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Evaluation of antimicrobial activity of callus of *Phyla nodiflora* (L.) Family Verbenaceae

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Abstract

The Methanolic extract of callus of *Phyla nodiflora (L.)* has been evaluated for antibacterial activity of *Staphylococcus aureus, Acinetobacter baumanii, Klebseilla pneumonia, Bacillus subtilis, Fusarium Spp.* and Candida. The regenerated callus was collected and subjected to maceration for 7 days with methanol. After maceration the menstrum was concentrated. The anti microbial screening was performed in the methanolic extract of the callus by agar diffusion methods using a paper disc. The sterilized (autoclaved at 120˚C for 30 min) medium was inoculated with the suspension of the microorganisms. The paper impregnated with the extracts (1000 µg/ml) was placed on the solidified medium. The petri dishes were pre incubated for one hour at room temperature and incubated at 37˚C for 24hr and 48 hr for antibacterial and antifungal activity, respectively (chloramphenicol (1000µg/ml) was used as a standard for anti bacterial and anti fungal activities respectively. The ethanol extract showed significant antibacterial activity due to the presence of bio-active compounds.

Keywords : Methanolic extract, *Phyla nodiflora, Staphylococcus aureus, Acinetobacter baumanii, Klebseilla pneumonia, Bacillus subtilis* and *Fusarium Spp.*

Introduction

Phyla nodiflora (L.) (=*Lippia nodiflora* (L.) belongs to Verbenaceae family, which is widely distributed in South Africa and Central America (Terblanche and Kornelius, 1996). It is a runner plant with scanty roots possessing various ethanobotanical and medical applications in adenopathy, chronic indolent ulcers, etc. (Kirtikar and Basu, 1975).

The aerial parts of this plant were used as anodyne, antibacterial, diuretic, emmenagogue, parasiticide, refrigerant and febrifuge agents (Agarwal and Kamal, 1997). Several researchers have reported various pharmacological properties including antispasmodic, hypotensive, anti-inflammatory, analgesic, antipyretic (Forestieri *et al.*, 1996), antibacterial, antinociceptive, antifungal, antioxidant and free radical scavenging activities (Shukla *et al.*, 2009). *P. nodiflora* extracts have been used to cure multiple skin diseases and hair afflictions (Abbasi *et al.*, 2010). Nodes and shoot tips of *Phyla nodiflora* had successfully propagated for induction of shoot multiplication. Nodes and shoot tips were cultured on MS medium supplemented with cytokinin, benzyl adenine and kinetin. The maximum numbers of shoots were produced in 3.0mgl^{-1} and an

average of 14.66 ± 1.30 shoots were produced from each explants. Maximum number of shoots 16.4 ± 4.00 was produced in half strength MS medium containing IBA (Evelyne Priya and Ravindhran, 2011).

Callus induction and accumulation of alkaloid in stem and shoot tip explants of *Phyla nodiflora* were cultured on different media such as MS, B5, SH, and WPM. Stem explants showed better callus biomass on MS medium supplemented with 1.5 mgl^{-1} naphthalene acetic acid and 1.0 mgl^{-1} benzyl amino urine. The alkaloid content was higher in regenerated callus than intact stems and shoot tip explants, which were analyzed by Gravimetric method, HPLC and TLC (Abdul Bakkrudneen *et al.*, 2011).

This plant is over-exploited due to its high medicinal value and hence, propagation of this plant by tissue culture may be mandatory, which offers a greater potential to deliver large quantities of disease-free, true-type healthy stock within a short span of time (Hussain *et al.*, 2001). In the present work an attempt has been made on the antimicrobial activities of Callus of *Phyla nodiflora* on the selected micro-organisms.

Materials and methods

Extraction of callus

The procured calli were first dried at room temperature for a few days and the material was then crushed to a fine powder. 500 mg of dried and powdered callus was placed in a 500 ml conical flask and 100 ml 70 % methanol was added. Then the conical flask was transferred to shaker for 8 hr. Extracts thus obtained was filtered through Buchner funnel and concentrated under vacuum. Then the concentrated extract was dried and stored in air-tight container for further analysis.

Antimicrobial assay

Preparation of culture media

Muller Hinton agar and broth, PDA agar and broth were prepared with sterilized water as per the instruction of the manufacturers.

Test organisms

Bacteria : *Staphylococcus aureus, Klebseilla pneumonia, Bacillus subtilis, Escherichia coli and Acinetobacter baumanii.*

Disc diffusion method

Anti-bacterial assay was performed by using the modified Kirby-Bauer disk diffusion susceptibility method (Bauer *et al.*, 1996). The bacterial strains were suspended in 4ml of normal saline (0.85%) and the density of the suspension was adjusted to approximately 108 CFU ml^{-1} using the 0.5 Mac Ferlands method. The surface of the sterile 3.8% Mueller Hinton agar (Himedia, India) in petri dishes was dried and the test strain was inoculated with a sterile swab to obtain a homogenous bacterial lawn. The sterilized 6mm discs (Himedia) containing 10µl of fraction was placed onto the agar, and the inhibition zones were measured (in mm) after incubation for 18 hr at 37◦C. DMSO was taken as negative control for organic extracts. Different organic solvent extracts (1% v/v) in DMSO did not affect the growth of microorganisms in accordance with our control experiments. The petri dishes are inverted and incubated for 24 hr at 37°C. Clear inhibition zones around the discs indicated the presence of antimicrobial activity.

