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PHARMACOGNOSTIC AND PHYTOCHEMICAL ANALYSES OF LEAVES AND SEED STORAGE OF *ABUTILON PAKISTANICUM* JAFRI AND ALI AN ENDEMIC PLANT OF PAKISTAN

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Abstract

There are vast varieties of medicinal plant in the world having therapeutical importance. With increasing popularity of herbal medicine as a curative measure, the need for correct identification and standardization of the plant is also increased. Present work was performed to study the pharmacognostic and phytochemical characters of leaves and exsitu seed conservation by seed storage of *Abutilon pakistanicum* Jafri &Ali. It is an endemic plant, found in the sub tropical regions of Pakistan and is used in traditional medicine for treating rheumatism. The leaves of *Abutilon pakistanicum* Jafri &Ali were investigated for pharmacognostic parameters. Phytochemical screening, macroscopic characters, physiochemical attributes and fluorescence analysis. The results revealed the presence of pharmacologically active compounds like alkaloids, flavonoids, terpinoids, saponins and phenolic compounds, leaf constant values and the extractive values obtained were found close to the values reported for other *Abutilon* species, while high ash value indicated presence of impurities in the crude drug. The investigation provides information for correct identification and authentication of plant species for further studies and medicinal evaluation of the species.

Key words: *Abutilon pakistanicum*, Pharmacognosy, Phytochemistry, Seed storage.

Introduction

Plants play an important role in providing basic needs like food, timber and medicine. These plants played important roles in disease cure for long time (Aseefa *et al*., 2010). Many medicinal plants are used by men against diseases and to improve health. Secondary metabolites produced from plants mostly include alkaloids, phenolic compounds, tannins, phytosterols and terpenoids have been exploited by man for their useful role in various ways (Balandrin *et al*., 1985). These compounds have been extracted with various solvent by different screening techniques (Ncube *et al*., 2008). It is believed that modern medicines have actually emerged from traditional medicines. Indeed, most of the medicines that are used to treat bacterial and other infections are isolated from plants and other natural resources (Sarwat *et al*., 2012). Most plants found in nature have received scientific and commercial attention. The development of medicines from plants is only possible by standardization of plant species with reference to their phytoconstituents, for commercialization, correct identification and to avoid adulteration (Hariharan & Subburaju, 2012).

Diagnostic characters and quality standard for various plants species have already been investigated by different workers. The roots of *Hibiscus rosasinensis* were examined by Soni *et al*., (2011) while Sharma *et al*., (2013) studied pharmacological and phytochemical profile of the whole plant of *Abutilon indicum*, the chemical constituents and pharmacological studies of *Solanum torvum* was performed by Yousuf *et al*., (2013).

Unfortunately this important natural pharmacy is under serious threat of being lost by various natural and artificial factors and immediately needs some conservation measure for their continuous provision in medicine. These naturally occurring wild species can be conserved through exsitu conservation by using seed bank or in vitro culture (Ishnava & Mohan, 2008). Seed banks act as an insurance policy for biodiversity. It is a process of conserving seed for future use (Bonomi, 2006). Seed storage through seed bank is an old phenomena, used by people in Iraq since 675 BC (Seabrook, 2007).

Abutilon pakistanicum Jafri & Ali belongs to the family Malvaceae. The plant is endemic to Pakistan. It is an erect under shrub with hairs on the stem and branches, leaves are orbicular, obtuse, entire or minutely toothed. The flower is pale yellow and the fruit is ovoid, schizocarp with 8-10 mericarps and 3 seeds per mericarp (Abedin, 1979).

Present study includes pharmacognostic and phytochemical investigation and seed storage of *A.pakistanicum*. No work has ever been done on the pharmacognostic studies and seed storage of this species. However, some compounds have been isolated such as glycoside named Pakiside A and B (Ali *et al*., 2010 a) and Abutilin, a flavonoid reported by Ali *et al*., (2010 b).Hussain *et al*.,(2005) isolated antioxidant flavonoid from *A. pakistanicum* and reported indigenous use of this species for the treatment of rheumatism and as demulcent, emollient and diuretics.

