Study of *Abutilon theophrasti* Stem Extract for its Antibacterial Activity

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Abstract

This work was performed to examine the antibacterial activity of stem extract of Abutilon theophrasti. A simple maceration process was utilized to obtain the crude extract with methanol as a solvent. The dried extract was done by using a rotary evaporator. The bacterial strains of gram-positive Bacillus cerus (MTCC-3381), Staphylococcus aureus (MTCC-443) and Staphylococcus epidermidis (MTCC-1771)) and gram-negative Klebsiella pneumonia (MTCC-7028), Escherichia coli (MTCC-10619) and Proteus vulgaris (MTCC-2412)) were chosen. Nalidixic acid (30 µg/mL) was used as a standard drug.

The crude extract was diluted by using DMSO to obtain the extracts with the concentrations of 100 µg/mL, 250 µg/mL and 500 µg/mL. The disk diffusion method was employed in this study. All inhibitions were observed by the concentration-dependent. The maximum inhibition zone of 18 mm was obtained for Staphylococcus aureus and Klebsiella pneumonia at 500 µg/mL. Escherichia coli had lower inhibition with an optimum zone of inhibition of 7 mm at 500 µg/mL.

Keywords: *Abutilon theophrasti*, methanol, disk diffusion method, stem extract, antibacterial activity.

Introduction

Since long ago, the predecessors have fulfilled the basic needs of human beings especially for the treatment of numerous diseases. Plants present a fundamental task in the establishment of the absolute traditional medicine tract. The History of Egyptian Medicine is an outpost in medicine dated about 2900 BCE, but the famous medical document is "Ebers Papyrus" dated about 1500 BCE.[1] This document talks about 700 medicines originating from plants. Until now, several herbal medicines are still used in the treatment of the illness such as cough, cold, headache etc.

Furthermore, the plants present a significant task as the origins for antibacterial ingredients from ancient times. Traditional medical practicians (Hakeem) utilized the plants to medicate numerous infectious illnesses.[2] Plants have many phytochemical compounds such as tannins, alkaloids, terpenoids and flavonoids that have contributions for antibacterial, anthelmintic, anticancer, antioxidant, anti-inflammatory and many others.[3] There is no hesitation that the plant kingdom still has many plant species with no reported specific medicinal content.

Abutilon theophrasti (Velvet plant) is a yearly plant that only grows from its seeds. The plant is native to Asian tropical countries. It can be found in high-altitude regions in the Kashmir valley.[4] Some other regions include Kupwara, Sopore and Qazi-Gund.[5] Its stem grows up to 5 m tall. The stem is branched in the upper part with velvety and smooth hairs.[6] *Abutilon theophrasti* is extensively utilized as traditional Chinese medicine. Diuretic, expectorant, anti-inflammatory, anthelmintic, analgesic, aphrodisiac, demulcent and emollients are associated with the *Abutilon theophrasti*.[7] The seeds conceive of mucilage and fatty acid. Therefore, they are utilized to medicate the constipation.[8] Phytochemical constituents of stem extract contain phenols, polyphenols and tannins.[9]

In the present study, their function as antibacterial activity from the stem extract of *Abutilon theophrasti* was conducted to expand their utilization as a medicinal plant.

Material and Methods

Collection of Plant Material: *Abutilon theophrasti* (Velvet leaf) was obtained from Lower Munda village, Qazigund district, Jammu & Kashmir, India. The longitude was 75.20˚ and latitude was 33.56˚. The Velvet plant was recognized and recorded at Herbarium central for Biodiversity and Taxonomy, University of Kashmir, India. A voucher specimen with No. 2113-KASH was reversed in Herbarium central for Biodiversity and Taxonomy, University of Kashmir.

Extraction: The collected plant stem was cleaned with tap water followed by drying in shade. The dried stem was completely extracted with the methanol solvent at embient temperature. The rotary evaporator was used to evaporate the extract to remove and separate the solvent from the extract. The extract was concentrated to make a dried extract using a rotary evaporator.

Test Culture: For the evaluation of the antibacterial activity, six bacterial strains of both gram-positive including *Staphylococcus aureus* (MTCC-443, SA), *Bacillus cerus (*MTCC-3381, BC) and *Staphylococcus epidermidis* (MTCC-1771, SE) and gram-negative including *Escherichia coli (*MTCC-10619, EC), *Klebsiella pneumonia* (MTCC-7028, KP) and *Proteus vulgaris* (MTCC-2412, PV) were used.

Evaluation of Antibacterial Activity: A disk diffusion method was performed for the assessment of antibacterial activity.[10] Muller-Hinton agar (MHA) was chosen as a medium. 70% ethanol, autoclave and ultraviolet radiation were taken to meet the sterilization requirements. The fresh bacterial culture was employed for the inoculum preparation. A suspension of McFarland standard no. 0.5 was prepared. The bacterial culture was wiped on the sterile agar plate surface. The dried plant extract was re-suspended to make the concentration of 100, 250, 500 µg/mL in dimethyl sulfoxide (DMSO) and dissolved by sonication. 5 mm sterile discs were fulfilled with 50 µL extract and put on the inoculated bacterial agar plate surface.