Antimicrobial activity of methanolic extract of callus (MEC) of *Phyla nodiflora*

The paper impregnated with the extracts (1000 µg/ml) was placed on the solidified medium. The petri dishes were pre incubated for one hr at room temperature and incubated at 37˚C for 24hr and 48 hr for antibacterial and antifungal activity respectively. Chloramphenicol (1000 µg/ml) was used as a standard

Table – 1. Antimicrobial activity of methanolic extract of callus (MEC) of *Phyla nodiflora*

Bacterial Strains	Concentration						
	1 (mg/ml)	5 (mg/ml)	10 (mg/ml)	15 (mg/ml)	20 (mg/ml)	Positive control	Negative control
S.aureus	-	-	11	13	17	22	-
A.baumanii	-	6	11	12	15	22	-
K. pneumonia	-	-	-	-	-	22	-
B.substilis	-	6	8	11	14	22	-
Fusarium spp.	-	-	6	10	12	22	-
C.albicans	-	6	8	12	18	22	-

for anti bacterial and anti fungal activities respectively. The ethanol extract showed significant antibacterial activity due to the presence of bio-active compounds.

Result and discussion

The antibacterial activity of methanolic extract of callus of *Phyla nodiflora* was shown in Table - 1. Five concentration of extracts were taken (1,5,10,15, 20 µg/ml) and tested against bacteria, namely, *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Bacillus subtilis, Candida albicans* and *Fusarium* species. Zone of inhibition for the following was measured and observed on increasing the concentration of extracts (Fig.- 1a - d). The maximum zone of inhibition was obtained against *Staphylococcus aureus* (Fig.- 1a).

Callus is undifferentiated and unorganized mass of parenchyma cells formed by the proliferation of parent tissue. Callus tissue is a good source of genetic variability and adventitious shoot formation. The callus so produced on the medium was green compact healthy fast growing and organogenic. Indirect shoot regeneration through callus phase was obtained from leaf explants. From the studies, it revealed that the active compounds from *Phyla nodiflora* is more potent in controlling *Candida albicans*, *Bacillus subtilis*, and

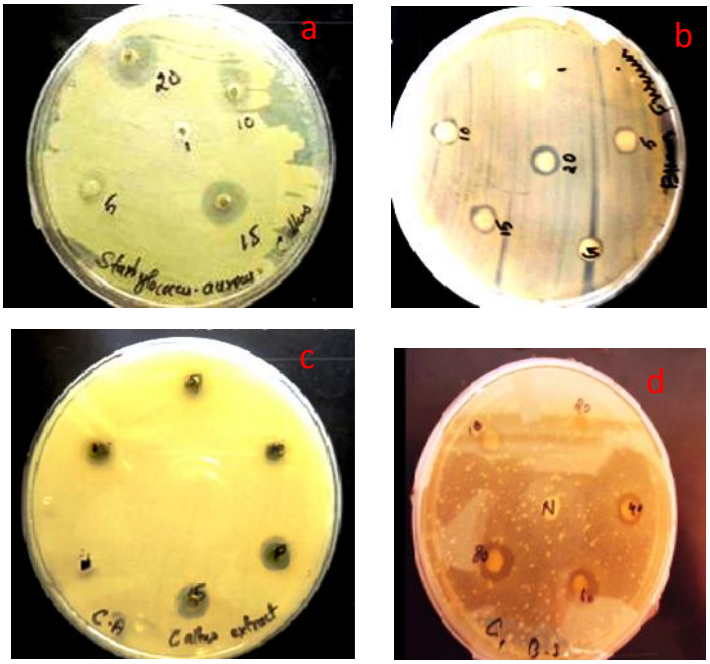

Fig.-1. Antimicrobial activity of MEC of *Phyla nodiflora*
(a) zone of inhibition of methanolic extract of callus from *Phyla nodiflora on Candida albicans.*(b) *taphylococcus aureus* (c) : *Fusarium species* (d) : *Bacillus subtilis*

Staphylococcus aureus. Thus the antimicrobial activity of the extracts on the test organisms may be due to the presence of the above phytochemical components. It is very clear that the active compound has a maximum zone of inhibition when compared with chloramphenicol as a standard. This protocol can be used for continuous

and rapid multiplication of *Phyla nodiflora*. These *in vitro* raised plants did not show any morphological abnormality when compared to original plants.

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