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EVALUATION OF ETHANOLIC EXTRACTS OF *IN VITRO* GROWN *BAUHINIA VARIEGATA* L. FOR ANTIBACTERIAL ACTIVITIES

MADHU KUMARI*

****Department of Botany, B.R.A.Bihar University, Muzaffarpur.***

ABSTRACT

Nodal segments from six years old plants of *Bauhinia variegata* L. were cultured on Murashige and Skoog's (MS) medium supplemented with different concentration and combinations of BAP and IBA. Explants culture in MS basal medium supplemented with 5mg/1 BAP showed highest rate of shoot multiplication. When *in vitro* shoots were inoculated on the MS basal medium supplemented with IBA 2mg/l, profussed rooting was observed. *Bauhinia variegata*, commonly known as Kachnar is considered as medicinal plant in Nepal and India. The ethanolic extract of this plant was found to have antimicrobial activity against *Bacillus subtilis* (MTCC8), *E coli* (MTCC1), *Staphylococcus aureus* (MTCC98), *Salmonella typhi* (MTCC737). The largest zone of inhibition (16 mm) was found against *B. subtilis*. The extract was found to be more effective against gram- positive than gram-negative bacteria.

KEYWORDS: ***Bahuina variegata,*** **nodal segment culture,** ***In vitro*** **multiplication, multiple shoot induction, plantlet regeneration, antimicrobial activity.**

DR. MADHU KUMARI
Department of Botany, B.R.A.Bihar University, Muzaffarpur.

INTRODUCTION

Plants have been considered a valuable source of natural products for maintaining human health, with more intensive studies devoted to natural therapies. The use of plant compounds for pharmaceutical purposes has gradually increased in India. People from developed countries now tend to use traditional medicines, which involve compounds derived from medicinal plants**.** Natural products of higher plants may give a new source of antimicrobial agents. Certain *Bauhinia* species have a long history of traditional medicinal applications[1]. Therefore, such plants have to be investigated to understand their properties, safely and efficiently. *Bauhinia variegata* L. lies in the family Ceasalpiniaceae, and is locally called Kachnar. It is a medium sized deciduous tree, found throughout India. Bauhinia variegata is a medicinal plant[2] is widely used in folklore medicine. Its bark, root, leaves, seeds and flowers are used for their medicinal properties. The root and barks are acrid, cooling, constipating, depurative, anthelmintic[3], anti-inflammatory and styptic. They are useful in diarrhea, dysentery, skin disease, leprosy, intestinal worms, wound ulcers, tumors[4], coughs and antidiabetes[5,6]. Root contains flavanol glyucosides[7]is reported to have anti-inflammatory activity.

Conventional propagation via seed is relatively slow as per increasing requirement, there is an urgent need for applying non-conventional methods of propagation of *Bauhinia variegata.* Micro-propagation ensures not only continuous supply of plant throughout the year but also prevent the destruction of the natural population of medicinal plant[8,9]. During past few years, there has been an increase interest for in vitro multiplications and germplasm conservation of rare, endangered, aromatic and medicinal plants[10,11]. Earlier reports on the cultivation and improvement programs of this taxon focus mainly on the development of efficient protocols for its regeneration through tissue culture methods. The available literature on the medicinal properties of this plant pertains mainly to the field grown plants. Although the plant was successfully raised under *in vitro* tissue culture condition, to best of our knowledge, there is no published work regarding to compare the medicinal value of the *in vitro* raised plant of *Bauhinia variegata.*

The main objective of our study is to establish an efficient, quick and reliable protocol for in vitro regeneration of *Bauhinia variegata* and study of antimicrobial properties of *in vitro* raised plant mass of *Bauhinia variegata.* The later may be scaled up for commercial production of plants for extraction of pharmaceutically important chemicals.

