

SEPARATION AND IDENTIFICATION OF BIO-ACTIVE COMPOUNDS FROM *ABUTILON INDICUM*.

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Abstract: Herbal medicines play an important role in primary health care in many developing countries. It is estimated that there are more than 45,000 species of medicinal plant present in our country. One such medicinal plant is *Abutilon indicum*. It is erect, woody and shrubby plant found commonly in tropical regions. The whole plant is used as curative for many diseases. The present study aims to determine the phytochemicals by qualitative and quantitative analysis of methanolic leaves of *Abutilon indicum*. This study also involves in separating and identifying phytochemicals using FTIR analysis and UV spectroscopy. The qualitative analysis of the leaves showed the presence of alkaloids, steroids, terpenoids, flavonoids, saponins, phenols, tannins, aminoacids, carbohydrates and reducing sugar. The quantification of the compounds like alkaloids, flavonoids and phenols were estimated. FTIR analysis shows the presence of three major peaks and UV Spectrometry shows the presence of one major peak and exhibited the presence of a major component in the methanolic extract of the leaves. The result confirms that the leaves of *Abutilon indicum* contains significant phytochemicals and are helpful to prepare natural drugs in scientific research.

Key words: *Abutilon indicum*, FTIR, Medicinal plant, Phytochemical screening, UV spectrometry

I. INTRODUCTION

Medicinal plants are the gift to human beings to lead a disease free healthy life (Archana sharma, *et. al.*, 2013). They play a significant role in maintaining our human health. Plant medicines are used worldwide in the traditional treatment for many diseases (Vyas, *et. al.*, 2011). The medicinal plants are useful for healing as well as for curing human diseases due to the presence of the phytoconstituents (Vijaya Packirisamy, *et. al.*, 2014). It is estimated that there are more than 45,000 species of medicinal plants present in our country. They are possessed to have various properties like antioxidant, anti inflammatory, anti cancer etc. Of these only 60% of plants are officially used by practitioners and 40% of plants are used traditionally. One such traditionally used plant is *Abutilon indicum*.

Abutilon indicum is a perennial erect shrub. It is an herbaceous weed belonging to the family Malvaceae and is distributed throughout the tropical regions (Archana sharma, *et. al.*, 2013). It is commonly known as "Thuthi" in Tamil and "Country Mallow" in English (Saini, *et. al.*, 2015). It is used as medicinal plant since ancient times. The whole plant and different parts of the plant are used to cure many human ailments. The leaves of this plant are used to cure ulcer, Inflammation, Rheumatism, Syphilis of penis, Piles and to relieve leg pains, Inflammation of bladder, Catarrhal bilious diarrhoea, Bronchitis, Gonorrhoea, Fevers (Saini, *et. al.*, 2015). It is used as antidote for the treatment of snake bites (Gautam *et. al.*, 2011). The decoction of the leaves are used for toothache, tender gums (Prakshanth *et. al.*, 2006) bilious diarrhoea, ear ache and also used as eye wash and mouth wash (Khadapadi and Bhajipale, 2010).

The present study involves the identification of the phytochemicals of leaves of *Abutilon indicum* in methanolic extract both qualitatively and quantitatively and to separate the components by using FTIR analysis and UV-Visible spectrophotometry.

II. MATERIALS AND METHODS:

2.1 Collection of plant sample

The fresh leaves were collected from Tiruchirapalli, Tamilnadu, India.

2.2 Preparation of the extract

The leaves of *Abutilon indicum* were washed thoroughly in tap water to remove dust particles. The leaves were then dried in shade at room temperature and coarsely powdered by a mechanical grinder. The dried powdered sample was soaked in methanol for 3 to 5 days. After 5 days, the extract was filtered using No.1 Whatman filter paper and stored in air tight container for further analysis.

2.3 Qualitative analysis of phytochemicals

Preliminary phytochemical screening was carried out by the method described by (Kokate, *et. al.*, 1986 and Harbourne, *et. al.*, 1980).

2.3.1 Test for alkaloids (Mayer's test)

To the 1ml of extract, 1 ml of Mayer's reagent (Potassium iodide solution) was added. Formation of whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

2.3.2 Test for steroids (Liebermann Burchard test)

To the 1ml of extract, 2ml of acetic anhydride and 2ml of concentrated sulphuric acid were added. Formation of violet to blue or green colour indicates the presence of steroids.

2.3.3 Test for terpenoids (Salkowski test)

To the 1 ml of extract, 2ml of chloroform and few drops of sulphuric acid were added. Formation of reddish brown ring indicates the presence of terpenoids.

2.3.4 Test for flavonoids (Alkaline reagent test)

To the 1 ml of extract, few drops of dilute ammonium solution and few drops of concentrated hydrochloric acid were added. A yellow colouration indicates the presence of flavonoids.

