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Abutilon indicum (Linn.) Sweet leaves, a Natural source of Saponin : a Specrtophotometric assay

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Abstract: *Abutilon indicum,* an important medicinal plant is a good source of various natural metabolites that has hepatoprotective, antitumor, anti-inflammatory, lipid lowering, antifungal, wounds healing and antibacterial properties. Present study was aimed to evaluate the saponin content in leaves of *A. indicum*, spectrophotometrically. *A indicum* leaves showed good saponin yield i.e. about 30 %. Spectrophotometric analysis of crude extracted from dried leaves, showed 296 mg of total saponin and 7.0 mg of steroidal saponin, diosgenin equivalent (DE) per gm of crude extract. This indicated that *A. indicum* plants leaves contained high level of saponin and could be regarded as potential natural source of it, which could feasibly be utilized for a variety of commercial purposes. In addition, they can be used in ethnomedicine as drugs against viral, bacterial, fungal infections and to synthesize sex hormones, oral contraceptives and drugs to cure various serious diseases of human being.
Key words: Diosgenin, saponins, *Abutilon indicum***,** spectrophotometer.

Introduction

Abutilon indicum (Linn.), a member of Malvaceae family commonly known as 'Kanghi' in Hindi and 'Atibala' in Sanskrit, is a perennial shrub and found as weed in almost all hot places of India. Plant has various biologically active secondary metabolites which conferred significant pharmacological and medicinal properties to this plant. Plant *A. indicum* is extensively utilized for the treatment of pharmaceutical disorders and ailments as it possesses wound healing, antioxidative, antitumor, antidiabetic, antifungal, antibacterial, larvicidal, hypoglycemic and hepatoprotective properties [1]. The juice from the leaves is effectively utilized in ulcer, diabetes, diuretic infection and gingivitis [2-4]. It has been reputed in Siddha system of medicine as a remedy to treat jaundice, piles, ulcer and leprosy [5].

Among the various secondary metabolites (phenolics, alkaloids, flavonoids, tannins, glycosides) which are reported to be found in *A. indicum* plants, saponins have an enormous significance in pharmaceutical industry. Saponins are generally a non-volatile, surface active compounds that are widely distributed in nature. Saponins are high-molecular-weight glycosides, consisting of a sugar unit(s) linked to a triterpene or a steroid aglycone. According to the structures of the aglycones, saponins can be classified into two types: triterpenoid and steroidal [6]. The therapeutic effects of a large number of folk medicines are thought to be associated with their saponin content [7]. Recently, plant saponins have received an increasing attention in consideration of the involvement of saponins in important biological activites and of their ability to act as natural drugs in many diseases. They have antitumor, cholesterol lowering, immune potentiating, anticancer, antioxidants, properties [8][9] as well as strong haemolytic, antimicrobial, insecticidal, and molluscicidal activities [10]. Beside this, plant saponins are reported to lower the risk of coronary heart diseases [11]. Saponins is used as a potential ointment in recovery process of wound healing [12] and hormone synthesis in pharmaceutical industry [13].

Recently, only few plants like *Dioscorea*, Fenugreek, *Yucca* has been exploited commercially as a natural resource of saponins and their derivatives; thus, there is a huge chance of extinction of these plants.

Therefore, there is a need time to find a new alternative natural source of saponins to release pressure on commonly used plants. Moreover, a lot of literature has been found on phytochemical and therapeutical usage of plant *A. indicum*, [1][14]-[18], but as per our knowledge no study has been conducted on analysis of saponins content with this plant. Keeping above facts in mind, present study was aimed to evaluate the total saponin content and steroidal saponin spectrophotometrically in dried leaves of *A. indicum*.

Material and methods

Plant material

The leaves of the plant *Abutilon indicum* were used as an experimental material and collected from Green house of The Institute of Science, Mumbai. Plant was authenticated in the Blatter Herbarium, Department of Botany, St. Xavier's College, Mumbai, where a Voucher specimen (no- K.V.S. 1888 of K.V. Shenoy) is deposited. The leaves were then shade dried and grounded to the fine powder.

Qualitative determination of saponins

Ten ml of distilled water was added to 100 mg of ground leaves in test tubes. The test tubes were then vigorously shaken for 2 min. The appearance of stable and persistent foam on the liquid surface indicated the presence of saponins [19].