MATERIALS & METHODS

Source of Explants

In order to obtain *in vitro* plants, a protocol was developed for rapid clonal propagation of *B. variegata* L through mature nodal explants. Nodals & internodal explants were collected from the campus of the department of Botany, B R A Bihar University, Muzaffarpur. These nodal segments were cuts into 1.5cm to 2.0cm length with single node & internode intact. These nodal & internodal cutting were washed with 5% (v/v) detergent solution (tepol) for 10minutes followed by rinsed with running tap water for several times. These nodal segments were surface sterilized with sterile with bavistin 0.2% (w/v) for 10 minutes and then washed with sterile distilled water. In the laminar chamber the nodal segments were further treated with 70% alcohol for one minute followed by 0.1% (w/v) mercuric chloride treatment for 5 minutes, aseptically explants were washed with sterile distilled water for 3 to 4 times dried using sterile blotting paper which was ultimately used as explants for raising *in vitro* cultures.

Growth Medium & Culture Condition

Murashige and Skoog's [12](MS) medium 1962 was used as nutrient source with or without growth hormones such as BAP, IBA either

individually or in combination for culture explants. Sucrose 3% and agar 0.8% (Hi media) were used as carbon source and gelling agent respectively. The pH of the medium was adjusted to 5.8 and autoclaved at 121°C for 20 minutes. The cultures were maintained in the culture room at 25°±C 2ºC, under white fluorescent light.

Shoot Organogenesis and Elongation

For multiple shoot induction shoot nodal explants were culture on MS medium supplemented with BAP (1- 5 mg/l) alone or combination with Kinetin (1-2 mg/l) (Table 1). Data on percentage of responding explants and number of shoots per explants were recorded after 25 days of initiation culture.

Antibacterial Assay

Preparation of Plant Extract

In vitro grown plants, callus, were weighted and grinned with help of motor & pestle. About 50gm of power grinded material was extracted in soxhlet apparatus with 250ml of ethanolic solvent[13]. The extracted solvent was removed from the extract under reduced pressure with rotary vacuum evaporator. The sticky greenish-brown substance was obtained was stored in refrigerator condition till use[14].

Culture of Microorganism

Both gram positive and gram negative bacteria were used as test organism for this study. The organisms like *Escherichia coli* MTCC 2845*, Salmonella typhi* MTCC 737*, Staphylococcus aureus* MTCC 98, *Bacillus subtilis* MTCC 441, were used for antibacterial assay. The bacteria were grown in nutrient broth media at 37ºC, for antibacterial assays. The disc diffusion method[15] was adopted for antimicrobial study of plant extract. Briefly, 20ml of media was transferred aseptically into each sterile petri dish and allowed to solidify. An over night grown inoculums (100µl) suspension was spread uniformly over the agar medium using sterile glass rod for uniform distribution of bacteria. The readily prepared sterile discs were loaded with different concentrations of about 10-20 µg of plant extract of *B. variegata*. The paper diffuse discs were placed on the medium and plates were incubated at 37°C for 24 hours. The antibacterial activity was recorded by measuring the width of the clear inhibition zone around the disc in millimeter.

Phytochemical Screening

The ethanolic extract of *in vitro* grown plants of *Bauhinia variegata* L were subjected to different tests [16,17] to identify the nature of chemical constituents present in the plant material.

RESULTS & DISCUSSION

Organogenesis is dependent on the factors like explants type physiological state of donor plant or organ and endogenous level of phytohormones[18]. It has been reported by various workers that he balance of auxin to cytokinin is a determining factor[19]. The media supplemented with BAP alone triggered shoot induction in nodal explants of *Bauhinia variegata* (fig-A). The appreciable frequency of shoots emerged from each nodal explants when it cultured on MS medium supplemented with high concentration of BAP (5mg/l). Further, increase in concentration of BAP reduced the frequency of shoot induction. BAP alone at the concentration of 5mg/l produced maximum shoot per explants. (fig-B)

