

In Vitro phytochemical and antibacterial studies on *Rhinacanthus nasutus* (L.) Kurz - a medicinally important plant

Abstract

The present study was aimed to evaluate and compare the phytochemical and antibacterial potential of mother plants (aerial parts), *in vitro* leaves and stems derived callus and *in vitro* callus mediated shootlets of *Rhinacanthus nasutus* (L.) Kurz. Preliminary phytochemical analysis and extraction was performed on stem and leaves segments derived calli (4 weeks old), aerial portions of mother plants and calli mediated shootlets of *R. nasutus*. Anti-bacterial activity of different extracts (25µg) (ethanol, chloroform, aqueous and ethyl acetate) of stem and leaves segments derived calli, aerial portions of mother plants and calli mediated shootlets were investigated by well-diffusion method against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bacillus subtilis* and *Escherichia coli*. The preliminary phytochemical studies confirmed that the leaves and stem derived calli mediated shootlets showed the higher degree of metabolite constituents and extraction value compared to the *in vitro* derived calli and *in vivo* leaves and stem (aerial portions). The ethyl acetate extracted solvents showed the maximum bio-efficacy compared with other solvents due to the presence of more compounds such as saponins, steroids, tannins, phenolics, triterpenoids, alkaloids, coumarins, anthraquinones, glycosides and flavonoids. The present study observation suggested that a possibility to establish high yielding genotypes by *in vitro* culture for production of medicinally important bioactive compounds. The methods developed in this work make possible for the low volume and high potential production of active principles under *in vitro* condition in short duration with less amount of explants utilization.

Keywords: *Rhinacanthus nasutus*, bio-efficacy, phytochemical, anti-bacterial

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Introduction

Due to the less side effects and safety, the importance of medicinal and aromatic plants has drawn attention from time to time.¹ *Rhinacanthus nasutus* (L.) Kurz is the best known member of the family Acanthaceae. In the ayurvedic system of medicine, *R. nasutus* is practiced as a traditional medicinal plant in the Indian sub continent, China and Southeast Asia including Thailand.² The literatures reported that the traditional medicine preparation from roots, stems and leaves of this shrub has long been used in Thai traditional medicine for treatment of various diseases such as eczema, pulmonary tuberculosis, herpes, hepatitis, diabetes, cancer, obesity, leprosy, eczema, scurvy, dhobi's itch hypertension and various skin diseases.^{3,4} Several reports of investigation states that leaves and stems of *R. nasutus* contains various types of flavonoids, benzenoids, coumarin, anthraquinone, quinone, glycosides, carbohydrate, triterpenes, sterols, and chlorophyll.⁵ The naphthoquinone; rhinacanthins (A-D, G-Q), rhinacanthone and lignan groups are the main bioactive compounds.⁶ The leaf petroleum ether fraction shows significant mosquitocidal activity.⁷ It has been reported that rhinacanthin-C, rhinacanthin-D and rhinacanthin-N isolated from *R. nasutus* possessed various biological activities.⁸⁻¹⁰

Naphthoquinone compounds have been reported to possess anti-proliferative activity against a panel of cancer cells. Rhinacanthone (3, 4-dihydro-3, 3-dimethyl-2H-naphtha [1,2-b]pyran-5, 6-dione) was isolated from the shrub, *R. nasutus*, which is essential for the tumor activity.¹¹ Due to its high demand resulted over exploitation from the wild and leads to depletion of this important medicinal plant

demands to develop an alternative protocol for the propagation two of *R. nasutus*. Johnson et al.¹² reported the micro propagation and calli mediated organogenesis of *R. nasutus*. To fulfill the commercial requirements and growing demand of this herb, an alternative source is needed to meet the demand. *In vitro* cell culture or cell line culture is an effective alternative pathway as it has already proved successful in many other cases.¹³ To date, only a few plant metabolites have been produced via cell culture production in large scale production. With reference to quantity and quality of secondary metabolites, few cell line cultures proved to possess more amounts of secondary metabolites than the differentiated mother plant.¹⁴ The main intention of this study is to evaluate and compare the phytochemical and antibacterial potential of mother plants (*in vivo*), *in vitro* leaves and stem derived callus and calli mediated plantlets of *R. nasutus*.

Materials and methods

Phytochemical analysis

The mother plants (leaves and stem aerial portions) and calli mediated shootlets of *R. nasutus* were air and shade dried for 15 d at room temperature and pulverized to powder using the electric homogenizer. *In vitro* derived callus (leaves and inter-nodal) were dried in the hot air oven and powdered using the electric homogenizer. The powdered (2 g) samples were extracted with 100ml of solvent (chloroform, ethanol, ethyl acetate and aqueous) for 8h by using the soxhlet apparatus.¹⁵ The preliminary phytochemical screening was performed by Harborne method.¹⁶

Antibacterial activity

The crude extracts of *R. nasutus* leaves, stem and calli mediated shootlets derived extracts were concentrated and subjected for their antibacterial activity against the selected pathogenic bacteria. Pure bacterial cultures viz., *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bacillus subtilis* (ATCC 10707) and *E. coli* (ATCC 35218) were maintained on nutrient broth at 37°C for 24 h. Antibacterial efficacy was performed by well diffusion method with 25µg/ml of extract and incubated for 24 h at 37°C.¹⁷ The inhibition zone and antibacterial activity against the pathogenic bacteria were recorded. Amoxicillin and tetracycline discs were used as a positive control. The solvents (chloroform, ethanol, ethyl acetate and aqueous) alone are used as negative control. The experiments were repeated in triplicate and the results were documented.

