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Original Paper

In vitro antioxidant activity of *Anisochilus carnosus* leaf, stem and callus

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Abstract

Anisochilus carnosus (Lamiaceae) is an annual herb growing at high altitudes, which is used in traditional medicine. The present study was carried out to determine the total phenolic concentration, total flavonoid concentration and antioxidant activity of ethanolic extracts of leaf and stem, and their respective calli. For callus development, healthy leaf and stem explants of *A. carnosus* were inoculated in Murashige and Skoog medium with auxin-type plant growth regulators 2,4-dichlorophenoxy acetic acid (2,4-D), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and naphthalene acetic acid (NAA). Callus initiation was started within one week of inoculation and profound callusing was observed in presence of 2 mg L^{-1} 2,4-D, IAA, and IBA. NAA alone did not induce any callus at lower doses, while combination of 2,4-D and 2,4-D + NAA produced callus at all tested concentrations. Maximum total phenolic concentration was found in the leaf callus, while the highest flavonoid concentration was found in the stem callus. The antioxidant activity of both leaf and stem calli extracts were consistent. In terms of antioxidant activity, callus extracts exceeded leaf and stem extracts. It can be concluded that the callus extract is an excellent source of phytotherapeutic antioxidants. Callus-derived secondary metabolites from *A. carnosus* have potential use in a variety of biological applications.

Key words: *Anisochilus carnosus*, antioxidants, flavonoids, phenolics, radical scavengers.
Abbreviations: 2,4-D, 2,4-dichlorophenoxy acetic acid; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; NAA, naphthalene acetic acid; DPPH, 1,1-diphenyl-2-picrylhydrazyl; ABTS, 2,2'-azino-bis(3-ethylbenzthiazoline-6 sulfonic acid).

Introduction

Medicinal plants are rich in natural chemical compounds with high therapeutic potential. *Anisochilus carnosus* (L.f.) Wall is a valuable medicinal plant that is extensively utilized in traditional medicine. It is widely distributed in Nepal, India, Sri Lanka, South China and Thailand (Suddee, Paton 2009). The plant root powder is used by the Thakar tribes of Maharashtra to relieve stomach problems (Kamble et al. 2008). Tribals in the Kolli hills of Tamilnadu apply leaf paste of *A. carnosus* with coconut oil for treatment of skin disorders (Xavier et al. 2015). The plant is known for its antiulcer properties, and ulcers are treated using a water decoction of the plant (Ha et al. 2011). Previous research on *A. carnosus* suggests that the species shows antimicrobial (Valarmathy et al. 2010; Vijaya et al. 2013; Shetty et al. 2017), antioxidant (Bhagat et al. 2011), anticancer (Bhagat et al. 2014; Bhagath et al. 2016), antiulcer (Ha et al. 2011) and hepatoprotective activities (Wall et al. 2010; Reshi et al. 2017). *A. carnosus* is an immunostimulant, being a constituent of "Kabasura kudineer", a well-known Sidha herbal decoction for therapeutic purposes (Balaji et al. 2020). Thus, "Kabasura kudineer" has been shown to reduce viral load in asymptomatic COVID-19 patients (Natarajan et al. 2021).

Phytochemical studies help to explore the future possibilities of medicinally important plants in pharmaceutical industries. Polyphenolic antioxidants have gained popularity recently due to their ability to combat free radicals associated to a variety of ailments (Li et al. 2014). Flavonoids are one of the major compounds that have gained a lot of attention in the pharmaceutical market. Ethanolic extract of *A. carnosus* contains the highest concentration of luteolin (0.372% w/w) (Bhagat et al. 2014). Carvacrol, camphor and α-cis-bergamotene are the major components in essential oil obtained from aerial parts of the plant (Senatore et al. 2003). Presence of luteolin, apigenin, luteolin-7-glucoside and apigenin-7-glucoside were reported from dry leaves (Bhagat et al. 2011).

