

ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF LEAF EXTRACT OF ABUTILON INDICUM PLANT

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1. ABSTRACT

The leaf extract of Abutilon indicum plant of saponin shows antioxidant activity by the Gas chromatography-mass spectroscopy (2, 2-diphenyl-1-picrylhydrazyl), and Chloroform, ethanol and aqueous extracts of the leaves of Abutilon indicum were investigated for antibacterial against salmonella typhi, Escherichia coli, and Staphylococcus aureus ect. Among the virus extract maximum antibacterial activity was by ethanol extract followed by chloroform extract while aqueous extract, showed no activity.

- **KEYWORD:** Abutilon indicum plant, leaf extract, 2, 2-diphenyl-1-picrylhydrazyl, Antioxidant assay, Traditional medicine value, Pharmacological Evidences, Chemical Constituents.

2. INTRODUCTION

The plants are integral parts of Nature. Medicinal plants have an almost endless variety of uses to human beings.^[1] The Medicinal plants are used since from billions century as remedies for human diseases and for disorders. Many Plants contain numerous phytochemicals with large therapeutic effects and the plants are more natural, beneficial, and safe as compared to synthetic drugs such as a drug which made from chemicals. One of the best and most useful medicinal plant is Abutilon indicum.^[2] The Abutilon indicum comes under the family Malvaceae, and this plant is commonly called as country mallow in (English), Titti Gidutingi Hettukisu Hetutti Shrimudri Urki in (Kannada), Petari in (Marathi), Kanghi (Hindi, and Atibala (Sanskrit).^[3] The A. Indicum plant is a hairy under – shrub with golden yellow flowers, found in hotter parts of India and this plant is erect, woody, and shrubby.^[4] Various diseases are cause by pathogenic microorganisms, such as bacteria,

viruses, parasites, or fungi, sometimes diseases can spread widely by directly or indirectly from one person to another.

- The shops contain saponins, flavonoids, alkaloids, hexoses, n- alkane fusions (C22- 34), alkanols, β - sitosterol, vanillic, p- coumaric, caffeic acid, fumaric acid, sesquiterpene lactones (Alantolactone and isoalantolactone) and amino acids. The factory *A. indicum* contains 0.15 of essential oil painting which substantially consists of α - pinene, caryophyllene, caryophyllene oxide, endosomal, farnesol, borneol, geraniol, geranyl acetate, rudiments and 18- cineole along with number of other minor ingredients.^[8,9,10 and 11]
- *Indicum* leaves has shown the presence of amino acids, glucose, fructose, and galactose.^[12] This factory has biologically active secondary metabolites which confer significant pharmacological and medicinal parcels to this factory.^[13]
- Among the colorful secondary metabolites similar as phenols, alkaloids, and flavonoids are set up in this factory, saponins have an enormous significance in pharmaceutical assiduity. Saponins are typically nonvolatile, and they're face active composites which are extensively distributed in nature.^[14]
- Saponins are secondary metabolites which are distributed along the factory area. Saponin acts as a chemical hedge or similar as a guard in the factory defense system to encounter the pathogens. Saponins are set up in factory apkins which are substantially vulnerable to fungal or bacterial attack. Saponins are dangerous but are answerable in water.^[15]

• **Phytochemicals**

phytochemical disquisition of the factory revealed the presence of chemical ingredients videlicet luteolin, chrysoberyl, apigenin 7- O- beta rhamnopyranosyl, quercetin, triacontanoicacid, magazine, methyl stigmasterol, glucopyranosideetc.^[16] Bioactivity guided insulation of *Abutilon indicum* yielded eugenol(4- allyl- 2- methoxyphenol), which was set up to retain significant analgesic exertion^[17] in acetic acid convinced writhing test. In some places, juice from the leaves of the factory is used in combination with the liquid excerpt of *A. cepa* to treat hostility. As a part of our continuing study on chemical and natural characterization of different shops, attempt was made this time to probe the antimicrobial

exertion of *A. indicum* against different Gram-positive, Gram-negative bacteria and fungi species.^[18,19]

