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# Physico-chemical, Fluorescent and Phytochemical analysis of *Anisochilus carnosus* (L.f.) Wall: a Lamiaceae herb from Maharashtra, India

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**Abstract**

*Anisochilus carnosus* (L. f.) Wall is one of the wild and aromatic lamiaceae members with significant medicinal potential. It is being used by Bhilla and Paliyar tribals from Maharashtra and Tamil Nadu states as indigenous traditional medicine. Present work is focused on the physico-chemical and fluorescent analysis of powdered drug material of the leaves of the plant and its phytochemical analysis. The study showed that the plant is rich in phytoconstituents like alkaloids, phenolics, flavonoids, terpenes and steroids. Further, HPLC analysis reveals the availability of caffeic acid, luteolin -7 glucoside, nepetin-7 glucoside, homoplantagenin, luteolin and ursolic acid with the highest content of homoplantagenin (4.20µg/g dry drug sample) followed by nepetin-7 glucoside (3.80 µg/g dry drug sample). The plant sample has rich diversity of phytoconstituents. The identified phytoconstituents are correlated with bioactivities of the plant to validate traditional medicinal claims of the plant.

Natural products and plant derived herbal remedies are getting increased attention since last two decades. Throughout the human history, many infectious diseases have been treated with herbal medicines. A number of scientific investigations have highlighted the importance and the contribution of several thousand medicinal plants. The wealth of Indian medicinal plants is well documented with their active principles and properties[9,23]. The medicinal plants play vital role in routine healthcare, holistic growth, health and well beings, especially in rural areas of India[29].

The use of all botanicals is well rooted in medical practice. Since ancient times, herbal healers collected information about herbs and developed well-defined pharmacopoeias to treat a variety of diseases and disorders. More than a quarter of all drugs used today contain

active ingredients derived from plants or plant products[18].

It's estimated that nearly 80 percent of the world's population use herbs as reliable solution for their primary health care. All over the world several botanicals are sold as dietary supplements because of their high efficacy and safety. It indicates that medicinal plants play a vital role for the development of new drugs[40]. Medicinal plants play a central role not only as traditional medicines but also as trade commodities, meeting the demand of distant markets. India has a very small share (less than 2%) in medicinal plants trade commodities of global market[8,51]. To compete with the growing market, there is urgency to expeditiously utilize and scientifically validate more medicinally useful plants. Keeping this view, present study was focused on physico-chemical, fluorescent and phytochemical analysis of *Anisochilus carnosus* a wild member of family lamiaceae.

*Anisochilus carnosus* is an annual, erect herb of mint family- lamiaceae, commonly called as *Kapuri* (in Marathi), *Karpoorada* gidda (in Kannada) and *Karpurvali* (in Telgu), a common inhabitant of higher altitudes[19,42]. Usually, the plant appears as a beautiful herb with height ranging from 0.3 to 0.6m. It was so far reported from Karnataka, Maharashtra, Rajasthan and Tamil Nadu as traditional medicine by tribal communities of respective states for different ailments[32]. The whole plant is used as diaphoretic, stimulant and expectorant; it was also use to cure liver disorders, cough, cold and skin diseases[2,20,41,48]. Leaves are used for cough, dropsy, indigestion and sores in the leg fingers[6,13,44].

*Collection of plant, identification and powder preparation :*

The plant material of *Anisochilus carnosus* (L. f.) Wall was collected form the Chikhaldara forest ranges of Amravati Division, Maharashtra State (India). After collection, the plant was identified taxonomically (fig. 1) using flora of Marathwada[39] and flora of Maharashtra state[49]. A voucher specimen (No. D- 1058) was submitted to Department of Botany, Shri Shivaji College of Arts, Commerce and Science, Akola (MS). The collected plant material was then shade dried for about 10 days and powdered using mortar and pestle. The fine powder was kept in air tight polythene bags until use.

*Physico-chemical, fluorescent and preliminary phytochemistry :*

The physico-chemical and fluorescence analysis was done as per standard methods[12,15,37]. To analyze preliminary phytochemistry, standard protocols were followed[11,17,27,50]. The powdered material was extracted in distilled water (AQ), methanol (ME) and Chloroform (ChE) and qualitative tests were done to check the presence of alkaloids, phenolics, flavonoids, terpenes, tannins, saponins, glycosides, volatile oils, carbohydrates, proteins and amino acids.