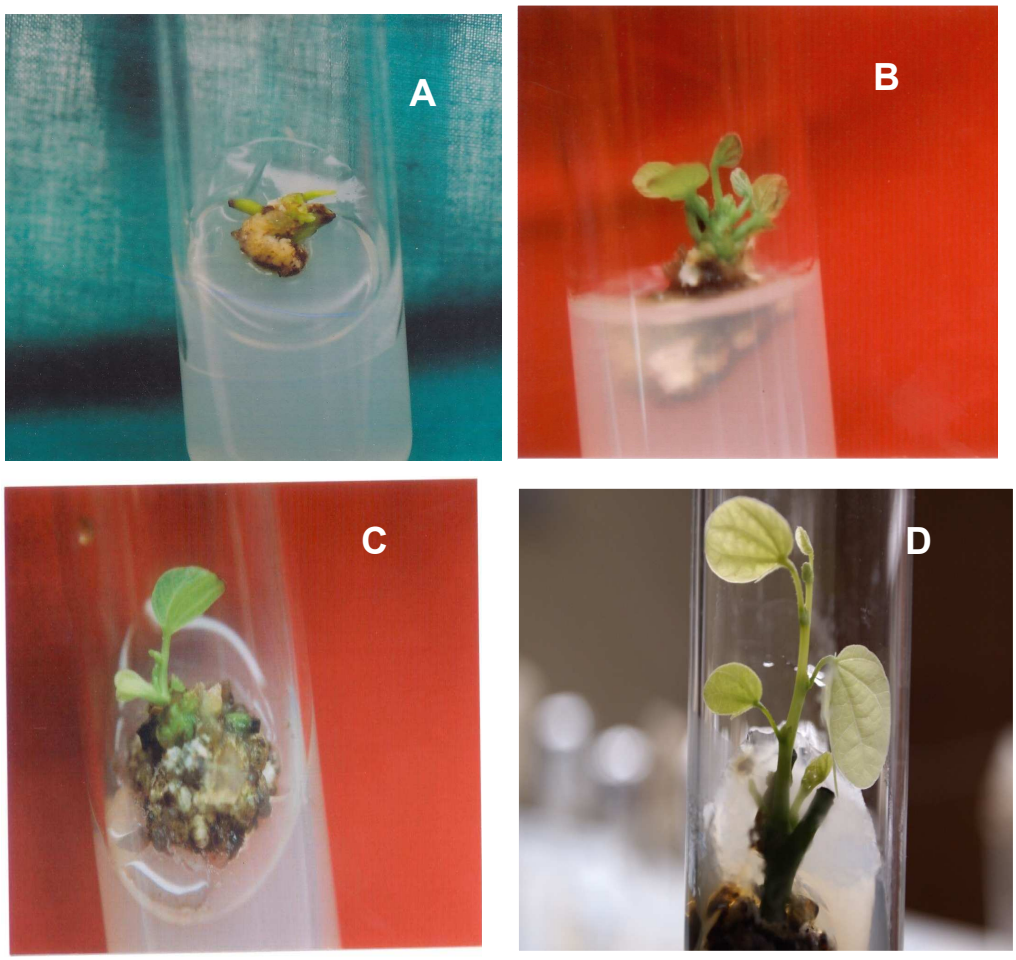

Figure 1

In vitro clonal propagation of Bahuina variegata L from internodal explants. A. shoot buds initiation on MS + BAP (2mg/l); B. multiple leafy shoot emergence in MS + BAP (5mg/l); C. shoots emerged from callus in MS + BA (2.5mg/l) + KN (0.5mg/l). D. Regenerated plantlets after 40 days old culture.

When the explants were cultured on MS medium with BAP (2.5mg/l) and kinetin (0.5mg/l) the callus as well as shoots induction take placed after 30 days (fig-C). When BAP was used in combination with IBA the inductions of shoot as well as root both take placed. The induction of roots from callus was observed on MS basal media containing only IBA. The frequency of root induction observed in different concentration of IBA (1mg to 2 mg/l) (Table-1). However, the concentration of IBA higher than this the frequency of root induction decreased. It is well known that root induction depends on the auxin concentration in tissue[20].

In vitro Rooting Plant Establishment

The *in vitro* grown plantlets (fig-D) were ready for acclimatization. After hardening it is transferred into the soil. The auxin IBA were used singly to induce rooting from *in vitro* raised shoot lets. A range of concentration was tested (1.0, 2mg/l) for rooting. The superior results on rooting were obtained on MS with IBA (2mg/l). The well rooting plant lets were transferred to plastic cups containing vermiculite for hardening and kept under controlled condition.