Material and Method

Collection of plant material: Plants were collected from the Medicinal Section of Botanic Garden, Centre for Plant Conservation, University of Karachi. The plant was identified with the help of Flora of Pakistan (Abedin, 1979) voucher specimens were deposited in Karachi University Herbarium, Centre for plant Conservation.

Preparation of extract: Five grams each of leaf powder was soaked in 50 ml ethanol and 50 ml of chloroform separately, the mixtures were left at room temperature with constant shaking for five days and then filtered with Whatman filter paper # 4. The extracts were evaporated to dryness at room temperature, and the residues were weighed and used for phytochemical tests.

Phytochemical investigations: Preliminary qualitative phytochemical screening for various metabolites such as phenolic compounds, tannins, glycosides, anthraquinone derivatives (Evans, 1997) alkaloids (Wagner,1993; Evans, 1997), fixed oils and saponins (Kokate, 1999), proteins (Gahan, 1984), amino acids (Yasuma & Ichikawa, 1953), carbohydrates (Ramakrishnan *et al*., 1994) and gums and mucilage (Whistler, 1993) were carried out with the plant extract.

Pharmacognosy: *Macroscopic studies.* Size, type of lamina, color, odor, taste, leaf base, petiole, venation, margin, apex, surface and texture of the leaf was studied.

Leaf surface study: This study included (a). Stomata Number and Stomatal Index. (b). Vein islets and vein termination number.

Determination of stomata number and stomatal index: Epidermis of fresh leaf was peeled off. With the help of a pair of forceps, the peel was removed and mounted in dilute glycerin and observed under microscope. Numerical data i.e. number of epidermal cells and number of stomata per square mm was recorded and Stomatal indices were calculated using following formula (Evans, 2002).

$$I=S/S+E \times 100$$

where as

I= Stomatal Index
S= Number of stomata per unit area
E= No. of epidermal cells per unit area

Vein islets and vein termination number: Pieces of plant leaves were taken from margin to midrib and cleared by putting in KOH overnight. The sections were stained with safranin and observed under microscope for the evaluation of vein islet and vein termination numbers per square millimeter.

Physio-chemical parameters: The tests were performed to calculate percentage of total ash, water soluble ash and acid insoluble ash values. Extracts were prepared by the method described by Ansari *et al*., (2006). The leaf powder was dissolved in different solvents for extractive values. Fluorescence was carried out for the powder as per standard procedure (Evans, 2002; Nikam *et al*., 2009).

Seed storage: Development of seed bank include, cleaning the seed samples, drying them to optimum moisture levels, testing their germination and their storage for conservation. Seeds were tested 3 times at an interval of 3 months for their moisture content and viability to classify them as an orthodox, intermediate and recalcitrant seeds (Hong & Ellis, 1995).

Seed cleaning: Collected seeds were passed through graded sieves for the removal of debris. Damaged and infected seeds were removed and healthy seeds were spread on tray left open for drying.

Seed moisture: Seed moisture content was determined by using the method given by International Seed Testing Association (Anon., 1981), with little modification. The seeds were kept in a pre- weighed containers and left on the lab bench for drying. They were weighed at different interval for constant value. Seeds were weighed again to determine loss in weight.

Moisture content was calculated on the wet weight base and expressed it as percentage using following formula (Anon., 1981).

$$\text{Percentage (\%) moisture content} = \frac{(w2 - w1) - (w2 - w3)}{(w2 - w1)} \times 100$$

where, w_1 is the weight of the container
w_2 is the weight of the container and sample before drying
w_3 is the weight of container and sample after drying

Seed viability test: Seed viability was tested with germination test.

Germination test: Seeds were taken randomly from the containers and placed on the moist filter paper (Whatman Grade 4) in a petri plate and covered with lid. They were evaluated for seedling development after 7 days and observations were recorded.