*Crude quantification of the major phytochemicals :*

Only alkaloids, Phenolics, Tannins and flavonoids compounds were quantified in powdered material of *A. carnosus*. The methods applied for the same are discussed below-

***Alkaloids :*** Powdered sample (5 ml) was mixed in 200 ml of 10% $CH_3COOH$ in $C_2H_5OH$ (ethanol) the flask was covered and mixed well and allowed to stand for 4 hrs. Then filtered the mix and the filtrate is heated in a water bath until reaches ¼ of the original value. To this, concentrated $NH_4OH$ was added till the complete precipitation and the precipitate was collected and washed with dilute NH4OH. Then the solution was filtered and the residue was weighed as crude alkaloid content[11].

***Phenolics****:* The total phenolics in the extract were determined using modified Folin-ciocalteu method[28]. To each sample solution (1.0 ml) and standard (Gallic acid) was added 5 ml of Folin-ciocalteu and 4 ml sodium carbonate (7 % w/v). The mixture were shaken and allowed to stand for 30 min in the dark at room temperature; after which absorbance was measured at 765 nm using a spectrophotometer. The amount of total phenolics was expressed as Gallic acid equivalent (GAE) in milligram per gram dry plant extract using the expression; C = c x (V/m); (where C= Total phenolics content of plant extract in mg/g GAE, c= concentration of Gallic acid established from calibration curve mg/g, V= volume of the extract (ml) and m= weight of pure plant extract (g).

***Tannins:*** Take 0.5 gm sample in 50 ml of distilled water; stir the solution for 1 hr. filter the mixture and take 5 ml of filtered sample in test-tube. To this add 2 ml of 0.1 M $FeCl_3$ in 0.1 M HCl and 0.008 M $K_4Fe(CN)6 .3H_2O$. Take the absorbance at 395nm wavelength and record changes within 10 minutes[1].

***Flavonoids :*** Take 10 gm of powdered sample and repeatedly extract it with 100 ml of 80% aqueous Methanol. Filter the solution and filter is then transferred into a water bath for evaporation into dryness. The residue is weighed as flavonoid content[7].

*Spectral and Chromatographic analysis :*

For the spectrophotometric analysis of *Anisochilus carnosus* leaf powder, eleven different standards were selected. These were- Caffeic acid, Rosmarinic acid, Luteolin, Luteolin-7- glycoside, Nepetin, Nepatine-7 glycoside, Homoplantagenin, Hispidulin, Salvigenin, Ursolic acid and Carnosic acid. These standards were procured from Sigma –Aldrich India ltd. The UV-spectra of each of these standards were recorded along with their retention time.

Chromatographic analysis was performed on Shimadzu make high performance liquid chromatography system, equipped with a diode array detector working in the range of 190–400 nm, a quaternary solvent delivery system, a column temperature controller and an autosampler. Analysis was carried out at 30°C on a C18 column (250mm×4.6mm, 5 µm). A linear gradient elution of eluents A (0.5%, v/v aqueous glacial acetic acid) and B (methanol) was used for the separation. The elution programme was optimized and conducted as follows: a linear gradient of 38–42% B with the range of 0.0–14.0 min, a linear gradient of 42–45% B with the range of 14.0–17.0 min, a linear gradient of 45– 48% B with the range of 17.0–17.1 min, a linear gradient of 48–50% B with the range of 17.1–32.0 min and a linear gradient of 50–85% B with the range of 32.0–

45.0 min. This was followed by a 10min equilibration period prior to the injection of each sample.

All the samples were milled into powder and oven-dried at 50°C until constant weight was reached. 1.0 g powder of each dried sample was extracted with 20 ml 70% (v/v) aqueous methanol in an ultrasonic bath for 2 h and then cooled at room temperature. The extract was filtered through glass wool for sample, cleaned up and diluted to 25 ml with 70% methanol. The sample solution was filtered through a 0.45µm membrane filter prior to HPLC analysis and the injection volume was 5µl [35-36].

*Physico-chemical and Fluorescent analysis:*

The physico-chemical study of *Anisochilus carnosus* was done, the results are presented in the table-1. The Moisture content of the plant recorded as 16.20%. The extractive values of the water, alcohol and chloroform extracts were 6.8% (fairly green), 8.5% (yellowish green) and 4.1% (grayish green) respectively. The ash values calculated as total ash (8.4%), acid insoluble ash (4.2%) and water soluble ash (6.5%).

The reaction of powdered drug with the routine laboratory chemicals was also analyzed. The results of reactions of different laboratory chemicals are presented in the table 2. This is one of the unique criteria to identify adulteration in the *Anisochilus* drug powder available in the market.

