Studies on callus induction, phytochemical constituents and antimicrobial activity of *Solanum nigrum* L. (Solanaceae)

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Abstract: *In vitro* callus induction of *Solanum nigrum* L. (Solanaceae) was carried out on MS medium supplemented with different concentrations of auxin and cytokinin utilizing leaves explants. The optimum culture conditions for callus formation and high frequency were obtained on MS medium supplemented with 3 mg/l NAA and 0.5 mg/l BA. The phytochemical screening of the crude plant extract and its callus revealed the presence of alkaloids, Saponins, tannis, flavonoids and phenolic. The results showed that the amount of flavonoids and total phenolic contents were higher in the mother plant than in the developing callusing. In addition, the crude extract of *Solanum nigrum* and its callus showed, mostly, high antimicrobial activity against gram positive and gram negative bacterial strains as well as fungi.

[Ewais EA, Desouky SA and Eshazly EH. **Studies on callus induction, phytochemical constituents and antimicrobial activity of *Solanum nigrum* L. (Solanaceae)**. *Nat Sci* 2015;13(6):133-138]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 17

Keywords: *Solanum nigrum*, auxin; cytokinin; reducing sugars; tannis; flavonoids and phenolic

1. Introduction

Plant tissue culture has been identified as an excellent surrogate method to overcome the problems connected with utilization and conservation of medicinal plants (Bajaj *et al*., 1988). A callus culture system offers many advantages as a model system for several biological investigations. Even callus has proved better for the synthesis of alkaloids in several cases (Bhat, 1995 and Mahadev *et a*l., 2014).

Alarge number of medicinal plants are explored from the natural flora for the production of commercial drugs. The past few decades have seen increasing scientific interest in both growth of plant tissue culture and the commercial development of this technology as means of producing valuable phytochemicals. The most important of these bioactive constituents of plants are alkaloids, tannins, terpenoids, saponins, quinones, flavonoids, steroids, cardiac glycosides, oils and phenolic compounds (Kala, 2014).

Solanum nigrum (black nightshade) is a medicinal plant belongs to member of the Solanaceae family of plants. The whole plant is anti-periodic, anti-phlogistic, diaphortic, emollient, febrifuge, narcotic, purgative and sedative. The leaves, stems and roots of these plants are used externally as a poultice; wash etc. in the treatment of cancerous soles, boils, leucoderma and wounds (Moerman, 1998). Extracts of the plants are analgesic, antispasmodic, anti-inflammatory and vasodilator. The plant has been used in the manufacture of locally analgesic ointments and the juice of the fruit has been used as an analgesic for toothaches (Chiej, 1984).

An efficient protocol was devised for rapid callus induction of *Solanum nigrum* L. from young leaves. MS medium (Murashige and Skoog, 1962) supplemented with different concentrations IAA (1-3 mg l^{-1}) with BAP (0.5 mg l^{-1}) and NAA (1-3 mgl^{-1}) with BAP (0.5 mg l^{-1}) for callus initiation. The growth of the calli derived from leaves increased with time of incubation and remained almost constant after 30 days (Yogananth *et al.,*2009).

High frequency of green compact callus induction of *Solanum nigrum* L. was obtained in leaf explants cultured on MS medium supplemented with 3.0 mg l^{-1} NAA and 0.5 mg l^{-1} BAP. Also, (Sridhar and Naidu, 2011) describes successful plant regeneration from in vitro derived callus of young leaves. In this respect. Lali (2012) found that the *Solanum nigrum* plant is contains reducing sugars, anthraquinones, terpenoids, flavonoids, tannins, saponins, alkaloids and cardiac glycosides.Als, some other investigators found that, the extract of *Solanum nigrum* have antimicrobial activity against bacteria and fungi (Venkatesan *et al*., 2009, Kaushik *et al*., 2009, Sridhar and Naidu, 2011 and Khizar *et al*., 2014).

Hence, in this study an attempt is made to a protocol for successful callus induction and to screen the phytochemical constituents of *Solanum nigrum* and its callus, in addition to evaluate their antimicrobial activity.

2. Material and Methods

2.1. Plant material

Seeds of *Solanum nigrum* L. were obtained from Botany& Microbiology Dept., Faculty of Science, Al-Azhar University, Assuit, Egypt.