Germination percentage is calculated as follows:

$$\text{Germination \%} = \frac{\text{Number of germinated seeds}}{\text{(Total number of tested seeds – Empty ones)}} \times 100$$

To calculate the speed of germination the mean time to germination (MTG) was calculated using the following equation (Daws *et al*., 2005):

$$MTG = \sum (ni \times \frac{di}{n}),$$

ni= number of germinated seeds at di days;
di=incubation period in days at ni
n = total number of seeds germinated in the treatment

Seed dormancy index was also calculated using following equation (Offord *et al.,* 2004).

$$\text{Dormancy index} = 1 - \frac{\text{Seed germinated \%}}{\text{Viability \%}}$$

Seed vigor test: 15–20 seeds from each sample were placed on filter paper, moistened with 4 ml of distilled water, in 3 different Petri dishes. Germinated seeds were removed after every 24 hours, Germination was considered when 2 – 4 mm of radical came out. Numbers of seedlings emerging daily were counted from day of planting the seeds in the medium till the time germination is complete. Thereafter a germination index (G.I.) was computed by using the following formula (Gupta, 1980).

$$GI = \frac{n}{d}$$

Where, n=number of seedlings emerging on day'd'
d = day after planting

The seed lot having greater germination index was considered more vigorous.

Seedling vigor test: Seeds were germinated as described above and the length of roots and shoots were measured. Seed vigour index was calculated by multiplying germination (%) and seedling length.

Results

Phytochemical evaluation: Phytochemical analysis of extracts revealed that alkaloids, phenolics, terpenoids, anthraquinones and saponins were present in both extracts with methanol and chloroform while glycosides and amino acids were absent in both extracts. Carbohydrates, tannins, gum, mucilage and flavonoids were found in methanol extract only, and oil in the extract of chloroform only (Table 1).

Pharmacognostic studies: The morphological features of the leaves indicated dark green color on upper surface and light green color on lower surface. The powder of leaf appeared green in color, coarse in texture, odorless with characteristic taste (Table 2).

Microscopy of leaf surface: Various quantitative microscopic features of leaf surface called leaf constants such as, vein islets number and vein termination number, number and type of stomata with stomatal index and type of trichome of *A.pakistanicum were* worked and are presented in Table 3 (Fig. 1, 2a, 2b and 3).

Physiochemical investigation of powder drug: The powder drug was investigated for physiochemical parameters like total ash value, water soluble ash, acid insoluble ash and extractive value (Table 4).

Fluorescence analysis of powder: Leaf powder was treated with different reagents and colour change was observed in daylight and ultra violet light at 366 nm (Table 5).

Moisture content: The moisture content of the seeds were calculated at different stages of drying, results are given in Table 6. The mean moisture content value for *A. pakistanicum* was 7.17%, which falls in the category of orthodox type of seeds which can be stored safely in the seed bank.

Table 1. Phytochemical screening of *Abutilon pakistanicum* in two different solvents.

Phytochemical test	Methanol	Chloroform
Alkaloid		
Mayer' test	+	+
Wagner' test	+	+
Carbohydrates		
Benedict's test	+	-
Saponin		
Foam test	+	-
Protiens/ Amino acid		
Biuret test	-	-
Nynhydrin test	-	-
Gum &Mucilage		
Alcohol 95% test	+	-
Fixed oil		
Spot test	-	+
Phenolic compounds		
Gelatin test	+	+
Lead acetate test	+	+
Glycoside test		
Borntrager's test	-	-
Terpenoids		
Salwoski's test	+	+
Anthroquinones	+	+
Flavonoids	+	-
Tenins	+	-

Table 2. Morphological character of *Abutilon pakistanicum* leaf.

Characters	Observations
Colour	Green
Odor	Odorless
Taste	Characteristic
Shape	Chordate
Size	2 – 5 cms
Surface	Pubescent
Leaf base	Symmetrical
Lamina	Simple
Apex	Acute
Margin	Almost entire
Venation	Reticulate
Texture	Rough

Table 3. Quantitative analysis of Leaf Constant of *Abutilon pakistanicum*

S. No.	Particulars	Value
1.	Stomata number	3.20
2.	Stomaral index	8.90
3.	Vein Islet number	4.5 -6.66
4.	Vein termination number	1.0 -3.00

Table 4. Physiochemical Evaluation of the crude drug *Abutilon pakistanicum*.