The original leaf powder color of the plant as such was pale yellow. When treated with iodine the color changes to light green. The treatment of 5% ferric chloride gives yellow color. With 1N NaOH, the powder color changes to green while with acetic acid it gives light brown color. With strong acids, the color becomes green. The powder extract with liquid ammonia and lead acetate gives brown and cream color respectively (table-2). However, when the same treated samples were visualized under UV-light, it appeared to have different colours. This could be used as standard marker to analyze market available powdered drug materials.

*Preliminary Phytochemical Analysis :*

For the qualitative phytochemical analysis, three different solvents (aqueous, methanolic and chloroform) were used for extract preparation. These extracts were analyzed for the presence of 10 different parameters according to the methods described elsewhere. Of all the extracts, methanolic extracts showed presence of all tested phytocompounds except alkaloids. The water extracts showed presence of phenolics, tannins, flavonoids, saponins, proteins and amino acids whereas the chloroform extract showed positive tests of alkaloids, phenolics and terpenes (table-3).

*Crude quantification of major phytochemicals of Anisochilus carnosus :*

The crude content of major phytochemical compounds in *Anisochilus carnosus* was determined using different methods[17,27]. The quantitative analysis of alkaloids, Phenolics, tannins and flavonoids was done. The content of alkaloids was found 0.85± 0.31 µg/g of dry sample, that of phenolics

1.47± 0.11 µg/g of dry sample, tannins 0.11± 0.10 µg/g of dry sample and flavonoids 1.20± 0.81 µg/g of dry sample. The extract showed presence of highest amount of phenolics and lowest amount of tannins (Table-4 and Fig. 2).

*HPLC analysis of Methanolic extract of A. carnosus :*

Spectrophotometric and chromatographic analysis of *A. carnosus* leaf powder extract was done. For the analysis aqueous methanolic extracts were prepared and the data was compared with the reference standards. Eleven reference standards were used for the analysis which was chosen on the basis of previous work done on different species of lamiaceae members. For spectrophotometric analysis in current study, eleven standard compounds were selected as reference standards (table-5).

The preparation of standard references and their UV and HPLC analysis method was described in the previous chapter. The retention time of these standards for HPLC analysis is given in the table 5. The content of each phytoconstituent in the sample of *A. carnosus* leaf powder drug is presented in the table-6. For the analysis of the phytoconstituents present in the experimental sample the author had used the similarity index, of retention time and absorption (λ-max). The content of the sample is presented in µ g/g of sample.

Total nine prominent peaks were observed in HPLC chromatogram of *A. carnosus* methanol leaf extract (fig. 3). Out of nine peaks, peak number 5 and 8 were remained unidentified. The other peaks showed presence of caffeic acid, luteolin-7-glucoside, nepetin-7 glucoside, homoplantagenin, luteolin and ursolic acid. The peaks with respective peak numbers, retention time and content of compounds on the basis of peak area are presented in the table -6. The highest content was noted that of homoplantagenin (4.20µg/g dry drug sample) followed by nepetin-7 glucoside (3.80 µg/g dry drug sample).

Simple plant extracts or decoctions are probably the crude forms of plant drugs of earlier generation[22,34]. In the beginning of the 21st century, several workers have estimated that plant materials are present in nearly 50 % of western medicines[34,43]. The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment[2,14]. The present work is focused on the physico-chemical, fluorescent and phytochemical analysis of a lamiaceae member *Anisochilus carnosus* to correlate its phytoconstituents with medicinal potential.

Moisture content, extractive values and ash values are significant in determining the purity and authenticity of the crude material and decide its applicability as drug material[15,31]. These physico-chemical values for *A. carnosus* indicate suitability of this plant as drug source. Further, the interaction of leaf powder with different lab chemicals visualized under normal sunlight and under UV- light could act as baseline marker to authenticate the powder available in the market and its purity[37]. Certainly, the powder with adulterants will not match physico-chemical and fluorescent properties presented in this study.

Fig. 1: Habit of *Anisochilus carnosus* (L.f.) Wall.