2.2. Seeds sterilization

Seeds of *Solanum nigrum* were sterilized by immersion in 70% Ethanol alcohol for 30 seconds and then immersed in concentrations 5 % of commercial Clorox (NaOCl 5.25%) under aseptic condition in laminar air-flow cabinet. Seeds were immersed in these concentrations for 5 minutes and then rinsed 3 times in sterilized distilled water.

2.3. *In vitro* seeds germination

In this experiment, basal MS medium (Murashige and Skoog, 1962) containing 3% sucrose was used. Culture medium was solidified using 0.8% agar added prior to autoclaving at 1.2 Kg /cm^2 for 15 min. The pH was adjusted to 5.7 by addition of 0.1N HCl or 0.1N KOH. Culturing was done in 300 ml glass jars containing 40 ml of medium. Cultures were incubated for 3 weeks under controlled conditions in the growth chamber at 26± 2°C by power air condition. Day/night schedule controlled 16 hours light and 8 hours darkness, controlled automatically. Illumination intensity; 1500 lux at top culture levels from white cooling fluorescent lamps (60 cm long) measured by lux meter.

2.4. Callus Induction

In vitro germinated seedlings of *Solanum nigrum* L (2 weeks old) were subjected as plant material in this study.The leaf explants from seedlings of *S. nigrum* were excised and cut into squares (10x10 mm) and cultured in the MS media. A total of 4 explants, as source of callus, were placed on each of the culture medium with different concentrations of auxin and cytokinin. Each treatment, which consists of 4 explants, was repeated at least 3 times. The percentage and day of callus formation, morphology, fresh weights of callus and intensity of callus growth were observed weekly. Callus induction percentage was determined after 8 weeks of culture. The calli were transferred to fresh medium every 4 weeks. All the cultures were maintained under a photoperiod of 16 h light and 8 h darkness at 26±2°C with a light intensity of 1000 lux provided by white fluorescent tubes.

2.5. Phytochemical studies and antimicrobial activities

The ethanolic extracts of the field grown leaves and *in vitro* prepared callus of *Solanum nigrum* were used in phytochemical studies and antimicrobial activities phytochemical studies and antimicrobial activities. Phytochemical study including preliminary phytochemical screening, including steam distillation of volatile oils (Balbaa*et al.,* 1981)Test for Alkaloids (Woo *et al.,* 1977) . Test for Glycosides (Treare and Evan 1985), Test for Cardiac Glycosides (Treareand and Evan, 1985), Test for Saponins (Kokate, 1994 and Kokate *et al.,* 2001), Test for phenols (Ahmad *et al.,* 2005), Test for Phytosterols (Fieserand Fieser, 1959) and (Brieskorn*et al.,* 1961) Test for Tannins (Treare and Evan, 1985), Test for Flavonoids (Geissmann, 1962) and (Khandeal, 2008). Total phenolics were determined with the Folin Ciocalteu as described by (Maurya and Singh, 2010), Total flavonoids by (Samatha *et al.,* 2012).

The antimicrobial activity was carried out following disc diffusion method (Arya *et al.*, 2010).

3. Results and Discussion

3.1. Callus induction

In this study, callus induction was evaluated on MS medium (Murashige and Skoog, 1962) with combinations of auxin (NAA) and cytokinin (BA). It was observed that all the concentrations of NAA (0.5, 1, 2and 3 mg l^{-1}) and BA (0.5 mg l^{-1}) were, with one exception, capable of inducing calli, with the highest percentage (87.4%) being obtained from 3 mg l^{-1} NAA and BA 0.5 mg l^{-1} supplemented MS medium (Table 1). This was followed by 2.5 mg l^{-1} NAA and BA o.5 mgl^{-1} with an induction percentage of 75%. The exceptional case was observed in media with 1 mg l^{-1} NAA and 0.5 mg l^{-1} BA.

Different concentrations of auxin and cytokine have varying effects on plant growth and morphogenic response. This is shown in the present experiment where by, it was observed that NAA and BA possess different intensities in inducing callogenic response. Accordingly, (NAA 3mg l^{-1}) and (BA0.5mg l^{-1}) resulted in better callogenesis response as compared to lowest concentration (NAA 0.5mg l^{-1}) and (BA0.5 mg l^{-1}). The calli were greenish white, compact when MS supplemented with (NAA 3mg l^{-1}) and (BA0.5 mg l^{-1}). It was observed (Table 1 & Fig. 1) that the highest callus fresh weight (17.7 g/explant) was obtained when leaves of *Solanum nigrum* cultured on the medium supplemented with (NAA 3mg l^{-1}) and (BA 0.5 mg l^{-1}), while the lowest (1.8 g /explant) fresh weight were obtained on the medium supplemented with 0.5 mg l^{-1} NAA and 0.5 mg l^{-1} BA. These results according with Kolar *et al.*, (2008) reported that the highest frequency of green, compact callus of *Solanum nigrum* was obtained on MS medium (Murashige and Skoog, 1962) supplemented with 2.0 mg g^{-1} IAA and 0.5 mg L^{-1} BAP using leaves explants.We have standardized

