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PRELIMINARY PHARMACOGNOSTIC ASSESSMENT, CALLUS FORMATION AND REGENERATION OF *ABUTILON SEPALUM* HUS. & BAQ.

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Abstract

Plant-derivative secondary metabolites have defensive properties against infecting agents that causes sickness. Moreover, they are helpful in impeding several noninfectious diseases. This investigation includes the preliminary analysis of bio-chemical compounds and callus formation by using leaves of *Abutilon sepalum* Hus. & Baq., (An endemic plant of Pakistan). The work on preliminary pharmacognostic evaluation, callus formation and regeneration of *Abutilon sepalum* Hus. & Baq., was not done earlier. The exploration of this plant for preliminary pharmacognostic evaluation, callus formation and regeneration is done first time in Pakistan.

Key words: Pharmacognostic assessment, Callus formation, Regeneration, *Abutilon sepalum*.

Introduction

Plants are useful for the support of climatic peace, supplement cycle, and other biological system forms. Furthermore, they give territories and sustenance, they are moreover essential for the stability of worldwide atmosphere and other environmental changes (Cross, 1995).

Traditionally used plants in medicine provided a variety of phytochemical compounds having curative properties (Chopra *et al*., 1992; Harborne & Baxter, 1995). These therapeutic effects of the plants are possible because of the occurrence of numerous secondary metabolites (Doss, 2009). Secondary metabolites such as alkaloids, phenols, tannins etc have been isolated from the plants with the help of different solvents (Ncube *et al*., 2008).

The formation of drugs for the remedy of diseases is possible by proper identification and appropriate standardization of the plant to avoid adulteration. Investigations for the standardization on many *Abutilon* species have been done by many researchers. Morphological and anatomical investigation on the leaves of *Abutilon indicum* have been done by Karthikeyan *et al*. (2012), whereas evaluation of phenolic contents of *Abutilon grandiflorum* was accomplished by Sikorska & Matlawska, (2008). Furthermore, Tian *et al*. (2012) worked on pharmacognostic studies of *Abutilon theophrasti*.

Tissue culture technique is accomplished and usually applied for the improvement in number of plants and witnessed appropriate in protecting endemic plant species. *In vitro* micropragation is not only a factorfor conserving the plant species in a confined place but also it propagates and increases in number of the required germplasm in a limited time period. *In vitro* methodology applied for the conservation and the re-establishment of cloned outcomes can play an integral part in the improvement of the biological environment (Paunescu, 2009).

Abutilon sepalum Hus. & Baq., (an endemic plant of Pakistan) belongs to family Malvaceae is an erect perennial shrub. Leaves hairy on both the surfaces lower more densely tomentose. Calyx is compactly hairy, fused to the center, corolla is slightly notched, calyx larger than the fruit (Abedin, 1979). This plant is reported in few places of Karachi includes Karachi University, P.C.S.I.R, Manghopir, Thatta and Haleji (Abedin, 1979).

There is no documented evidence before on pharmacognostic and micropropagation evaluation of *Abutilon sepalum* Hus.& Baq. The purpose of the present analysis is to estimate several pharmacognostical, callus formation and regeneration parameters. These records are supposed to be valuable achievement for the verification and quality control for the usage in herbal medicine.

Material and Method

Plant material: The leaves were collected from Karachi University, additionally it was identified with the help of Flora of Pakistan (Abedin, 1979). Voucher specimens were submitted in Karachi University Herbarium, Centre for Plant Conservation. The leaves were dehydrated in the shade and ground into powder with the help of grinding machine.

Macroscopic evaluation: Fresh plant leaves were selected for several evaluations like color, shape andsize.

Phytochemical examinations: Qualitative analysis of phytochemicals was accomplished by means of procedures given by Raaman (2006). Phenolic compounds, Glycosides and alkaloids (Evans, 1997), proteins (Gahan, 1984), carbohydrates (Ramakrishnan *et al*., 1994), amino acids (Yasuma & Ichikawa, 1953).

Physicochemical analysis: Physicochemical parameters such as ash value, water soluble, acid insoluble investigations were achieved as per standard methods (Anon., 1998).