- Numerous bioactive compounds have isolated from different part of the plant. Whole plant contains caffeic acid, fumaric acid, β -sitosterol, vanillic acid, p-coumaric acid, abutilon A, (R)-N-(1'-methoxycarbonyl-2'-phenylethyl)-4-hydroxybenzamide, hydroxybenzoic, galacturonic, p- β -D-glycosyloxybenzoic etc. Oil of the plant consists of farnesol, bornyl acetate, geranyl acetate, element, and α -cineole. Upstanding part contains vanillic acid, caffeic acid, p-hydroxybenzoic acid, β -sitosterol, fumaric acid, p-coumarin, p- β -D-glucosyloxybenzoic acids, gluco-vanilloyl glucose, amino acids like threonine, serine, leucine, aspartic acid, histidine etc. Leaves contain terpenes, hydrocarbon, flavonoids, amino acids, ketone, aldehyde, adipic acids like stearic, palmitic, linoleic, oleic etc. Root contains endospermol, α -pinene, caryophyllene, caryophyllene oxide. Flower contains flavonoids like apigenin 7-O-beta-glucopyranoside, quercetin 3-O-beta-glucopyranoside, luteolin, chrysoeriol, luteolin 7-O-beta-glucopyranoside, chrysoeriol 7-O-beta-glucopyranoside, quercetin 3-O-alpha-rhamnopyranosyl (1 \rightarrow 6)-beta-glucopyranoside. Fruits contain flavonoids and alkaloids. Seed contains water-soluble galactomannan, cis-12,13-epoxyoleic (veronica) acid, 9,10-methylene octadec-9-enoic (sterculia) acid, as well as 8,9-methyleneheptadec-8-enoic (malvalic) acid.^[20]

- Operations of antibacterial compounds in the food industry, antimicrobial compounds have implicit to use as bio-preservatives and bio-insecticides and also they've a potentiality for developing genetically modified crop plants with increased pest resistance. Operation of plant antimicrobial compounds for controlling growth of foodborne pathogens is having the range of exertion against the microorganisms.^[21] Antibacterial agent depends on its use and its effectiveness. The US Food and Drug Administration regulate antibacterial detergents and antibacterial substances.^[22]

• Uses in traditional Medicine and Reported activities

As laxative, emollient, and for the treatment of piles 3,4. The folk practitioners also use this plant for curing blood dysentery, fever, allergy, and as aphrodisiac.^[23] Antibacterial and antifungal activities have been reported for the roots.^[24]

- **Previously isolated class of constituents:** Alkaloids, Flavonoids, Sterols, Triterpenoids, and glycosides.^[25]

Abutilon indicum plant: Image

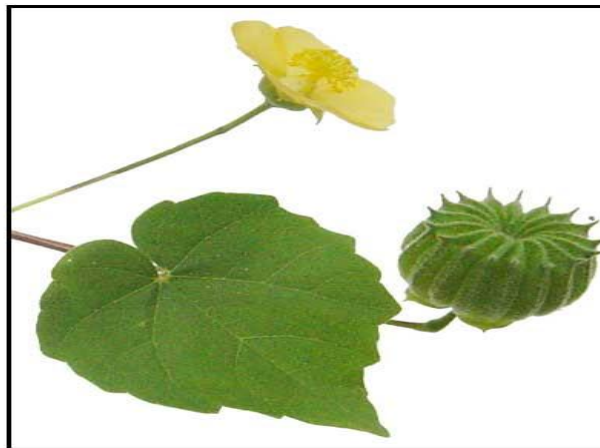


Fig. 1

- **Phyto-Pharmacological evidence of abutilon indicum**
- **Anti-inflammatory and anti-proliferative activity**
- **Antioxidant and antimicrobial activity**
- **Anticancer activity**
- **Wound healing activity**
- **Anti-Arthritic Activity**
- **Analgesic and Sedative property**
- **Anti-diabetic Activity**
- **Anti-diarrheal activity**
- **Anti-convulsant activity**
- **Larvicidal activity**
- **Anti-asthmatic activity**
- **Diuretic activity**
- **Immunomodulatory activity**
- **Anti-estrogenic activity**