a ri98+epeatable protocol for callus induction (Fig. 1). Callus production is one of the important steps for continuous production of secondary production for controlling different human diseases.

Table 1. Callus induction from the leaf explants of *Solanum nigrum* cultured in MS medium supplemented with different concentration of NAA and BA

Plant growth regulators (mg L^{-1})		Percentage of callus Formation %	Callus color Morphology	Mean F.wt. of callus (g/jar)	Mean D.wt. of callus (g/jar)	Mater content	Degree of callus formation
BA	NAA						
0.5	0.5	12.5	Brownish	1.8	0.085	4.7	+
	1.0	25	Brownish	2.8	0.098	35	+
	1.5	37.7	Brownish Watery	7.7	0.31	4	++
	2	62	Yellowish Friable	8.2	0.26	31	++
	2.5	75	White, friable	9.5	0.37	3.8	+++
	3	87.4	Greenish, white, compact	17.2	0.5	2.9	+++

Callus growth rating value = (+) poor, (++) moderate, (+++) profuse and (-) no callus formation
+-6509/4+-0

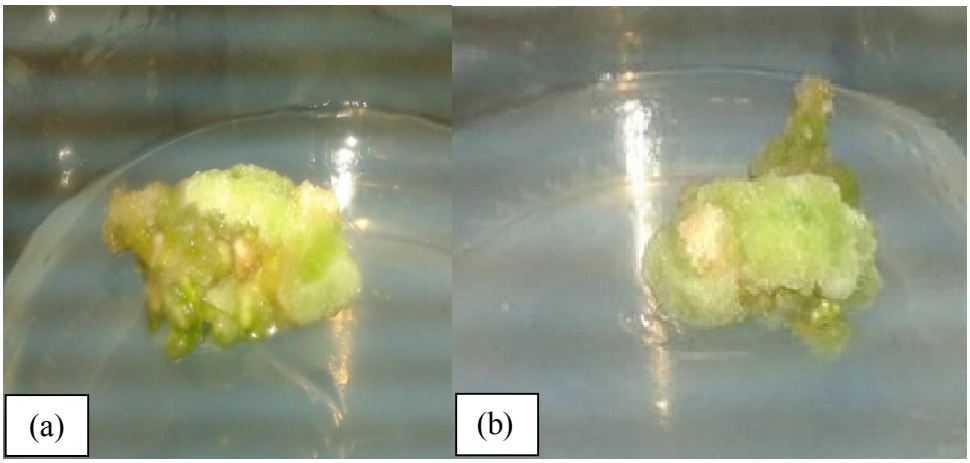

Figure 1. Callus induction from leaves explants *Solanum nigrum*, granulated (a) and friable (b) after 4 weeks of culture in MS medium supplemented with different concentrations of NAA and BA

3.2. Chemical analyses of the mother plants of *Solanum nigrum* and its callus

3.2.1. Preliminary Phytochemical Screening

The preliminary phytochemical screening revealed that alkaloids, glycosides, cardiac glycosides, saponins, phenol, tannins and flavonoids were present in *Solanum nigrum* plants whereas, glycoside saponins, phenol and flavonoids were found in callus of the plant. In addition, sterol was absence in both callus and the mother plant (Table 2).

It was realized that these results of this study were partially in accordance with the observation obtained by (Venkatesan*et al.* 2009) who reported that the phytochemical screening of the crude extract of *Solanum nigrum* revealed the presence of alkaloids, reducing sugars, tannis, flavonoids, phlobatannis and steroids. Similarly, Lali (2012) reported that phytochemical analysis of petroleum ether, chloroform, benzene, methanol and ethanol extracts from *Solanum nigrum* showed the presence of reducing sugars, anthraquinones, terpenoids, flavonoids, tannins, saponins, alkaloids and cardiac glycosides.