Standardization parameter	%w/w (Mean ± SEM)
Total ash	31.89 ± 2.90
Acid insoluble ash	0 1.63 ± 0.60
Water soluble ash	31.71 ± 0.05
Methanol soluble extractive value	11.56 ± 1.20
Chloroform soluble extractive value	03.16 ± 0.70

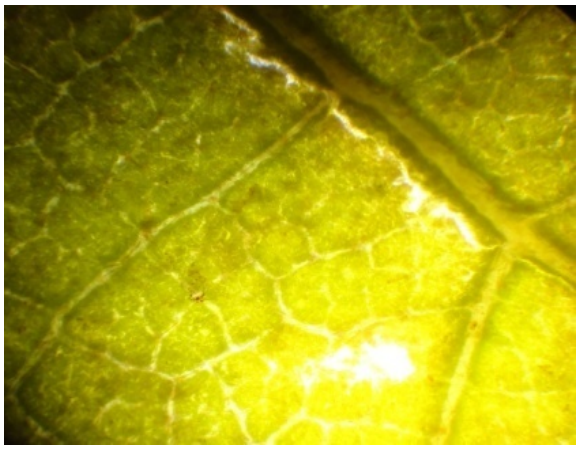

Fig. 1. Venation pattern of *Abutilon pakistanicum* leaf.

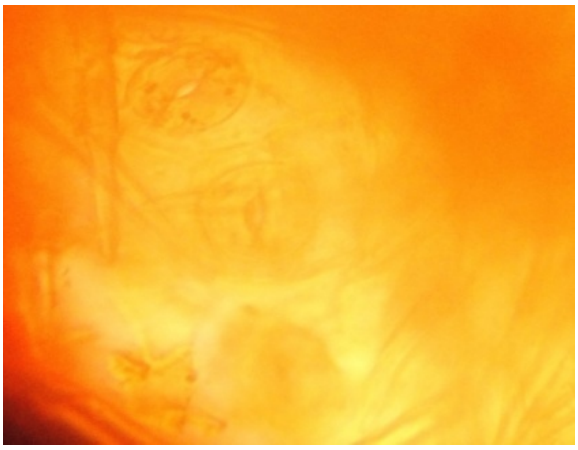

Fig. 2b. Epidermis showing stomata.

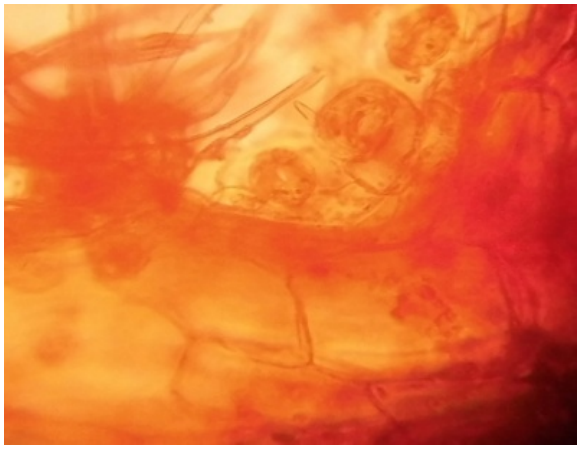

Fig. 2a. Epidermis with stomata of *Abutilon pakistanicum* leaf.

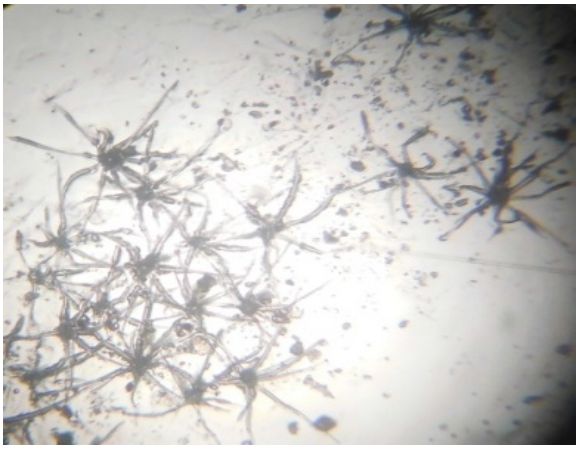

Fig. 3. Trichome of *Abutilon pakistanicum* leaf.