Anti-inflammatory and Anti proliferative activity: The Anti-inflammatory and Anti proliferative activity of ethanolic leaf extract of Abutilon indicum for potential chemo preventive agent has been evaluated by Kuladhar et al. The ethanolic leaf extract of A.

indicum is revealed good anti-inflammatory activity (IC₅₀: 8.89 µg/mL) based on 5-Lipoxygenase (5-LOX) inhibition assay.^[26]

Antioxidant and Antimicrobial activity: Dharendra Kaushik et al designed to evaluate the antioxidant and antimicrobial activities of chloroform fraction of alcoholic extract of whole plant of *Abutilon indicum* extract was screened for antioxidant and free radical scavenging effects at various concentrations.^[27]

Anticancer activity: Srikanth P et al study medicinal plants namely *Abutilon indicum* and *Blume mollies* were chosen to screen for potential antioxidant properties and cytotoxic activity. The extract was also screened to assess antioxidant activity using FRAP, 1, 1-Diphenyl-2-picrylhydrazyl [DPPH] radical scavenging activity and Nitric Oxide radical inhibition estimated using Griess Illusory reaction with slight modification. These extracts show antioxidant properties as well as inhibitory effect on cancer cells with the increased concentration and duration.^[28]

Wound healing activity: P Ganga Suresh et al evaluated the wound healing activity of *Abutilon indicum* Linn. All the extracts were obtained subjected to phytochemical studies. The progressive changes in the wound area were monitored by tracing the wound margin every day. From the result, it is concluded that the petroleum ether extract of “*Abutilon indicum*” Linn had greater wound healing activity than the Ethanolic extract.^[29]

3. Scope

Abutilon indicum is an herbal plant. This plant belonging to the family of (Malvaceae). This plant has been extensively used as a traditional medicine as laxative, emollient, analgesic, anti-diabetic, and other diseases. This herbal plant is better than synthetic drug from chemical, the herbal plant has no side effect, *A. indicum* plant have all pharmacological activity.

4. Methodology

- 1. Collection:** The plant leaves of *Abutilon indicum* (Malvaceae) were collected from in India.
- 2. Saponin extraction:** *A. indicum* leaves were collected from Vellore quarter. The leaves were washed with the distilled water for about two to three times, and also, it was cut into small pieces and shade dried for several days. The leaves were predicated using motor

and pestle and stored in an watertight vessel. The powered leaves were mixed in methanol and acetone in the rate 15(V/ V) to prize saponins. 10 ml of the detergent was added to 1 g of the power, and it was allowed to soak in the detergent for about 24 hrs. also, the admixture was subordinated to centrifugation at 2000 rpm for 10 twinkles at 4 °C. The admixture was filtered using sterile Whatmans sludge paper number 1, and also, detergent was filtered again using hypesludge containing 0.2 µ cellulose acetate membrane.^[30]

3. **Gas chromatography-mass spectrometry:** (GS- MS) analysis GC was performed in VIT sophisticated logical lab. 5 mg of crude saponin excerpt (CSE) was dissolved in 1 ml of methanol and was anatomized in GC- MS. The instrument used was GC- MS JEOL (GCMATE II GC- MS, Agilent Technologies 6890N Network GC system for GC). The column(HP5) was fused silica 50 m ×0.25 mm. Analysis conditions were 20 twinkles at 100 °C, 3 twinkles at 235 °C for column temperature, 240 °C for injector temperature, helium was the carrier gas and split rate was 54. The sample(1 µl) was faded in a split less injector at 300 °C. Run time was 30 twinkles. The composites were linked by GC coupled with MS. The molecular weight and structure of the composites were caught on by matching with reference composites available in the National Institute Standard and Technology.^[31]
4. **2, 2-diphenyl-1-picrylhydrazyl (DPPH) reduction assay:** DPPH solution of 1 mg/ml concentration dissolved in methanol was used for this study. 200 µl of DPPH solution was added to all the test tubes. 100 µl of methanol was used as blank. 100 µl of ascorbic acid (1 mg/ml) was used as standard, 100 µl of CSE at varying concentrations (0.25 mg/0.1 ml to 2.5 mg/0.1 ml) was used as test. These tubes were incubated then incubated in the dark region for about 30 minutes. Then, absorbance was observed in an ultraviolet spectrophotometer at 517 nm. The radical scavenging activity (inhibition of DPPH free radical in percent) was calculated using the following formula:

$$\underline{\% \text{ inhibition} = ([\text{Ac}-\text{At}]/\text{Ac}) * 100.}$$

Where **Ac**: Absorption of the blank sample; **At**: Absorption of the test sample. Percentage of inhibition concentration was calculated from the graph plotting inhibition percentage against extract concentration.^[32]

- 5. Antioxidant assay (DPPH reduction assay):** CSE demonstrated increasing percentage of antioxidant activity, with lowest inhibition of 15.7% at 0.25 mg/ml concentration, to a maximum of 96.17% inhibition at 2.5 mg/ml concentration. Fig. 2 shows the graphical representation of the DPPH assay results.

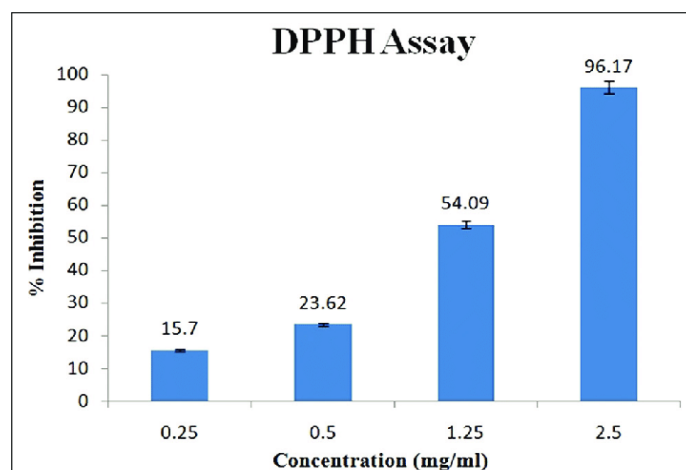


Figure 2

- 6. Antibacterial activity:** The antibacterial activities of these extracts were tested against *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi*. The antibacterial sensitivity pattern for the isolates was studied by the disc diffusion method.^[33,34]

- Methods**

Muller Hinton agar media was prepared, and the plates were swabbed with 24hrs. cultures of separate bacteria grown in nutrient broth overnight. Sterile discs of 6 mm periphery were saturated with 25µl of each excerpt independently. Blank slice saturated with DMSO was used as negative control and discs of chloramphenicol (30 µg) as positive control. The plates were also incubated at 37°C for 24 hrs. Inhibition was recorded by measuring the periphery of inhibition zone at the end of 24h. Each trial was repeated in trifectas. 6 mm periphery were saturated with 25µl of each excerpt independently. Blank discimpregnated with DMSO was used as negative control and discs of chloramphenicol (30 µg) as positive control. The plates were also incubated at 37°C for 24 hrs. Inhibition was recorded by measuring the periphery of inhibition zone at the end of 24h. Each trial was repeated in trifectas.

Table 1: Antibacterial activity of the leaves of *Abutilon indicum* against bacterial pathogens by disc diffusion method.