3.2.2. Active constituents

The results in table (3) showed that the amount of phenolic and flavonoids contents were higher significantly in the mother plants than in callus. The total phenolic contents were recorded,

(314.4±1.09 mg g^{-1} Gallic acid) and (152±0.84 mg $g.^{-1}$ Gallic acid) in the mother plant and in callus, respectively and total flavonoids were recorded (290.4±0.5mg g^{-1} rotund) and (105±0.685mg g^{-1} rotund) in the mother plant and in callus, respectively. However, these results are contrary to the findings of Yogananth *et al*., (2009) who reported that the solasodine content in callus extracts of *Solanum nigrum* were higher compared to that the field grown leaves extracts.

In this respect, Ravi et al., (2009) reported that ethanolic extract of *Solanum nigrum* produced higher significantly anti-inflammatory and anticonvulsant. These authors added that flavonoids present in plant extract might be a responsible active constituent for this activity.

Table 2 The preliminary phytochemical screening of the mother plant of *Solanum nigrum* and its callus

Group	Test	The mother plant	Callus
Glycosides	Glycosides test	+ve	+ve
	Modified borntrager,s test	+ve	+ve
Alkaloids	Wagner's test	+ve	-ve
	Dragndorrf test	+ve	-ve
Cardiac glycosides	Legal's test	+ve	-ve
Saponins	Foam test	+ve	+ve
	Blood hemolysis test	+ve	+ve
Phenolic compound	Ferric chloride test	+ve	+ve
Sterol	Salkawskis test	-ve	-ve
	Libermannburchard's test	-ve	-ve
Tannins	Lead acetate test	+ve	-ve
	Gelatin test	+ve	-ve
flavonoids	Shinoda's test	+ve	+ve
	NaOH test	+ve	+ve
Triturbine	Xanthoproteic test	+ve	-ve
Volatile oil	Stem distillation	+ve	-ve

(+ve) mean present, (-ve) mean absent

Table 3. Total active constituents of the mother plant of *Solanum nigrum* and its callus

	Mother Plant	Callus
Total flavonoids (mg g^{-1} rotund)	290.4±0.5	105±0.68
Total phenolic acids (mg $g.^{-1}$ Gallic acid)	314.4±1.09	152±0.84

The results of antimicrobial activity of ethanolic extract of *Solanum nigrum* was shown in table (4). The ethanolic extract of both the mother plant and its callus showed, generally, great antimicrobial activities against 7 types of bacteria and 2 fungus species tested. It was observed that the mother plant extract was potent antimicrobial against all tested organisms, with two exceptions, than callus extract. The exceptional cases were observed with *Staphylococcus aureus* and *Bacillus subtilis* where callus extract had more activity than mother plant extract. The reason for being the mother plant extract more potent is it contains alkaloids, tannins and volatile oils that are absent in callus extract.

The data present antimicrobial activity results confirmed the finding of (Khizar *et al.* (2014) who reported that the possible potential of antimicrobial as well as antifungal activity of fruits of *Solanum nigrum* extracts.

4. Conclusion

In conclusion, the presence of glycoside, saponins, phenol and flavonoids, in both the mother plant extract and its callus extract, suggest that these constituents are responsible for the antimicrobial activity. In the present study of *in vitro* propagation of *Solanum nigrum* through callus induction we have achieved a clear and a simple protocol for phytochemical screening and antimicrobial activity. Callus culture system offer many advantages as model system for biological investigations. In view of the medicinal properties and increased demand of this plant in pharmaceutical industry, the outline protocol offers a simple system for mass propagation of this important medicinal plant.

Table 4. Antimicrobial activity of ethanolic extract of *Solanum nigrum* (mother plant and its callus) by disc diffusion method. Inhibition zone (mm). Values are means of 3 readings

No	Microorganism	Extract of Mother plant (5 mg/ml)	Extract of callus (5mg/ml)
1	**Gram positive organism**		
	Streptococcus faecalis	19.1	14.1
	Staphylococcus aureus	20.7	22.1
	Staphylococcus epidermidis	21.3	15.2
	Bacillus anthracis	20.1	16.1
	Bacillus subtilis	18.1	19.0
2	**Gram Negative organism**		
	Escherichia coli	20.1	12.5
	Pseudomonas aeruginosa	21.5	16.1
3	**Fungal species**		
	Microsporium gypsum	14.2	11.5
	Aspergillus niger	13.7	10.1

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5/27/2015