Fluorescence study: Reaction of powdered leaf material with several reagents was studied under visible light and ultraviolet light (366 nm).

***In vitro* callus formation:** Leaf pieces were placed in a sterilized MS medium (Murashige & Skoog, 1962) supplemented with growth regulators having different concentrations of 2,4- D (0, 0.1,0.5, 1.0, 2.0, 3.0,4.0 mg/L), BAP (1.0,2.0,3.0,4.0 mg/l) and KIN(1.0,2.0,3.0,4.0 mg/l) along with 3% sucrose and 2.5 gm/l gellan gum, the solidifying representative agent under laminar flow hood. The disinfection stage was conducted by autoclaving at temperature 121°C for 20 minutes at 15 psi. Jars having explants and media were maintained under photoperiodic condition (16hr light, 8hr dark) complemented with temperature at 25±2°C for 6 weeks.

Callus regeneration: After 6 weeks the callus was introduced to regeneration medium MS along with various concentrations of BAP (0.1, 0.5, 1.0, 2.0, 3.0 mg/l) and in combination with NAA (1 BAP+0.05 NAA, 1 BAP+ 0.1NAA, 1 BAP +0.2NAA mg/l).

Result

Macroscopic and microscopic studies: Macroscopic characters of leaves like color, odor is characterstic. Leaf characters like shape, size, apex, base and some microscopic nature of stomata and stellate trichome found are given in Table 1.

Phytochemical analysis: Preliminary qualitative phytochemical examination showed that the alkaloids, phenolics, carbohydrates, protein and amino acid were present in methanolic extract whereas chloroform extract did not show presence of any phytochemical (Table 2).

Physicochemical estimation: Physicochemical parameters like ash value, moisture content of leaves, extractive value, water soluble and acid insoluble contents are given in Table 3.

Fluorescence analysis: Changes in the coloration of leaf powder on reaction with different reagents like HCl, H_2SO_4, $FeCl_3$, NaOH and with water under visible light and ultraviolet light (366nm) is shown in Table 4.

***In vitro* callus formation:** MS media in combination with various concentration of phytohormone 2,4-D were used for the formation of callus. After 4 weeks, the callus developed is shown in Table 5.

Callus regeneration: The formed callus is then subjected to MS medium containing different concentrations of BAP. The regeneration of plantlet from callus is given in Table 6.

Discussion

Due to side effects of chemicals present in the drugs, the world is refocusing towards natural resources. Usage of plant in medicine is as primitive as human existence. Phytochemical present in plants have therapeutic properties. Natural products having minimum side effects are becoming a priority over synthetic drugs. A strict pharmacognostical investigation is useful to analyze and determine genuineness of sample (Tyler *et al*., 1976).

Morphological study shows that *Abutilon sepalum* Hus. & Baq., have simple, pubescent leaves with characteristic odor and somewhat sour taste (Fig. 1). It contains stellate type of trichomes (Fig. 2) and anisocytic stomata (Fig. 3).

In the given sample, for the extraction of metabolites, methanol was appeared to be effective as compare to chloroform (Eloff, 1998). Phytochemicals such as alkaloids, phenols, glycosides are present in the drugs have therapeutic consequences such as anti-carcinogenic, anti-bacterial and antioxidant properties (Djurdjevic *et al.,* 2006).

Table 1. Morphological characteristics of the leaves of *Abutilon sepalum* Hus.& Baq.

Parameters	Observation
Color	Green
Odor	Characteristic
Taste	Bit Sour
Indumentum	Tomentose
Size	3.5-17cm × 2.8-14.7 cm
Apex	Acute – acuminate
Margin	Irregular dentate
Venation	Reticulate
Leaf Base	Cordate
Lamina	Broadly ovate
Form	Simple
Arrangement of leaves	Alternate
Trichome type	Stellate
Stomata type	Anisocytic

Table 2. Phytochemical evaluation of *Abutilon sepalum* Hus.& Baq.

