identification of individual compounds from the plant crude extracts *Abutilon indicum* also *in vivo* studies are needed for better understanding of their mechanism of action as antioxidant.

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