2.3.5 Test for saponins (Froth test)

To the 1 ml of extract, 5 ml of distilled water was added and shaken vigorously. Formation of froth indicates the presence of saponins.

2.3.6 Test for phenols (Lead Acetate test)

To the 1ml of extract, 1 ml of lead acetate solution was added. Formation of precipitate indicates the presence of phenols.

2.3.7 Test for tannins (Lead acetate test)

To the 1ml of extract, 1ml of lead acetate was added. A formation of white precipitate indicates the presence of tannins.

2.3.8 Test for tannins (Ferric chloride test)

To the 1ml of extract, 1ml of ferric chloride solution was added. Formation of blue, black or brownish green colour indicates the presence of tannins.

2.3.9 Test for cardiac glycosides (Keller killiani test)

To the 1ml of extract, add 5ml of distilled water and evaporate it to dryness. Then to the Sample add 2ml of glacial acetic acid containing trace amount of ferric chloride solution. Then add 1ml of concentrated sulphuric acid to the sides of the tube. Formation of brown ring underlaid with blue colour indicates presence of cardiac glycosides

2.3.10 Test for aminoacids (Ninhydrin test)

To the 1ml of sample, add 3 to 4 drops of Ninhydrin solution was added and boiled in water bath for 10 minutes. Formation of purple or blue colour indicates the presence of amino acids.

2.3.11 Test for proteins (Biuret test)

To the 1ml of extract, 1ml of 40% sodium hydroxide solution and 2 drops of 1% copper sulphate solution were added. Formation of violet colour indicates the presence of proteins.

2.3.12 Test for carbohydrates (Barfoed test)

To the 2ml of extract, 1ml of Barfoed's reagent was added and boiled in water bath for few minutes. Formation of reddish brown precipitate indicates the presence of carbohydrates.

2.3.13 Test for reducing sugars (Fehling's test)

To the 1ml of extract, equal quantities of Fehling solution A and B were added and heated. Formation of brick red precipitate indicates the presence of reducing sugars.

2.4 Quantitative estimation of phytochemicals

2.4.1 Alkaloid determination

5 gm of sample was added to 200 ml of 10% acetic acid in ethanol in a beaker. The beaker was tightly covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. The entire solution was precipitated by the drop wise addition of concentrated ammonium hydroxide solution. The precipitate was collected and washed with dilute ammonium hydroxide and filtered. The residue is alkaloid, which was dried and weighed (Harbourne, *et. al.*, 1980).

2.4.2 Flavonoid determination

10 gm of sample was added to 100 ml of 80% aqueous methanol in a beaker. The whole solution was filtered through Whatman filter paper No.42 (125mm). The filtrate was then evaporated to dryness and weighed (Harbourne, *et. al.*, 1980).

2.4.3 Determination of total phenols

Few grams of sample were boiled with 50 ml of ether for the extraction of phenols for 15 minutes. To the 5ml of extract, 10 ml of distilled water, 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were added. The samples were left for 30 minutes. This was measured at 505 nm (Harbourne, *et. al.*, 1980).

III. Fourier Transform Infrared Spectrometer

ATR model FTIR spectrometer was used for the analysis of methanolic extract of *Abutilon indicum*. The spectrum was focused in the mid region. The spectrum was recorded using Attenuated Total Reflectance (ATR) bench measurement (Kathishwaran, *et. al.*, 2010).

IV. UV-VISIBLE Spectrometry:

The powdered leaves were dissolved in methanol and scanned in entire UV visible range (200-800 nm).

V. RESULTS AND DISCUSSION

The qualitative phytochemical analysis of the leaves of *Abutilon indicum* is summarized in the Table 1. The quantification of important phytochemicals of this plant is summarized in Table 2. The qualitative analysis shows the presence of alkaloids, steroids, terpenoids, carbohydrates, flavonoids, phenols, tannins, saponins, and amino acids. Phytochemicals such as saponins, terpenoids, flavonoids and alkaloids have hypoglycemic activities (Cherian and Augusti, 1995). The leaves show the presence of high amount of tannins and they play a major role in the treatment of intestinal disorders like diarrhoea and dysentery (Akinpelu and Onakoya, 2006). The leaves also have flavanoids which can act as antioxidants. The result of FTIR analysis shows the presence of three major peaks with three principle components in methanolic extract of leaves Fig 1. The UV spectrum shows the presence of one major peak in Fig 2. The other peaks were seemed to be retention peaks and they are of less important. It helps to undertake further studies on isolation and identification of specific phytochemicals for pharmacological studies.

Table 1. Qualitative results of the leaves of the *Abutilon indicum*

Tests	Methanol
Alkaloid	+
Steroids	+
Flavanoids	+
Terpenoids	+
Saponins	+
Phenols	+
Tannins	+
Cardiac glycosides	-
Aminoacids	+

Proteins	-
Carbohydrates	+
Reducing sugars	-

Table 2. Quantitative results of the leaves of the *Abutilon indicum*

Tests	Methanol
Alkaloid	13.56 ± 4.08
Flavonoid	9.13 ± 0.02
Phenols	14.53 ± 1.35

Fig 1. Results of FTIR analysis of the leaves of *Abutilon indicum*.