Extraction of saponin

Saponins were extracted from the dried leaves of *Abutilon indicum*, following the method of Obadoni and Ochuko [20] with minor modifications. 2 gm grounded powder was dispersed in 100 ml of 20% aqueous ethanol. The suspension was continuously stirred for 4 h at about 45^0C over water bath. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were concentrated by using rotary evaporator in 40 ^{0}C to gets 40 ml approximately. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The aqueous layer was re- extracted with 30 ml of n-butanol. The n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was evaporated. After evaporation, the samples were dried in the oven at 40°C to a constant weight. The saponin content was calculated using the following formula:

% saponin = [final weight of sample/initial weight of extracts] × 100

After weighting, the dried fractions containing saponins were store in freezer at 4^0C.

Spectrophotometric analysis of saponins

Total saponin content and steroidal saponin in crude extract were analyzed spectrophotometrically following the method of Hiai et al [21] and Baccou et al [22], respectively, with some modifications. For each assay, a separate diosgenin standard calibration curve was plotted (fig 1&2).

Quantitative determination of total saponins

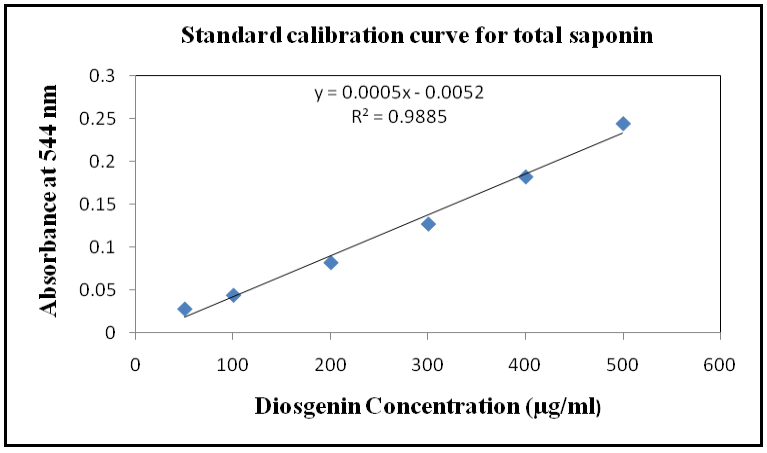

Figure 1. Calibration curve of diosgenin standard for total saponin content estimation

10 mg crude saponin extracts were dissolved in 5 ml of 50% aqueous methanol. 250 µl of aliquot was transferred to test tubes into which an equal volume of vanillin reagent (8 %) was added followed by 72% (v/v) sulphuric acid. The mixture was mixed and placed in a water bath adjusted at 60 °C for 10 min. The tubes were cooled on an ice-cold water bath for 3 to 4 min and absorbance of yellow color reaction mixture was measured at 544 nm using a UV–Vis spectrophotometer (UV-1800 Simadzu) against a blank containing 50% aqueous methanol instead of sample extract. The saponin concentrations were calculated from standard curve and expressed as mg diosgenin equivalents (DE) per g crude extract (fig-1).

Quantitative determination of steroidal saponins

For steroidal saponin estimation, a separate diosgenin standard curve was prepared with 1 to 5 µg/ml diosgenin dissolve in 2 ml ethyl acetate (Fig. 2). Steroidal saponin concentrations were calculated using this standard curve (R^2=0.992) and expressed as mg diosgenin equivalents (DE) per gm crude extract.

For assay, crude saponin extract was dissolved in ethyl acetate (1mg/ml) of which 100 µl aliquot was used for estimation. The test tubes with 100 µl aliquot, 1.9 ml of ethyl acetate, 1 ml of anisaldehyde–ethyl acetate reagent (0.5:95.5, v/v) and 1 ml sulphuric acid–ethyl acetate reagent (50:50, v/v) was mixed well and incubated in a water bath at 60 °C for 10 min. After cooling for 10 min at room temperature, absorbance was observed at 430 nm using UV–Vis spectrophotometer. Ethyl acetate was used to set baseline for the measurement of absorbance. As a reagent blank, 2 ml ethyl acetate was placed in a tube and assayed in similar manner. The extract was evaluated in triplicate.