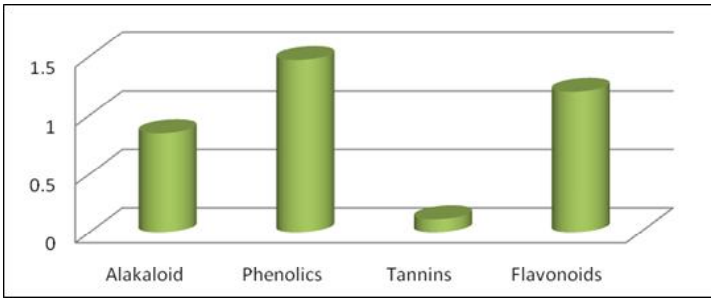


Fig. 2: Content of major phytochemicals in *Anisochilus carnosus* extract (µg/g dry wt.)

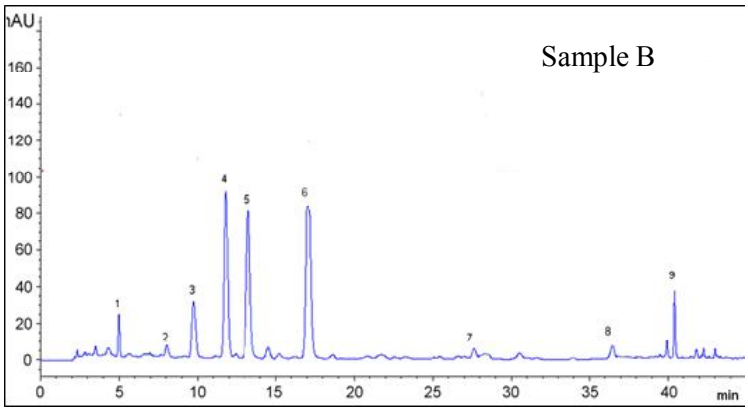


Fig. 3: HPLC Chromatogram of *Anisochilus carnosus* powder extract.

Table-1. Physico-chemical characterization of *Anisochilus carnosus*

| Moisture content | Extractive values | In % | Ash value | In % |
|---|---|---|---|---|
| 16.20% | Water | 6.8% (Fairly Green) | Total ash | 9.7% |
| | Alcohol | 8.5%(Yellowish green) | Acid insoluble ash | 4.2% |
| | Chloroform | 4.1%(Gray) | Water soluble ash | 6.5% |

Table-2. Effects of various chemicals on powder drug of *A. carnosus* and fluorescence analysis

| Sr. No. | Colour test | Visual observations | |
|---|---|---|---|
| | | Under normal Sunlight | Under UV light |
| 1 | Powder As Original | Pale yellow | Yellow green |
| 2 | Powder + Iodine | Light green | Green |
| 3 | Powder + 5% Ferric Chloride | Yellow | Pale yellow |
| 4 | Powder + 1 N NaOH | Green | Dark green |
| 5 | Powder + Acetic acid | Light brown | Green |
| 6 | Powder + 50% $H_2SO_4$ | Green | Dark green |
| 7 | Powder + 50% Conc. HCl | Green | Dark green |
| 8 | Powder + Liquid Ammonia. | Brown | Light brown |
| 9 | Powder + NaOH + Lead acetate | Creamy | Brown |

Table-3. Preliminary phytochemical analysis of various extracts of *A. carnosus* leaf powder (n=3)

| Parameters tested | *Anisochilus* extracts | | |
|---|---|---|---|
| | AQE | ME | ChE |
| Alkaloids | — | - | + |
| Phenolics | + | + | + |
| Tannins | + | + | - |
| Flavonoids | + | + | - |
| Saponins | + | + | - |
| Glycosides | - | + | - |
| Terpenes | - | + | + |
| Proteins and amino acids | + | + | - |
| Carbohydrates | - | + | - |
| Volatile oil | - | + | - |

Note: AQE = Aqueous extract, ME = Methanol extract and ChE= Chloroform extract

Table-4. Quantitative phytochmical analysis (crude content in µg/g of dry sample) (n=3)

| Sr. No. | Phytochemicals | crude content in µg/g of dry sample |
|---|---|---|
| 1 | Alkaloids | 0.85 ± 0.31 |
| 2 | Phenolics | 1.47 ± 0.11 |
| 3 | Tannins | 0.11 ± 0.10 |
| 4 | Flavonoids | 1.20 ± 0.81 |