In vitro plant tissue culture is one alternative for producing important plant active compounds since it ensures independence from geographical conditions by eliminating the need to rely on wild plants (Espinosa-Leal et al. 2018). The extracts of callus and cell suspension cultures, which yield promising amounts of polyphenols, notably flavonoids, could be exploited as an alternative source of antioxidants (Mamdouh, 2022). Although *A. carnosus* is a significant medicinal plant with a wide range of phytoconstituents, no studies have specifically carried out for the production of secondary metabolites from

their *in vitro* cultures. There have been no studies on the antioxidant properties of callus extracts of the plant. There is no other literature on the estimation of phenolics and flavonoids from the callus extracts of *A. carnosus* to date. In this study, the total phenolic and total flavonoid contents of leaf, stem and their calli extracts were determined spectrophotometrically, and an effective approach for callus generation was developed.

Materials and methods

Plant material

Healthy plants were collected from their natural habitat of Oonjappara, Nadukani (L10.0540, E76.6030; altitude 43 m), a village in Ernakulam district, Kerala, India. The plant was identified by a taxonomist at the Deptartment of Botany, St. Albert's College, Ernakulam, Kerala and a voucher specimen (Voucher number 504) was deposited in the St. Albert's College Herbarium.

Explant preparation

Healthy leaf and stem segments were collected, and surface sterilized for 15 min in running tap water, 10 min in Tween 80, 5 min in 70% ethanol, and 10 min in 5% bavistin. The explants were treated with 0.1% $HgCl_2$ for 3 min inside a laminar air flow. The explants were rinsed three times with sterile double distilled water.

Callus induction and proliferation

Sterilized explants were inoculated in MS (Murashige and Skoog) medium supplemented with different concentrations and combinations of auxin-type plant growth regulators: 2,4-dichoro phenoxy acetic acid (2,4-D), naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA) and indole-3-butyric acid (BA). The pH of the medium was adjusted to 5.8 before autoclaving at 121 °C for 20 min. Callus cultures were maintained in a growth chamber at 25 ± 2 °C. Initiated callus was then transferred to fresh medium containing the same components at 21-day intervals. Callus was harvested after fifth subculture and dried in an oven and powdered using a kitchen grinder.

Preparation of extracts

The dried powered leaves, stems and their respective calli were separately macerated with 70% ethanol (1 : 40, w/v) for 3 days at room temperature. The extracts were kept on an orbital shaker for 5 h each day. After three days of complete extraction (the same solvent was used throughout the extraction to extract the crude product), the extract was filtered using Whatman No.1 filter paper. Filtrate was kept in hot air oven at 50 °C for complete removal of solvent. The crude extract obtained after solvent evaporation was weighed and kept in an airtight container at –18 °C.

Determination of total phenolic concentration

The phenolic estimation of the extracts were performed by spectrophotometric method using Folin-Ciocalteau reagent (Roby et al. 2013). Gallic acid was used as an external standard. An extract sample (10 mg) of each extract was accurately weighed and made up to 1 mL with dimethyl sufoxide. To 0.2 mL of sample, 5 mL of Folin-Ciocalteau reagent was added (diluted with water 1 : 10 v/v). After 5 min of incubation, 4 mL of 7.5% sodium carbonate solution was added to it. The mixture was stirred and incubated at room temperature for 2 h. After incubation, the absorbance was measured at 750 nm using an UV-VIS spectrophotometer (Agilent, Cary 60). A standard curve was prepared using gallic acid (100 to 1000 μg mL^{-1}) for the calculation of total phenolic concentration. The values obtained were interpreted using a standard graph of gallic acid to estimate the milligram equivalents of gallic acid using the following equation based on the calibration curve:

$$y = 0.001 \text{ x} + 0.046,$$

where y was the absorbance.