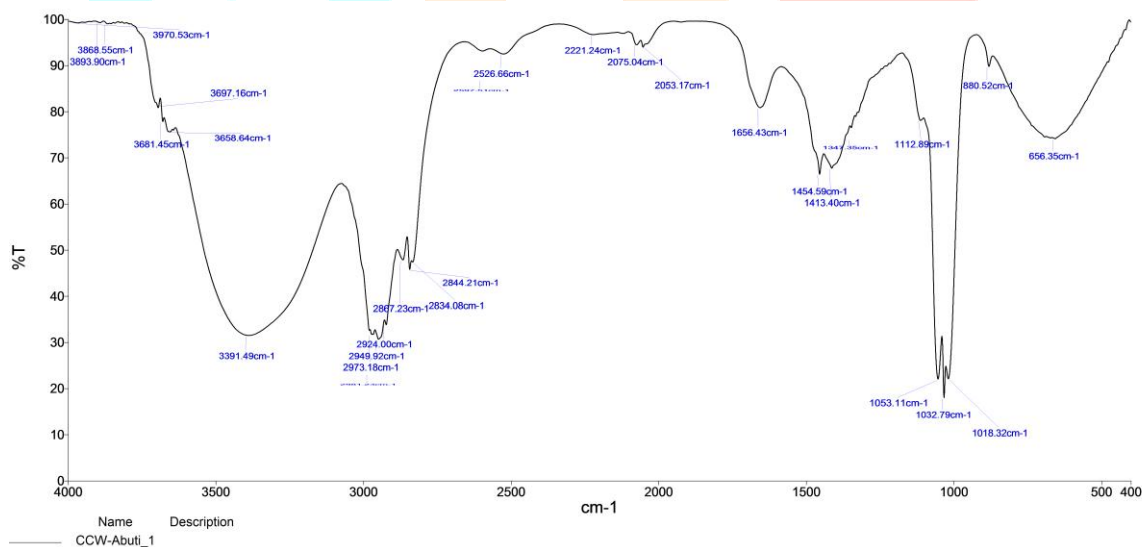
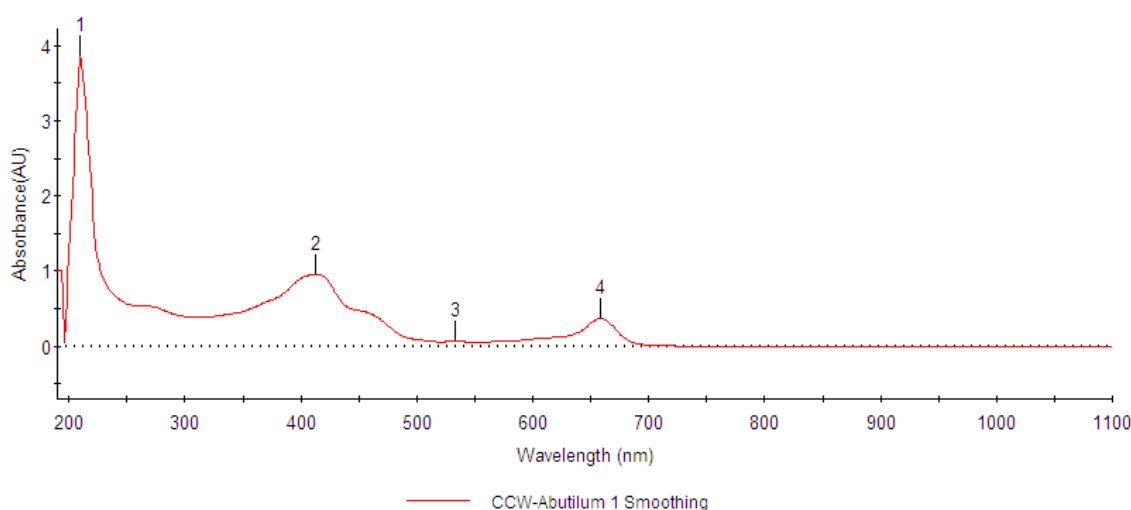


Fig 2. Results of UV-Visible spectrometry of the leaves of *Abutilon indicum*.

VI. CONCLUSION

The qualitative and quantitative analysis shows that the leaves of the *Abutilon indicum* contains significant phytoconstituents such as alkaloids, steroids, flavonoids, terpenoids, phenols, tannins, carbohydrates, and aminoacids. The FTIR analysis and UV spectra show the major peaks in the leaves of *Abutilon indicum*. Further, the work is in progress to analyse the pharmacological profile in the arena of medicine.

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