Table 5. Fluorescence analysis of leaf powder of *Abutilon pakistanicum*.

S. No.	Sample	Colour in UV day light 365 nm	
1.	Drug as such	Green	Green
2.	Drug + con.H2So4	Light brown	Brown
3.	Drug + dil.H2SO4	Brown	Brownish green
4.	Drug + con. Hcl	Bright green	Green
5.	Drug + dil. Hcl	Brown	Yellow brown
6.	Drug + con.HNo3	Light brown	Brown
7.	Drug + dil .HN03	Green brown	Golden brown
8.	Drug + Methanol	Bright green	Bright green
9.	Drug + Chloroform	Brown green	Yellow brown
10.	Drug + 10% NaOH	Bright green	Bright green

Seed Bank

Table 6. Seed moisture content data.

Replicate number	Weight of seed before drying (g)	Weight of seed after drying %	Difference of weight	Moisture content %
1.	1.20	1.10	0.1	8.33
2.	1. 58	1.50	0.08	5.06
3.	1.365	1.24	0.125	9.15
4.	1.397	1.315	0.082	5.86
5.	1.621	1.50	1.210	7.46

Mean moisture content = 7.17 %

Seed viability: The results of seed germination showed 73.84% normal germination (seed viability), 76.25% seed viability. Mean germination time of 4-5 days and seed dormancy index was 0.04 (Table 7).

Seed vigor test: Emergence of radicals was observed and germination indices were calculated. It was observed that seeds did not germinate for first three days, radicals started to come out after 96 hours of germination. Most of the seeds germinated within 120 to 144 hours, after that germination rate decreased. Germination index was calculated as 9.37 (Table 8). Results of vigor test showed normal seedling development with vigor index of 579.64 (Table 9).

Discussion

The phytochemical tests are helpful in the detection of pharmacologically important compounds because every plant contain number of compounds of pharmacological importance (Ming *et al*., 2005). The phytochemical screening of *Abutilon pakistanicum* revealed presence of active compounds like alkaloids, carbohydrates, saponins, gum and mucilage, fixed oil, phenolic compounds, tannins, terpinoids, flavonoids and anthraquinones. All these compounds are important medicinally. Most of these compounds are detected in the methanolic extracts while some from chloroform extract, the result may be due to the weak polarity of chloroform because the nature of the solvent, which include polarity that influenced the rate of composition and diversity of the extracted compound (Eloff, 1998 ; Ncube *et al*., 2008). The phytochemicals found from plants are useful in taxonomic distinction (Bate-Smith, 1962), as well as for the detection of pharmacologically important compounds of plants (Sugumaran & Vetrichelvan, 2008). Alkaloids have important therapeutic value (Tyler, 1999). Saponins are found to be a significant antifungal agent (Sodipo *et al*., 1991). Similarly tannins also have curative property in the treatment of human disease (Asquith & Butler, 1986).The biological activity of flavonoids was also been reported by Havesetin (2002). Number of compounds of medicinal importance has been reported by several workers from different *Abutilon* species. Presence of considerable amount of mucilage in the genus *Abutilon* is reported by Ahmed *et al.,* (1993) and presence of two new flavonoids were reported from *Abutilon pakistanicum* by Ali *et al*., (2010 a & b).

Table 7. Seed germination data.