Determination of total flavonoid concentration

The total flavonoid concentration of extract was determined and measured by aluminium chloride (AlCl3) colorimetric assay (Chang et al. 2002). Quercetin was used as the reference flavonoid (0.01 to 1 mg mL^{-1}). In this method, 1 mL methanol, 0.1 mL aluminium chloride, 0.1 mL potassium acetate solution, and 2.8 mL distilled water were added to 0.5 mL of the sample. After incubation the absorbance was measured at 415nm using an UV-VIS spectrophotometer (Agilent, Cary 60) against the blank. A calibration curve was constructed using quercetin as the reference standard. The values obtained were interpreted using a standard graph to obtain the milligram equivalents of quercetin.

Antioxidant activity

The radical scavenging activity of leaf, stem and their respective callus extracts were determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay according to Chang et al. (2001) with some modifications. DPPH (0.1 mM) solution was prepared by dissolving 4 mg of DPPH in 100 mL of methanol. Different concentrations (12.5, 25, 50, 100 and 200 μg mL^{-1}) of 1.5 mL samples from stock solutions were made up to a final volume of 20 μL with dimethyl sufoxide. To this 1.48 mL of 0.1 mM DPPH solution was added. As a control, an equal amount of distilled water without the test chemical was used. After incubation of the reaction mixture in the dark at room temperature for 20 minutes, the absorbance of each sample was determined at 517 nm using an UV-VIS spectrophotometer (Agilent Carry 60). DPPH (3 mL) was used as a control and percentage inhibition was then calculated as follows:

$$\text{Percentage of inhibition} = A_{\text{control}} - A_{\text{test}} \times 100 / A_{\text{control}},$$

where A_{control} is the absorbance of the control (with all reagents and without any test compound) and A_{test} is the absorbance of the test compound.

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) test is an another popular in vitro method for determining the antioxidant capacity of extracts (Shah, Modi 2015). For reagent preparation, 0.2 mL of 17 mM potassium persulfate was added to 50 mL of 20 mM ABTS solution. To 0.2 mL of samples with varying concentrations (125 to 2000 µg mL^{-1}) from a stock concentration of 10 mg mL^{-1}, 0.16 mL of ABTS solution was added. It was then made up to a final volume of 1.36 mL using 1 mL distilled water. The mixture was then incubated for 20 min before being spectrophotometrically analyzed at 734 nm. Ascorbic acid (10 mg mL^{-1}) was utilized as the reference antioxidant, and distilled water without the test chemical was employed as the control. All analyses were done in triplicate. The percentage inhibition was calculated using the previous formula.

Statistical analysis

All experiments were performed in triplicate and the data were presented as mean ± SD. Data were statistically analyzed using SPSS Version 25. Statistical significance between samples were analyzed using ANOVA and DMRT. The K-S test for normality and Leven's test for variances were carried out. The Welch test was also carried out for ABTS. IC50 values were calculated using ED 50 PLUS V1.0 Software.

Results

Callus induction and proliferation

Plant growth regulators (2,4-D, IBA, IAA, and NAA) in various concentrations and combinations were employed to produce callus from healthy leaf and stem segments. All tested 2,4-D concentrations and 2,4-D (4 mg L^{-1}) + NAA (6 mg L^{-1}) treatments produced callus from both leaf and stem explants (Table 1). Different media led to the formation of calli that had unique characteristics (Fig. 1). Among the media, MS medium supplemented with 2 mg L^{-1} 2,4-D was the best for callus initiation and showed the highest percentage of callus response from both stem and leaf explants. Stem explants that were inoculated into a medium supplemented with 2 mg L^{-1} 2, 4 D produced callus within a week of inoculation. There was no callus response observed from leaf and stem explants in basal MS media. Lower concentration of IAA, IBA, and NAA did not induce callus from either of the explants, but all the tested concentrations of 2,4-D induced callus from both leaf and stem explants. The MS medium containing 2 and 5mg L^{-1} 2,4-D produced profound callus from stem explants (Fig. 1A & B). Medium supplemented with 6 mg L^{-1} NAA initiated callus from stem explants after two weeks of inoculation, but the callus become brown after subculturing (Fig. 1C). A combination of 2,4-D and NAA also led to the development of callus, but this did not occur until two weeks after the initial inoculation. The stem produced a friable, compact, green callus (Fig. 1A), and the leaf produced a watery, yellowish callus (Fig. 1E).