S. No.	Name of the test	Methanol	Chloroform
1.	Mayer's test (Alkaloids)	+	-
2.	Keller Killiani's test (Glycosides)	+	-
3.	$FeCl_3$test (Phenolic compounds)	+	+
4.	Fehling's test (Carbohydrates)	+	+
5.	Ninhydrin test (Amino acid)	+	-
6.	Biuret test (Proteins)	+	-
7.	Lead acetate (Phenolic compounds)	+	-

+ = Present, - = Absent

Table 3. Physico-chemical parameters for *Abutilon sepalum* Hus.& Baq.

Quality parameters	Results (% w/w)	
	Methanol	Chloroform
Extractive value	2.5 ± 0.1	1.66 ±.0.02
Moisture content	4.87 ± 0.014	
Ash value		
a. Total ash value	10.71 ± 0.16	
b. Water soluble ash	2.42 ± 0.13	
c. Acid insoluble ash	1.39 ± 0.20	

± SEM = Standard error of mean

Table 4. Fluorescence analysis of *Abutilon sepalum* Hus.& Baq.

S. No.	Reagents	Visible light	Uv (366nm)
1.	Dried leaves powder as such	green	Green
2.	Powder + D.H_2O	Green	Dark green
3.	Powder + 1N HCl	Light brown	Dark brown
4.	Powder + 5% $FeCl_3$	Dark green	Dark brown
5.	Powder + 75% Ethanol	Green	Dark green
6.	Powder + 1:1 H_2SO_4	Light green	Dark green
7.	Powder + 10% NaOH	Yellowish green	Dark green

Table 5. Effect of different concentrations of phytohormones for callus formation from leaf explant of *Abutilon sepalum* Hus & Baq.

Growth regulators mg/l	Callus color	Degree of callus formation
MS	-	-
MS + 0.1 2,4-D	Off- white	+
MS + 0.5 2,4-D	Creamy green	+
MS + 1 2,4-D	Cream	++
MS + 2 2,4-D	Creamy green	+++
MS + 3 2,4-D	Dark cream	++
MS + 4 2,4-D	Creamy brown	++
MS + 1 BAP	-	-
MS + 2 BAP	Cream	++
MS + 3 BAP	Yellow green	+++
MS + 4 BAP	Creamy yellow	++
MS + 1 KIN	-	-
MS + 2 KIN	Cream	+
MS + 3 KIN	Cream	++
MS + 4 KIN	Creamy brown	++

+ Slight callusing. ++ Considerable callusing. +++ Profuse callusing

Table 6. Effect of phytohormones on shoot formation from callus of *Abutilon sepalum* Hus.& Baq.

S. No.	Media concentrations (mg/l)	No. of shoots	Shoot length (cm)
1.	MS	-	-
2.	0.1 BAP	2.15 ± 0.23	2.1±0.36
3.	0.5 BAP	9.26 ± 0.46	2.7 ± 0.27
4.	1 BAP	12.17 ± 0.35	3.9 ± 0.97
5.	2 BAP	6.34 ± 0.69	3.4 ± 0.23
6.	3 BAP	10.27 ± 0.26	2.3 ± 0.93
7.	1 BAP + 0.05 NAA	8.41 ± 0.37	4.3 ± 0.51
8.	1 BAP + 0.1 NAA	15.34 ± 0.58	4.8 ± 0.63
9.	1 BAP + 0.2 NAA	11. 26 ± 0.32	3.2 ± 0.19

Physico chemical constituents like Ash value, is estimated about 10.71%. Increased Ash value more than 22% demonstratesthat the sample may contain high contents of silica, oxides or may be due to adulteration (Mulla & Swamy, 2010; Purohit *et al*., 2005), Moisture contents observed is 4.8% which indicates that the sample have less chances to be contaminated. In samples more than 14% moisture content have high chances of microbial growth and activity (Essiett *et al*., 2011). Acid insoluble value is 1.39% which determines the presence of Calcium Oxalate in the sample (Patel *et al*., 2010).Water soluble estimation of the crude drug is 2.42% which indicates the digestibility of plants when eaten (Essiett *et al*., 2011). Fluorescence analysis is another aspect for pharmacognostic determination in the drugs. Because of this authenticity and adulteration of the sample can be recognized (Selvam & Bandyopadhyay, 2005).