Results

The preliminary phytochemical studies confirmed that the occurrence of more quantity of secondary metabolites in the calli mediated plantlets than mother plants *in vivo* leaves and stem (aerial portions) and *in vitro* derived calli. The phytochemical study documented the high quantity of steroids, triterpenoids, glycosides, coumarins, anthraquinones, saponins, alkaloids, flavonoids, phenolic compounds, tannins, sugar etc in calli mediated shootlets compared to *in vitro* derived calli and *in vivo* aerial portions (mother plants). Different kinds of solvents were used for extraction, of which ethyl acetate extracts of *R. nasutus* showed maximum metabolites occurrence (13/13) compared with other extracts (Table 1). Similarly the ethyl acetate extracts of *R. nasutus* showed the maximum bio-efficacy (3/5) due to the presence of more metabolites (Table 2).

Table 1 Preliminary Phytochemical studies on the aerial portions, *in vitro* derived calli (stem and leaves) and calli mediated shootlets of *R. nasutus*

Phyto constituents	Aerial portions (Mother Plants)				<i>in vitro</i> Derived calli				calli Mediated shoot lets			
	C	E	EA	A	C	E	EA	A	C	E	EA	A
Alkaloids	+	+	+	+	+	+	++	+	+	+	++	+
Flavonoids	-	+	+	+	-	+	++	+	-	+	++	+
Phenolics	+	+	+	+	+	+	++	+	+	+	++	+
Steroids	+	+	+	-	+	+	++	-	+	+	++	-
Coumarins	-	-	+	-	-	-	+	-	-	-	+	-
Catechins	-	+	+	-	-	+	+	-	-	+	+	-
Tannins	+	-	+	-	+	-	++	-	+	-	++	-
Saponins	-	+	+	-	-	+	++	-	-	+	++	-
Triterpenoids	+	+	+	+	+	+	+	+	+	+	+	+
Sugars	+	+	+	+	+	+	+	+	+	+	+	+
Proteins	+	+	+	+	+	+	++	+	+	+	++	+
Anthraquinones	-	+	+	-	-	+	+	-	-	+	+	-
Glycosides	+	+	+	+	+	+	++	+	+	+	++	+
Total	8	11	13	7	8	11	13	7	8	11	13	7

C, chloroform; E, ethanol; EA, ethyl acetate; A, aqueous

Table 2 Anti-bacterial activity of aerial portions, *in vitro* derived calli (stem and leaves) and calli mediated shootlets of *R. nasutus* (25µg)

Microorganisms	Zone of inhibition in Mm											
	aerial portions (Mother Plants)				<i>In vitro</i> Derived calli				Calli mediated shootlets			
	C	E	EA	A	C	E	EA	A	C	E	EA	A
<i>S. aureus</i>	14	16	20	6	15	18	20	5	17	19	26	10
<i>P. aeruginosa</i>	5	7	7	0	9	10	12	0	11	14	16	0
<i>K. pneumoniae</i>	6	15	18	4	9	19	24	7	12	28	28	11
<i>B. subtilis</i>	8	11	13	7	13	18	17	10	17	26	24	16
<i>E. coli</i>	11	6	10	4	16	9	15	4	18	12	17	5

C, chloroform; E, ethanol; EA, ethyl acetate; A, aqueous

Discussion

Various compounds known as pharmaceuticals, antimicrobials, secondary metabolites such as coumarins, flavonoids, anthocyanins, tannins, anthraquinones, naphthoquinones, sterols, triterpenes, carotenoids and nicotine have been produced using tissue culture technique. In the present study the secondary metabolites have been produced using tissue culture techniques; and the metabolites were compared for the degree of presence and efficacy of the extracts of aerial portions, stem and leaf derived calli and calli mediated shootlets of *R. nasutus*. The result of the present study revealed that the antibacterial efficacies of ethanol, chloroform, ethyl acetate and

aqueous extracts of leaves and leaves segment derived calli, stem and stem derived calli and calli mediated shootlets extracts of *R. nasutus* was diverse in effectiveness which may be attributed due to the presence of the secondary metabolites. Results of the present study are directly correlated with the previous observations.¹⁸⁻²³ The various extracts of aerial portions and calli mediated shootlets showed the inhibition against the selected pathogenic bacteria. The earlier observations on *Balisospermum montanum* and *Alternanthera sessilis* aerial portions and *in vitro* derived calli and calli mediated shootlets extract demonstrated significant antibacterial activity.^{14,15}

The result of the present study revealed that the antibacterial efficacies of acetone, ethanol, chloroform and ethyl acetate extracts of

mother plants, *in vitro* derived calli and calli mediated shootlets of *R. nasutus* with varied frequency which may be attributed to the presence of the secondary metabolites (Table 1). The ethyl acetate extracts of *R. nasutus* calli mediated shootlets showed the maximum bio-efficacy compared with other solvents due to the presence of more compounds such as saponins, steroids, tannins, phenolics, triterpenoids, alkaloids and flavonoids (Table 1 & 2). Results of the present study are directly correlated with the previous observations.^{12-14,17-25} The ethyl acetate extracts *R. nasutus in vitro* calli derived shootlets showed the inhibition against the selected pathogenic bacteria. This study observations were supplemented by earlier observations on *Baliospermum montanum*, *Hypericum perforatum* and *Mimosa hamata* leaf and callus extract demonstrated significant antibacterial and antimicrobial activity.^{13,17,25} The methods developed in this work enable high potential production of active principles with low volume input under *in vitro* condition in short duration with less amount of explants without any seasonal influence. The results of the present study demonstrated that *in vitro* cultivation of calli mediated shootlets significantly enhanced production of secondary metabolites compared to micro propagated plants. The results of the present study observation indicated the possibility to establish high yielding genotypes by *in vitro* culture for production of medicinally important bioactive compounds.

Conflicts of Interest

The authors declare no conflict of interest.

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