Total phenolic and flavonoid concentration

The total phenolic and total flavonoid concentration of the leaf, stem, and their calli were determined using spectrophotometric methods. Leaf callus had larger total phenolic content than that in intact leaves (Table 2). There was significant difference in total phenolic concentration among different extracts ($F = 1265.4$, $p < 0.01$). The post-hoc test revealed that there were four subgroups among the extracts that were mutually significantly different at 5% level of significance. There was no significant difference observed between leaf and stem callus extracts. The highest mean value of total phenolic concentration was for leaf callus and the lowest for leaf callus produced in complete darkness.

However, the total flavonoid concentration of stem callus was significantly higher than that of leaf callus (Table 2).

Table 1. Effect of different plant growth regulators 2,4-D, IAA, IBA and NAA on the production of callus from leaf and stem explants of *Anisochilus carnosus*. Results are means ± SD

Growth regulator	Concentration (mg mL^{-1})	Callus-forming explants (%)	
		Stem	Leaf
2,4-D	1	54.80 ± 2.85	46.23 ± 3.60
	2	99.00 ± 0.70	87.20 ± 2.36
	3	76.69 ± 1.28	71.60 ± 1.92
	4	84.46 ± 1.50	65.10 ± 1.13
	5	95.46 ± 0.47	76.96 ± 0.99
	6	85.43 ± 1.29	80.03 ± 1.29
IAA	1	_	_
	2	_	_
	3	_	_
	4	45.76 ± 2.21	33.26 ± 2.14
	5	52.86 ± 3.69	59.63 ± 1.50
	6	61.60 ± 1.05	50.63 ± 0.82
IBA	1	_	_
	2	_	_
	3	_	_
	4	26.56 ± 0.51	30.93 ± 2.20
	5	32.10 ± 1.31	27.70 ± 1.26
	6	59.30 ± 1.06	40.26 ± 2.47
NAA	1	_	_
	2	_	_
	3	_	_
	4	_	_
	5	64.90 ± 3.81	44.03 ± 2.34
	6	71.46 ± 1.22	63.53 ± 2.28
2,4-D + NAA	1 + 6	64.23 ± 1.30	63.83 ± 1.67
	2 + 6	63.20 ± 2.40	40.96 ± 1.43
	3 + 6	74.20 ± 3.79	73.43 ± 2.65
	4 + 6	87.20 ± 0.94	88.83 ± 2.17
	5 + 6	85.43 ± 1.08	81.30 ± 0.58