Plants started producing fresh shots and roots after one week of transplanting. Later they

were transported to the field condition and the survival rate was 50% (Table-2). The efficient micro propagation technique described here may be highly useful for raising disease free quality planting material of *Bauhinia variegata* for commercial and off season cultivation which not only help in improving economic condition of the farmer but also fulfill the market demand of herbal industries.

TABLE 1
Effects of Cytokinins and Auxin and their Interaction on Shoot Proliferation from Nodal Explants of Bauhinia variegata.

Growth regulator	Concentration (mg/l)	% of response	Induction of shoots per explants	Induction of roots per explants
BAP	**0.5**	**0**	**0.0**	0.0
	1	**0**	**0.0**	0.0
	2	**1**	**1**	0.0
	3	**40**	**1-2**	0.0
	4	**60**	**2-3**	0.0
	5	**70**	**4-5**	0.0
BAP+ KN	**1 + .5**	**70**	**2-3**	0.0
	2.5+ .5	**74**	**3**	0.0
	1+ 1	**65**	**0**	0.0
IBA	**.5**	**0.0**	**0**	0.0
	1	**40**	**0**	1-2
	2	50	0	1-2

Table-2
Data on Survival of Transferred Regenerated Plants.

	No. of Plants	Plants Survived	% Response
Transferred for Hardening	**30**	**20**	66.6
Transferred for Acclimatization	20	10	50

In this investigation, during the process of transfer to field the survival percentage was found to be fifty percent. There are various reasons for low survival of regenerated plants after transfer to natural soil. Gangopadhyay et al.[21] (2002) reported that *in vitro* derived roots are often physiological functioning when in contact with the soil.

Table 3

Shows the antibacterial effect of ethanolic extract of Bauhinia variegata. The Result obtained in the present study revealed that EBV possesses potential antibacterial activity against all the four tested bacterial organisms (E. coli, S. typhi and S. aureus B. subtilis).

S.No.	Microorganisms	Zone of Inhibition(mm) 10µg	Zone of inhibition(mm) 20µg
1	***Escherichia coli(-ve)***	**6mm**	10mm
2	***Salmonella typhi* (-ve)**	**5mm**	8mm
3	***Staphylococcu aureus (+ve)***	**7mm**	12mm
4	***Bacillus subtilis* (+ve)**	**9mm**	16mm

The EBV showed a broad spectrum of activity against all the bacterial strains at the tested concentration of 10 - 20µg/disc. EBV exhibited greater zone of inhibition for *Bacillus subtilis* (16mm). The least activity was observed for *S. typhi* (5mm) at 10 µg/disc.

The plants and their extracts used against microbial infections due to the presence of secondary metabolites such as phenols, flavonoids, tannins, terpenoids etc[22,23] have documented the use of natural products as new antibacterial drugs.

Phytochemical Screening

The phytochemical tests revealed the presence of flavonoids, saponin, Alkaloids, fatty acid, Tannins, Glycosides in ethanol extract of *in vitro* grown plants of *Bauhinia variegata* L. The results of phytochemical screening are given in Table 3.

Table 4

Phytochemical Screening

S.NO	Name of the Phytoconstituents	Ethanolic Extract of *Bauhinia variegata* Plants
1	**Alkaloids**	**+**
2	**Flavonoids**	**++**
3	**fatty acid**	**+**
4	**Saponins**	**+**
5	**Glycosides**	**++**
6	**Tannins**	**+**

(++) presence of compound, (+) moderately presence of compound

The result justify the medicinal use of *Bauhinia variegata* L. as an anti-infectious diseases, as a natural sources. Thus this plant could be utilized as a source of antimicrobial drugs, with little or no side effects as compared to synthetic drugs.

CONCLUSION

The ethanolic extract of *Bauhinia variegata* L. was found to be sensitive to different microorganisms, thus this plant could be utilized as a natural source of antimicrobial drugs. Further studies are needed to isolate, characterize, and elucidation of the phytochemical compounds of the in vitro grown plants for formulation of antimicrobial drugs.

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