It was observed that no undifferentiated mass of cells was formed on hormone free medium. Effective profused callus was formed on MS medium in combination with 2mg/l 2,4-D (2,4-Dichlorophenoxyacetic acid) and MS along with 3 mg/l BAP (Figs. 4,5). Callus formation is an effective tool for the rapid production of plants (Abe and Futsuhara, 1986; Rout *et al*., 2009; Kazmi *et al*., 2015). Munazir *et al*. (2010) also worked on callus induction. From callus in comparison with direct regeneration of plant, more plantlets are produced (Figs. 6,7) (Hussain *et al*., 2010). It is observed that no shoot induction was started in hormone free MS medium from the callus. BAP alone or in combination with NAA enhanced the formation of shoots and shoot length from callus (Rout *et al*., 2011). Low concentrations of BAP support the formation of shoots from callus (Abohatem *et al*., 2011).The application of BAP is found to be favorable for shoot induction in various plants which are medicinally important like *Anethum graveolens* (Jana & Shekhawat, 2011), *Cadaba heterotricha* (Abbas & Qaiser, 2010), *Andrographis paniculata* (Martin, 2004), *Saussurea involucrata* (Guo *et al*., 2007). MS medium along with 1 mg/l BAP proved to be good for the regeneration of plantlets from callus, and also combination of 1mg/l BAP and 0.1 mg/l NAA. Therefore as a consequence of present studies a procedure is developed for the preliminary pharmacognostic evaluation and regeneration of plantlets from callus.

Fig. 1. *Abutilon sepalum* Hus. & Baq.

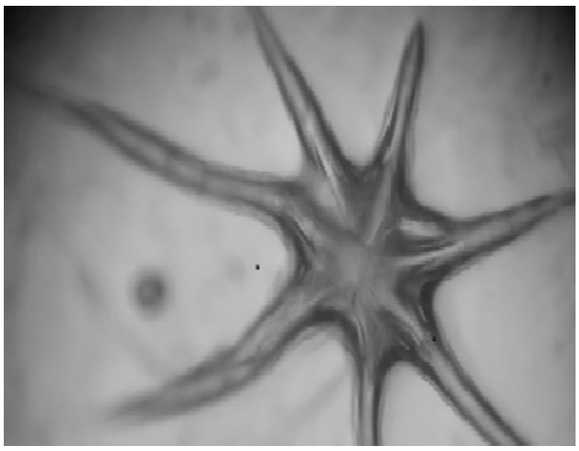

Fig. 2. Stellate trichome.

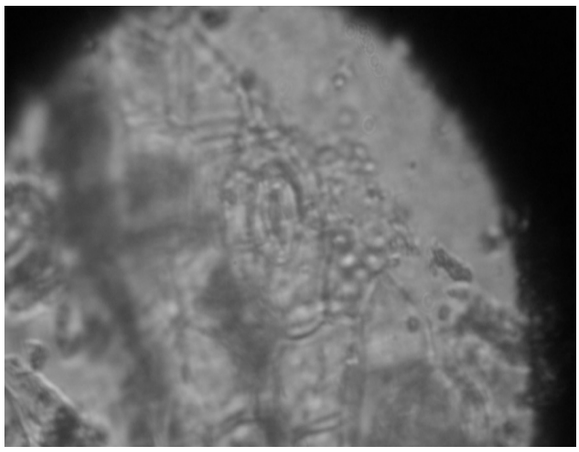

Fig. 3. Anisocytic stomata.