Replicates	Days	1	2	3	4	Total
Number of seeds		20	20	20	20	80
Date		No. of germinated seeds	No. of germinated seeds	No. of germinated seeds	No. of germinated seeds	No. of germinated seeds
19-02-13	1	0	0	0	0	0
20-02-13	2	0	0	0	0	0
21-02-13	3	0	0	0	0	0
22-02-13	4	1	1	3	2	7
23-02-13	5	6	4	3	4	17
24-02-13	6	5	3	2	1	11
25-02-13	7	4	2	1	1	8
26-02-13	8	1	0	2	2	5

Germination = 73.84%, MGT = 4-5 days, Seed Viability = 76.25 %, Seed dormancy Index = 0.04

Table 8. Seed vigor test.

Replicates	Hours	1	2	3	4	Total
Number of seeds		20	20	20	20	80
Date		Emergence of radicals	Emergence of radicals	Emergence of radicals	Emergence of radicals	
20-02-13	24	0	0	0	0	0
21-02-13	48	0	0	0	0	0
22-02-13	72	0	0	0	0	0
23-02-13	96	1	1	3	2	7
24-02-13	120	6	4	3	4	17
25-02-13	144	5	3	2	1	11
26-02-13	168	4	2	1	1	8
27-02-13	192	1	0	2	2	5

Germination Index = 9.37

Table 9. Seedling vigor test.

Replicates	Mean root length (cm)	Mean shoot length (cm)	Vigor index
1.	4.2	3.7	583.33
2.	4.6	4.0	635.02
3.	2.6	4.7	539.03
4.	4.0	3.6	561.18

Mean vigor index =579.64

The initial pharmacognostic identification is achieved by macroscopic studies. Sugumaran & Vetrichelvan (2008) also conducted macroscopic studies of leaf as the basis of pharmacognostic standardization.

Quantitative determination of some pharmacognostical parameters like stomata number, stomatal index, vein islet and vein termination values are helpful in the estimation of the purity of drug. Stomata number and stomatal index are an aid in the evaluation of crude drug (Evans, 2002) and Nabrin *et al.,* (2000) reported the significance of characters like stomata size stomatal index and size of pore in differentiating the taxa at specific and inter specific level. The stomata number and the stomata index of *A. pakistanicum* were found as 3.2 and 8.9 respectively while Chakraborthy (2009) and Karthikeyan *et al*., (2012), reported the stomata number and stomata index of *A.indicum* as 3.10 and 13.1 respectively. Similarly vein islet and vein termination number of *A.pakistanicum* were estimated as 4.5 – 6.66 and 1.0 - 3.0 whereas for *A. indium* these values were reported as 2 – 4.5 and 3.4 – 4.5 respectively (Chakraborthy, 2009: Karthikeyan *et al*., 2012), these values shows close relation between two species of genus *Abutilon.* These features are also considered important in the species identification of crude drug.

Total ash values shows the presence or absence of foreign particles like metallic silt or silica in the crude drug (Kumar *et al.*, 2012) Total ash value for *A. pakistanicum* was estimated as 31.8 % w/w and acid insoluble ash value was found to be 1.63% w/w which shows contamination with siliceous material whereas the water soluble ash value which was 31.71 % w/w is used to detect the presence of material exhausted by water, our results are in accordance with that of Nayak *et al*., (2010), and Subha *et al*., (2011), they conducted ash analysis for standardization of crude drug.

Seed storage is an important technique of storing good quality seeds for future planting. Factors like moisture content and loss of germination should be monitored in seed bank for exsitu conservation in order to minimize loss of genetic erosion (Phartyal *et al.,* 2002). The moisture content of *A.pakistanicum* was calculated as 7.17%, which favours seed deposition in seed bank. Results agrees with the findings of Barner (1975), who came up with the conclusion that moisture content of 4 – 8 % is safe for seed storage. According to International Board for plant generic Resources (Anon., 1976) for orthodox species the moisture content of 4 – 8 % is considered safe. The germination percentage of 73.84%, for *Abutilon pakistanicum* is close to the value calculated for 15 species of Irish threatened flora, which was 71% (Walsh *et al*., 2003). The seed dormancy was found lower than the stated threshold value that indicated dormancy. The high vigour index of the examined species shows seeds are more vigour.

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