DMSO served as a negative control and nalidixic acid (30 µg/mL/disc) served as a positive control. The bacterial agar plates were kept in incubation overnight at 37˚C. The inhibition zone diameter was measured in millimeters (mm). Three replicated measurements were performed for each sample and average values were counted.

Results and Discussion

The successful isolation of phytochemical compounds from the plant materials is mostly conditional on the solvent type used in the extraction procedure. Methanol is the best-known solvent to extract polar compounds and some non-polar compounds. In this study, methanolic extract of plant stem displayed different inhibition zones against different selected bacterial pathogens (Fig. 1). The results of the inhibition zone of *Abutilon theophrasti* stem extract against some pathogenic bacteria were exhibited in table 1.

The maximum inhibitory concentration was shown for both gram-positive (SA) and gram-negative (KP) bacteria. A maximum inhibition zone of 18 mm was obtained for SA and KP at 500 µg/mL concentration of the extract. For some pathogenic species, 250 µg/mL extract exhibited an inhibition zone of 15 mm. However, the inhibition zones of 10 mm were shown at 100 µg/mL concentration.

PV bacterial strain was also inhibited by the stem extract. 100 µg/mL, 250 µg/mL and 500 µg/mL of concentration showed an inhibition zone of 6 mm, 10 mm and 15 mm respectively. On the other hand, BC bacterial strain showed the inhibition zones of 8 mm, 12 mm, 13 mm with the same selected concentrations respectively. The inhibition zones for SE bacterial strain at 100 µg/mL, 250 µg/mL, 500 µg/mL of extract concentration were 9 mm, 10 mm and 12 mm respectively. Moreover, EC bacterial strain owned low inhibition by stem extract with the inhibition zones of 6 mm, 6 mm and 7 mm with the concentration of 100 µg/mL, 250 µg/mL, 500 µg/mL respectively. The nalidixic acid at 30 µg/mL as a standard drug displayed the inhibition zones of 25 mm, 19 mm, 20 mm, 22 mm, 25 mm and 20 mm for SA, KP, PV, BC, SE and EC respectively.

The antibacterial assay was examined for six bacterial strains (SA, BC, EC, SE, KP and PV). Methanol was used as a solvent in this study to make a crude extract. Three extract concentrations (100 µg/mL, 250 µg/mL and 500 µg/mL) were made. Nalidixic acid at 30 µg/mL was used as a standard drug.

Methanol is having a high polar index (5.1), hence it is a good solvent to extract polar compounds. It also tends to extract some non-polar compounds. Keeping this in mind, methanol was chosen as a solvent for the extraction. Some information about the phytochemistry of *Abutilon threophrasti* reveals the presence of phenol, polyphenols, terpenes, triterpenes, alkaloids, tannins, roseoside and cholesterol.

Literature gives sound information about the phytochemistry of *Abutilon theophrasti*, reveals the presence of phenols polyphenols, terpenes, triterpene, alkaloids, tannins, roseoside, cholesterol.[11–13] The present antibacterial investigation can attribute to these phytochemicals. Such compounds can inhibit the growth of bacteria, protein and forming complex compounds against extracellular proteins that disturb the bacterial cell membrane integrity.

Table 1
Inhibitory zone diameter of *Abutilon theophrasti* stem extract against pathogenic bacteria

Concentration (µg/mL)	Maximum of Inhibitory Zone (mm)					
	SA	KP	PV	BC	SE	EC
500	18	18	15	13	12	7
250	15	15	10	12	10	6
100	10	10	6	8	9	6
DMSO	0	0	0	0	0	0
NA	25	19	20	22	25	20