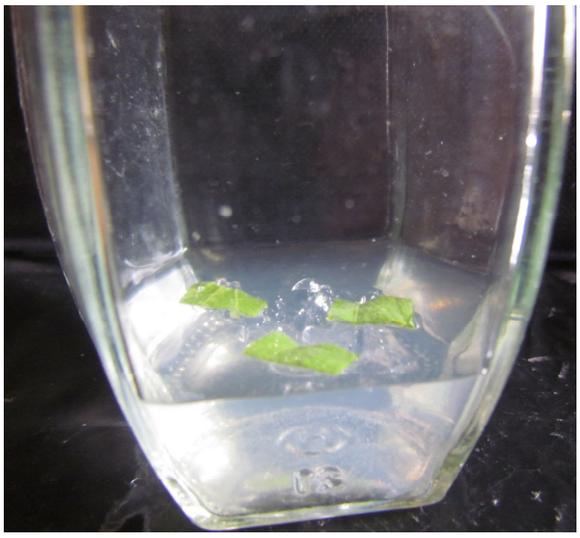

Fig. 4. Callus initiation.

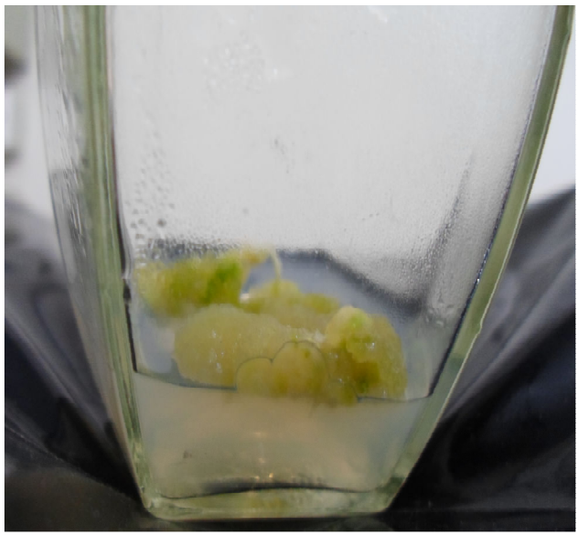

Fig. 5. Callus formation.

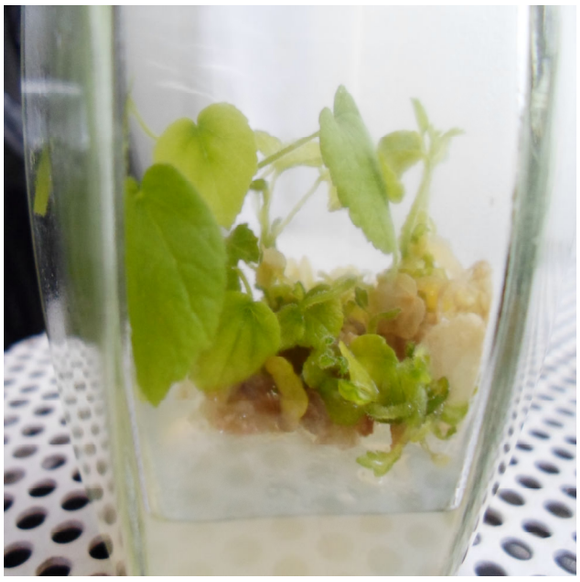

Fig. 6. Regeneration from Callus (MS + 1 mg/l BAP).

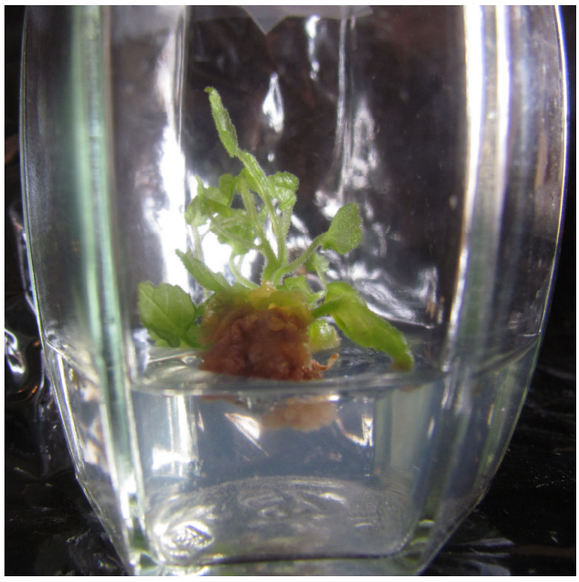

Fig. 7. Plant regeneration (MS + 1 mg/l BAP + 0.1 mg/l NAA).

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