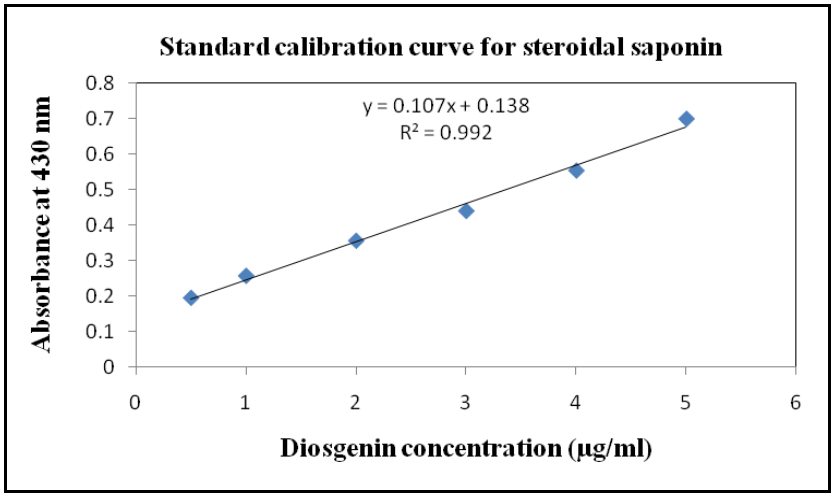

Figure 2. Calibration curve of diosgenin standard for steroidal saponin estimation.

Results and discussion

The qualitative froth test of leaf aqueous extract of *A. indicum* revealed the presence of saponins. The saponins present in the samples are most likely to be the triterpenes since plant is a dicotyledonous species. In traditional medicines, medicinal plants play central role by providing ingredients for drugs. In the present study, we observed an appreciable amount of saponin and diosgenin in plant leaves. The saponin content in *A. indicum* leaves was 30 % (table 1). Astuti et al [23] has reported 28.14±0.22 mg/g crude of saponins content from *Anredera cordifolia* leaves while Ezeabara et al [24] reported 0.43 – 0.72 % saponin content in six species of *Citrus*.

Table 1: Saponin yield and spectrophotometric estimation of total saponins and steroidal saponin in *A. indicum* crude extract. DE= Diosgenin equivalent

S.No.	Saponin yield (%)	Total saponin (mg DE/gm)	Steroidal saponin (mg DE/gm)
1.	30 ± 1.0	296 ± 9.0	7.0 ± 0.2

The results pertaining to spectrophotometric assay for total saponin and steroidal saponin are presented in table 1. The total saponin and steroidal saponin was estimated by using diosgenin standard curves, separately (Figs. 1&2). The crude extract of *A. indicum* leaves contained a considerable amount of total saponin and steroidal diosgenin that were 296 ± 9 mg DE/gm and 7 ± 0.2 mg DE / gm, respectively (table-1). Goel et al [25] reported total saponin content of 45.75, 25.65 and 48.26% (w/w) in the seeds of *Achyranthus aspara*, *Tribulus terrestris* and *Albizia lebbeck* respectively. Ncube et al [26] have studied total steroidal saponin and total saponin as diosgenin (DE) equivalents per dry matter in leaves of *T. violacea*, *H. hemerocallidea*, *D. robusta* and *M. plumbea* in summer, autumn, winter and spring seasons. Saxena et al [27] observed 1.3 to 2.5% diosgenin in non cryogenic and cryogenic seeds of fenugreek. In the present study, we observed 2% diosgenin in crude extract of saponin. Saponin has been reported to have a wide range of pharmacological and medicinal activities to treat diabetes, cancer, hepatitis, cardiovascular as high blood pressure, high cholesterol, and physical stress and has been used for the tonic and stimulating activities in medical herbs [28]. In addition, they are used as insecticide, antioxidant, antibiotic, antiviral and show hepatoprotective, anti-inflammatory, estrogenic and antitumor properties[8- 10][29].The presence of high amount of saponin as well as steroidal saponin diosgenin in leaves of *A. indicum* might be accountable for reported pharmacologic effects of the plant extract and also substantiate the ethno- medicinal use of this plant for drug formulation.

Statistical analysis

The statistical analysis was done by calculating the mean standard deviation (±). All the experiments were performed in triplicate.

Conclusion

This work justifies utility of this plant in ethnomedicine and various clinical and therapeutics. It is clear from the present work that this plant has an appreciable amount of saponin and diosgenin in its leaves. The presence of 30 % total saponin and 2 % steroidal saponin (of total saponin) in the studied plant indicates quite good quantum of this important molecule. Therefore, it can be concluded that the plant *A. indicum* may be a good natural alternative for manufacturing steroidal hormones and oral contraceptives to control human population; drugs to treat cancer, diabetes and heart diseases. Moreover, it also releases pressures from plants which are widely utilized to produce saponins and derivatives for medico- clinical utility. Additionally, plant leaves could also be used as insecticide, antifungal, antiviral, antibiotic and larvicidal agents against various pathogenic organisms which cause various fatal diseases in human as well as plants.

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