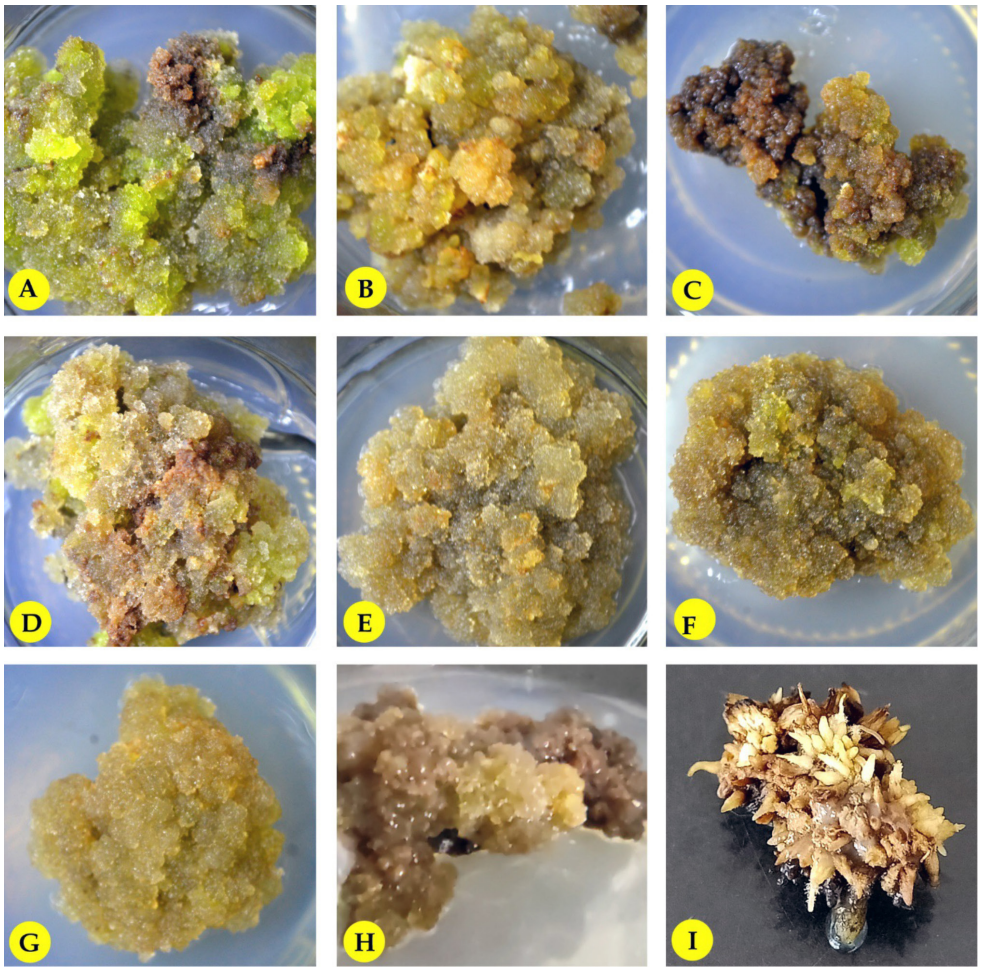

Fig. 1. Production of callus from leaf and stem explants of *Anisochilus carnosus*. A, stem callus at 2 mg L^{-1} 2,4-D. B, stem callus at 5 mg L^{-1} 2,4-D. C, stem callus at 6 mg L^{-1} NAA. D, stem callus at 4 mg L^{-1} 2,4-D + 6 mg L^{-1} NAA. E, leaf callus at 2 mg L^{-1} 2,4-D. F, leaf callus at 6 mg L^{-1} NAA. G, leaf callus at 6 mg L^{-1} IAA. H, leaf callus produced under complete darkness. I, adventitious structure developed from callus.

Under complete darkness, leaf callus produced significantly less flavonoid compounds. There was a significant difference in total flavonoid concentration between the extracts (F = 14145, $p < 0.01$). The post hoc analysis revealed that at the 5% level of significance, the mean values of all the extracts were significantly different from one another. The mean value of total flavonoid concentration of the callus that developed in complete darkness was lowest, whereas that of the callus that developed on the stem was highest.

Antioxidant activity of leaf, stem and callus

The DPPH and ABTS assays were used for the determination of free radical scavenging activity of leaf, stem and their calli (Table 3). The IC50 values were calculated from percentage inhibition of the extracts. All extracts showed antioxidant activity in the DPPH and ABTS assays and leaf callus exhibited higher antioxidant activity than that of intact leaves. Antioxidant activity of the extracts was in the order of leaf callus > stem callus > leaf callus proliferated under complete darkness > stem > leaves. When compared to other extracts, the existence of large phenolic concentration in leaf callus may explain its higher antioxidant activity, and high flavonoid concentration may influence the radical inhibition of stem callus.

The data for DPPH and ABTS assays did not significantly deviate from normality ($p > 0.05$). ANOVA showed that there were significant differences in DPPH and ABTS among different extracts ($p < 0.01$). The post hoc test results showed that the mean values of all the extracts are mutually significantly different at 5% level of significance.