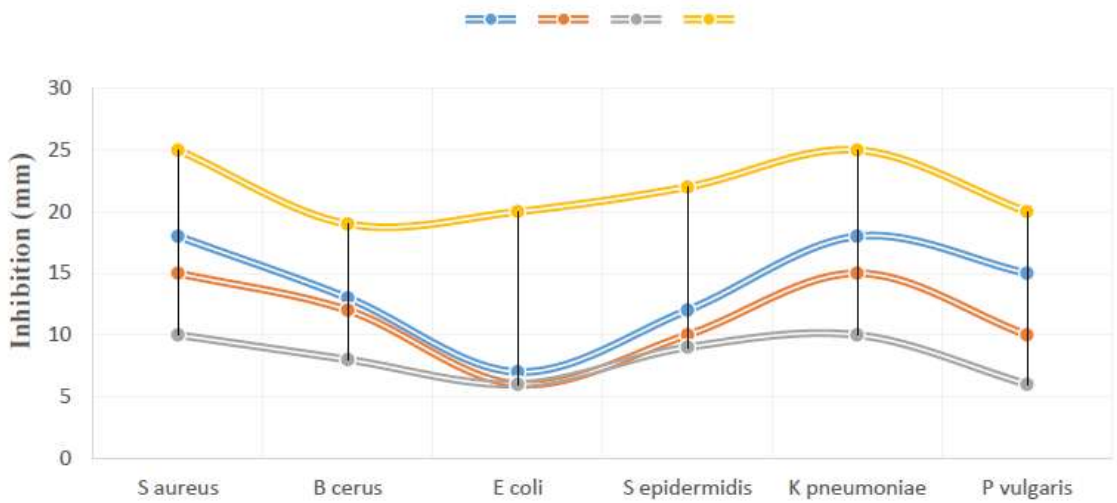

Figure 1: Antibacterial activity of *Abutilon theophrasti* stem extract

Tian and coworkers[14] observed the antibacterial activity of flavonoids from *Abutilon theophrasti* leaves against SA, *Salmonella* and EC which confirmed a better and perfect antibacterial activity supporting this study. Besides, the methanolic root extract of *Abutilon theophrasti* has antibacterial activity and the work predominantly supports this study.[5]

Conclusion

This work confirms that the crude stem extract of *Abutilon theopharsti* has the potential antibacterial activity for several gram-positive bacterial strains (BC, SA and SE) and gram-negative (KP, EC and PV) bacterial strains with the possibility of some phytochemical constituents with the antibacterial contribution.

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References

1. Newman D.J., Cragg G.M. and Snader K.M., The influence of natural products upon drug discovery, *Nat. Prod. Rep.*, **17**, 215–234 **(2000)**

2. Khan K.U. et al, Exploration and local utilization of medicinal vegetation naturally grown in the Deusai plateau of Gilgit, Pakistan, *Saudi J. Biol. Sci.*, **25**, 326–331 **(2018)**

3. Miliauskas G., Venskutonis P.R. and Van Beek T.A., Screening of radical scavenging activity of some medicinal and aromatic plant extracts, *Food Chem.*, **85**, 231–237 **(2004)**

4. Hassan M., Habib H., Kausar N., Zargar M.A. and Mir R.A., Evaluation of root and leaf of Abutilon theophrasti medik for antifungal activity, *Asian J. Pharm. Clin. Res.*, **9**, 75–77 **(2016)**

5. Hassan M., Mir R.A. and Habib H., Evaluation of antibacterial activity of Abutilon theophrasti medic, *Int. J. Adv. Res. Sci. Eng.*, **7**, 1519–1524 **(2018)**

6. Warwick S.I. and Black L.D., Genecological variation in recently established populations of Abutilon theophrasti (velvetleaf), *Can. J. Bot.*, **64**, 1632–1643 **(1986)**

7. Khadabadi S.S. and Bhajipale N.S., A review on some important medicinal plants of Abutilon spp, *Res. J. Pharm. Biol. Chem. Sci.*, **1**, 718–729 **(2010)**

8. Dinu M. et al, Proximate composition and some physico-chemical properties of abutilon theophrasti (velvetleaf) seed oil, *Rev. Chim.*, **61**, 50–54 **(2010)**

9. Tian C., Wang M., Liu X., Wang H. and Zhao C., HPLC quantification of nine chemical constituents from the five parts of Abutilon theophrasti Medic, *J. Chromatogr. Sci.*, **52**, 258–263 **(2014)**

10. Rios J.L., Recio M.C. and Villar A., Screening methods for natural products with antimicrobial activity: A review of the literature, *J. Ethnopharmacol.*, **23**, 127–149 **(1988)**

11. Saranraj P., Sivasakthi S. and Deepa M.S., Phytochemistry of pharmacologically important medicinal plants – A Review, *Int. J. Curr. Res. Chem. Pharm. Sci*, **3**, 56–66 **(2016)**

12. Tian C., Wang M., Liu X., Wang H. and Zhao C., HPLC quantification of nine chemical constituents from the five parts of Abutilon theophrasti Medic, *J. Chromatogr. Sci.*, **52**, 258–263 **(2014)**

13. Mamadalieva N.Z., Sharopov F., Girault J.P., Wink M. and Lafont R., Phytochemical analysis and bioactivity of the aerial parts of Abutilon theophrasti (Malvaceae), a medicinal weed, *Natural Product Research*, **28**, 1777–1779 **(2014)**

14. Tian C. et al, Abutilon theophrasti medic, Episperms as a total flavonoids fraction for pharmaceutical applications: In vitro antioxidant, antibacterial, anti-inflammatory activities, extraction technology and HPLC-MS profiles, *Ind. Crops Prod.*, **134**, 100–106 **(2019)**.

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