Name of the bacterial pathogen	Inhibition zone(mm)				
	DMSO* (Control)	Chloroform extract	Ethanol extract	Aqueous extract	Chloramphenicol (control)
<i>Bacillus subtilis</i>	-	13	14	-	27
<i>Staphylococcus aureus</i>	-	17	25	-	25
<i>Klebsiella pneumoniae</i>	-	8	14	-	30
<i>Pseudomonas aeruginosa</i>	-	15	25	-	23
<i>Escherichia coli</i>	-	15	17	-	22
<i>Salmonella typhi</i>	-	20	18	-	22

5. RESULT AND DISCUSSION

In vitro antioxidant activities of powders obtained from different extracts of *A. indicum* L. through superoxide anion generation by xanthine-/xanthine oxidase assay, linoleic acid peroxidation assay and by DPPH photometric assay were given in Table. 1. It appears from the table that powder obtained from different solvent extractions of *A. indicum* L. leaves had in vitro antioxidant activity, but maximum activity was found in the ethanol extract followed by ethyl acetate extract. Inhibitions in xanthine oxidase, linoleic acid peroxidation and DPPH in ethanol extract were found 92.7%, 91.4% and 96.7% respectively. Results were comparable with quercetin where inhibitions in xanthine oxidase, linoleic acid and DPPH came 100%, 85.4% and 96.4% respectively.^[35,36,37] It is also known that antioxidant activity of a plant may vary with nature of extraction solvents. Dentet al. used ethanol, methanol, and acetone as extraction solvents of dry sage leaves and observed that ethanol extract had maximum antioxidant activity.^[35] Qassim et al. studied effect of extraction solvents on polyphenols and antioxidant activity of medicinal halophytes and noted that aqueous methanol extracts of coastal halophytes had comparatively higher antioxidant activity, chloroform extract was moderately active against all organisms tested except for *Klebsiella pneumoniae* (8mm). Aqueous extract was found to possess no activity against any of the bacteria tested. The results of the plant extract tested against various bacteria were in concordant with the positive control (chloramphenicol).^[37,38]

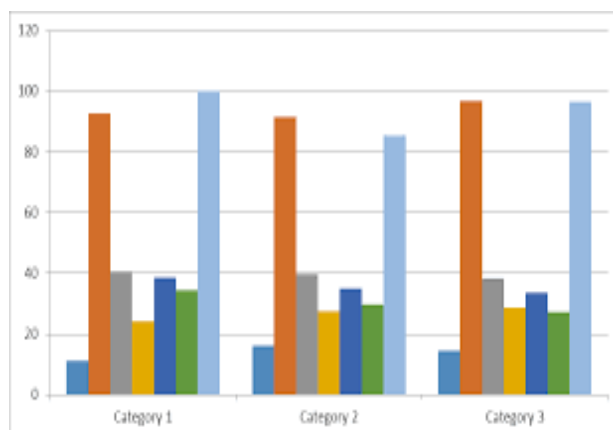


Figure 1: Showing % inhibition of xanthine oxidation, linoleic acid Peroxidation and Scavenging capacity of DPPH by different solvent extract of *A. indicum* L. leaves.

- Category 1: Xanthine oxidase (% inhibition)
- Category 2: Linoleic acid peroxidation (% inhibition)
- Category 3: DPPH (% inhibition)

- Petroleum ether
- Ethanol
- Ethyl acetate
- Methanol
- Acetone
- Chloroform
- Quercetin

Secondary metabolites of plants are usually responsible for their biological activities. It is known that season has significant effect on synthesis of secondary metabolite in plants thereby changing their biologic activity.^[39,40] It is, therefore, worth to investigate the seasonal effect on in vitro antioxidant activity of *A. indicum* leaves. Work in this direction is now in progress in our laboratory.

6. SUMMARY AND CONCLUSION

Grounded on the results attained from this work, it's apparent that the CSE from *A. indicum* leaves has implicit for natural operations as an antibacterial agent and as an antioxidant agent. Although farther in vivo studies and toxin studies are needed to confirm this report, a significant antibacterial exertion like that of the standard antibiotic, with a significant *A. indicum* leaves. *Abutilon indicum* have numerous further pharmacological parcels like,

hepatoprotective, crack mending, and immunomodulatory, analgesic, antimalarial, antimicrobial, hypoglycemic exertion. The main chemical ingredients are carbohydrates, steroids, glycosides, flavonoids, tannins, and Phenolic composites. Hence this review composition, trouble has been taken to collect and collect the details notes on *Abutilon indicum* which will be useful to the society to venture into a field of indispensable systems of drug.

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