Discussion

In the past ten years, a number of studies on callus cultures have been published in an effort to provide new phenolic and flavonoid compound sources from medicinal plants. An earlier study discovered that treatment with different types and combinations of plant growth regulators were the most effective and the fastest way for production and accumulation of some special phenolic molecules in the

Table 2. Total phenolic and flavonoid concentration of *Anisochilus carnosus* leaves, stem and their respective callus. Results are means ± SD. Significant difference among different extracts are indicated by different letters ($p < 0.01$)

Sample	Total phenolic concentration (µg gallic acid equivalents mg^{-1})	Total flavonoid concentration (µg quarcetin equivalents mg^{-1})
Leaves	81.32 ± 0.79 b	53.64 ± 0.21 b
Stem	83.70 ± 1.01 c	88.26 ± 0.05 d
Leaf callus	116.50 ± 0.02 d	62.53 ± 0.46 c
Stem callus	80.56 ± 0.55 b	115.57 ± 0.50 e
Leaf callus produced in dark	72.18 ± 1.24 a	42.83 ± 0.64 a

Table 3. Antioxidant activity (IC_{50} values) of leaf, stem and callus extracts of *Anisochilus carnosus*. Results are means ± SD. Significant difference among different extracts are indicated by different letters ($p < 0.01$)

Sample	DPPH (µg mL^{-1})	ABTS (µg mL^{-1})
Leaves	128.24 ± 2.16 f	1448.51 ± 15.33 e
Stem	107.68 ± 2.30 e	1239.30 ± 6.94 d
Leaf callus	71.22 ± 2.47 b	861.55 ± 19.81 b
Stem callus	81.43 ± 2.70 c	972.90 ± 5.28 c
Leaf callus produced in dark	94.67 ± 1.51 d	1665.42 ± 35.41 f
Ascorbic acid	25.18 ± 1.35 a	396.37 ± 9.23 a

calli of goji berry (Karakas 2020). Basil leaf derived callus at different growth regulator concentrations showed increased free radical scavenging activity when treated with NAA and/or thidiazuron (Nazir et al. 2019). The present study investigated the antioxidant content of the leaf, stem, and callus of *A. carnosus* using the colorimetric method. The callus produced from the leaf and stem explants is a potent source of phenolic and flavonoid contents with good antioxidant properties. Plant growth regulators have a great influence on callus development, and it is apparent that callus accumulates phenolics during repeated sub-culture. These phenolics play a major role in the free radical scavenging activity of the callus extracts. These antioxidants obtained from plant and their callus may have therapeutic and protective effects on humans. The antioxidant activity of *A. carnosus* aqueous and methanolic extracts was found to be significantly high, and it was suggested that, in order to prevent or delay the onset of ageing and age-related oxidative stress-related degenerative illnesses, leaves of the plant may be an effective natural antioxidant source (Bhagat et al. 2011). The plant has a diverse range of phytoconstituents and therefore might have the ability to cure diverse ailments starting from cough cold to cancer (Shirsat, Koche 2022). The total flavonoid and total phenol concentration of the extracts had a considerable impact on their free radical scavenging activities.

In a recent survey on utilization of uncultivated green leafy vegetables, it was found that *A. carnosus* is cooked with pulses and consumed as a leafy vegetable. Also, the plant is used to treat cough and stomach ulcers (Soujanya et al., 2021). Therefore, there is an urgent need to apply non-conventional methods for future commercial supply of *A. carnosus* (Subrahmanian et al. 2017). Hepatoprotective activity of leaf and leaf callus extracts have been reported in an earlier study and the plant's leaf and leaf callus contain secondary metabolites with hepatoprotective properties, and bioefficacy study of the extracts confirmed the considerable hepatoprotective activity of the plant (Reshi et al. 2018).

In the present study, various auxins-type growth regulators (2,4-D, IAA, IBA, and NAA) were tested for callus production from healthy leaf and stem explants on MS medium. 2,4-D, when used at a lower concentration than the other auxins, was found to be the most effective for callus production from both leaf and stem explants. These findings are consistent with earlier studies, which found that young explants cultured on MS medium supplemented with 2,4-D (1 to 3 mg mL^{-1}) demonstrated a high percentage of callus induction and proliferation (Reshi et al. 2018). However, a previous study found that the best callusing response from a leaf explant occurred in MS medium supplemented with 2,4-D at a concentration of 5 mg L^{-1} (Reshi et al. 2017). In the present study it was observed that MS medium supplemented with 5 mg L^{-1} 2,4-D resulted in adventitious structures from callus after the third subculture (Fig. 1E). On basal MS medium, no callus formation from leaf or stem explants was seen. In the present study, it was observed that, in contrast to leaf-produced calli, which were watery and yellow (Fig. 1E), stem-produced calli in all PGR treatments were green (Fig. 1A). Stem-derived callus yielded higher amounts of total flavonoid concentration than callus obtained from leaves. However, regarding total phenolic concentration, stem derived callus produced lower levels compared to leaf derived callus. When the total phenolic concentration, total flavonoid concentration, and antioxidant activity of callus extracts were taken into consideration, it was found that they had the untapped potential to be an effective production alternative for secondary metabolites with pharmaceutical significance.

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References

Balaji A., Prince N., Bhavani S., Nadu T. 2020. "Kapasurakudineer" - a review of its broad spectrum bioactivity. *Eur. J. Mol. Clin. Med.* 7: 7756–7779.

Bhagat J., Lobo R., Kumar N., Mathew J.E., Pai A. 2014. Cytotoxic potential of *Anisochilus carnosu*s (L.f.) wall and estimation of

luteolin content by HPLC. *BMC Complem. Alternat. Med.* 14: 421.

Bhaga J., Lobo R., Parmar V., Ballal M. 2011. *In vitro* free radical scavenging potential of Indian habitant *Anisochilus carnosus* (L.F.) wall. *Chinese J. Nat. Med.* 9: 456–460.

Bhagath J.K., Lobo R., Ballal M., Polu P.R., Lobo R. 2016. Anti-neoplastic activity of *Anisochilus cornosus* (L .f) wall on HeLa cell line. *Pharm. Lett.* 8: 186–190.

Chang C.C., Yang M.H., Wen H.M., Chern J.C. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.* 10: 178–182.

Espinosa-Leal C.A., Puente-Garza C.A., García-Lara S. 2018. *In vitro* plant tissue culture: means for production of biological active compounds. *Planta* 248: 1–18.

Ha R., Azmathulla M., Koshy R.K., Mohan M. 2011. Effect of methanolic extracts of leaves *Anisochilus cornosus* on gastric and duodenal ulcers in rats. *Int. J. Res. Pharm. Biomed. Sci.* 2: 1643–1650.

Kamble S.Y., More T.N., Patil S.R., Pawar S.G., Bindurani R., Bodhankar S.L. 2008. Plants used by the tribes of Northwest Maharashtra for the treatment of gastrointestinal disorders. *Indian J. Trad. Knowl.* 7: 321–325.

Karakas F.P. 2020. Efficient plant regeneration and callus induction from nodal and hypocotyl explants of goji berry (*Lycium barbarum* L.) and comparison of phenolic profiles in calli formed under different combinations of plant growth regulators. *Plant Physiol. Biochem.* 146: 384–391.

Li T., Shen P., Liu W., Liu C., Liang R., Yan N., Chen J. 2014. Major polyphenolics in pineapple peels and their antioxidant interactions. *Int. J. Food Prop.* 17: 1805–1817.

Mamdouh D. 2022. Optimization of callus and cell suspension cultures of *Lycium schweinfurthii* for improved production of phenolics, flavonoids, and antioxidant activity. *Horticulturae* 8: 394.

Natarajan S., Anbarasi C., Sathiyarajeswaran P., Manickam P., Geetha S., Kathiravan R., Prathiba P. 2021. Kabasura Kudineer (KSK), a poly-herbal Siddha medicine, reduced SARS-CoV-2 viral load in asymptomatic COVID-19 individuals as compared to vitamin C and zinc supplementation: findings from a prospective, exploratory, open-labeled, comparative, randomized controlled trial, Tamil Nadu, India. *Trials* 22: 623.

Nazir M., Tungmunnithum D., Bose S., Drouet S., Garros L., Giglioli-Guivarc'h N., Abbasi B.H., Hano C. 2019. Differential production of phenylpropanoid metabolites in callus cultures of *Ocimum basilicum* L. with distinct *in vitro* antioxidant activities and *in vivo* protective effects against UV stress. *J. Agric. Food Chem.* 67: 1847–1859.

Reshi N.A., Shankarsingh S.M., Vasanaika G.H. 2018. Evaluation of hepatoprotective potential of leaf and leaf callus extracts of *Anisochilus cornosus* (L) Wall. *Int. J. Phytomed.* 10: 156–161.

Reshi N.A., Sudarshana M.S., Girish H.V. 2017. *In vitro* micropropagation of *Anisochilus cornosus* (L) Wall. *J. Appl. Pharm. Sci.* 7: 98–102.

Roby M.H.H., Sarhan M.A., Selim K.A.H., Khalel K.I. 2013. Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and marjoram (*Origanum majorana* L.) extracts. *Industr. Crops Prod.* 43: 827–831.

Subrahmanian S.S., Krishnapriya A., Narayanayyar V. 2017. In vitro propagation of *Anisochilus cornosus* (L.f .) Wallich – a medicinal plant. *IAETSD J. Adv. Res. Appl. Sci.* 4: 245–247.

Senatore F., Lentini F., Venza F., Bruno M., Napolitano F. 2003. Composition and antibacterial activity of the essential oil of *Anisochilus cornosus* (Linn. fil.) Benth., a Tamil plant acclimatized in Sicily. *Flavour Fragrance J.* 18: 202–204.

Shah P., Modi H.A. 2015. Comparative study of DPPH, ABTS and FRAP assays for determination of antioxidant activity. *Int. J. Res. Appl. Sci. Eng. Technol.* 3: 636–641.

Shetty V., Lobo R., Kumar N., Lingadakai R., Pai G.C., Balaraju G., Ballal M. 2017. Antimicrobial activity of *Anisochilus cornosus* (L.F.) wall against the human gastric pathogen *Helicobacter pylori. Asian J. Pharm. Clin. Res.* 10: 292–295.

Shirsat R., Koche D. 2022. Physico-chemical, fluorescent and phytochemical analysis of *Anisochilus cornosus* (L.f.) Wall: a Lamiaceae herb from Maharashtra, India. *Indian J. Appl. Pure Biol.* 37: 1–12.

Soujanya K., Kumari B.A., Jyothsna E., Kiran V.K. 2021. Traditional knowledge on uncultivated green leafy vegetables (UCGLVS) used in Nalgonda District of Telangana. *Int. J. Bioresour. Stress Manage.* 12: 278–285.

Suddee S., Paton A. 2009. A revision of *Anisochilus* Wall. ex Benth. (Lamiaceae). *Kew Bull.* 64: 235–257.

Valarmathy K., Gokulakrishnan M., Kausar M.S., Paul K. 2010. a Study of antimicrobial activity of ethanolic extracts of various plant leaves against selected microbial species. *Int. J. Pharma Sci. Res.* 1: 293–295.

Vijaya P.P., Syed Ali M., Saranya R.S., Yogananth N., Anuradha V., Kalitha Parveen P. 2013. Antimicrobial activity and characterization of biosynthesized silver nanoparticles from *Anisochilus carnosus. Int. J. Nano Dimens.* 3: 255–262.

Wall L., Kumar A.S., Samanta K.C., Chipa R.C. 2010. Hepatoprotective activity of alcoholic and aqueous extracts of extracts of leaves of *Anisochilus cornosus* (Linn.)Wall. *Int. J. Pharm. Life Sci.* 1: 99–104.

Xavier T.F., Kannan M., Auxilia A. 2015. Traditional medicinal plants used in the treatment of different skin diseases. *Int. J. Curr. Microbiol. Appl. Sci.* 4: 1043–1053.

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