Table-5. Spectral and HPLC analysis of standards used during experimentation

| Sr. No. | Standard used | Retention time RT (min) | Peak wavelength (µ-max) nm |
|---|---|---|---|
| 1 | Caffeic acid | 5.18 | 217, 239, 328 |
| 2 | Rosmarinic acid | 5.57 | 328 |
| 3 | Luteolin -7 glycoside | 11.2 | 269, 340 |
| 4 | Nepetin-7 glycoside | 13.5 | 239, 322 |
| 5 | Homoplantagenin | 17.5 | 276, 332 |
| 6 | Luteolin | 27.4 | 258, 346 |
| 7 | Nepetin | 28.30 | 270 |
| 8 | Salvigenin | 34.6 | 277, 358 |
| 9 | Hispidulin | 37.5 | 274 |
| 10 | Carnosic acid | 37.7 | 282, 363 |
| 11 | Ursolic acid | 41.9 | 257 |

Table-6. HPLC analysis of methanolic extract of *Anisochilus carnosus*

| Sr. No. | Compounds identified in sample | Retention time RT (min) | Content (µg/g dry wt. of sample) |
|---|---|---|---|
| 1 | Caffeic acid | 5.18 | 0.65 |
| 2 | Unidentified | 7.60 | 0.22 |
| 3 | Luteolin -7 glucoside | 11.2 | 1.20 |
| 4 | Nepetin-7 glucoside | 13.5 | 3.80 |
| 5 | Unidentified | 15.62 | ND |
| 6 | Homoplantagenin | 17.5 | 4.20 |
| 7 | Luteolin | 27.4 | 0.20 |
| 8 | Unidentified | 36.6 | ND |
| 9 | Ursolic acid | 41.9 | 1.25 |

The preliminary phytochemistry showed that the plant is rich is phytoconstituents like alkaloids, phenolics, flavonoids, tannins, terpenes, saponin, steroids and glycosides. These secondary metabolites as per their availability in the plant, gives diverse medicinal properties to that plant. Due to its phyto-chemical richness, the plant is being used to treat cough, cold, liver disorder, skin diseases and certain gastric problems by different tribal communities across India[2,6,13,20,41,44].

In the present study, authors quantified the alkaloids, phenolics, flavonoids and tannins (table 4 and fig.2). further, the HPLC analysis of methanol leaf extract of *A. carnosus* showed presence of caffeic acid, luteolin -7 glucoside, nepetin-7 glucoside, homoplantagenin, luteolin and ursolic acid with the highest content of homoplantagenin (4.20µg/g dry drug sample) followed by nepetin-7 glucoside (3.80 mg/g dry drug sample) (table-6 and fig. 4). Earlier few workers have reported identification and isolation of bioactive compounds from this plant [10,24]. Availability of these phytochemicals in the powdered drug material is rightly correlated with the medicinal potential. The level of phenolics and flavonoids in the plant extract is directly correlated with the antioxidant and anticancer potential of the plant[25-26,33,38].

Cytotoxicity of ethanolic extract of *A. carnosus* was opined to have potential anticancer activity due to availability of luteolin[5] and the anticancer activity of ethanolic leaf extract of *A. carnosus* might be due to presence of phytosterols, terpenes and flavonoids[16]. Ethanolic extract of leaves of this plant was reportedly have hepatoprotective and antioxidant potential[52] and that alcoholic extract of *A. carnosus* showed antimicrobial activity against human pathogen Helicobacter pylori and other bacterial strains[45,47]. In 2020, this plant was reported to have significant antimicrobial activity and proposed this plant as a novel candidate with therapeutic potential against multi-drug resistant *Staphylococcus aureus*[21]. However, still there are some compounds identified in the methanol extract of *A. carnosus* like caffeic acid, nepetin-7- glucoside, luteolin-7- glucoside, ursolic acid which are not explored for their medicinal values. These compounds have been reported from other plants of this family having antiviral[4], anti-inflammatory[30] properties and could be use to reduced apoptosis and cure cardiovascular disorders[46]. Thus, *A. cornosus* appears to have rich chemical diversity which is responsible for its varied medicinal properties.

From the above discussion it is clear that, *A. carnosus* is an aromatic plant with tremendous medicinal potential. The physico-chemical and fluorescent analysis of crude drug material of the plant could be the standard to check the adulteration in market available drug powder. It has diverse range of phytoconstitues and therefore responsible to cure diverse ailments starting with cough cold to cancer. However, still there are few compounds isolated from this plant which should be properly evaluated for their bioactivities and drug candidacy which might help further to develop new drug molecules.

*Authors contribution:*

DK developed the concept, monitored

the work and edited the manuscript. RG and NK designed the experiments and conducted the analysis. RS and SR helped in plant collection, phytochemical analysis and writing the first draft of this manuscript.

*Declaration of Competing Interest:*

The authors declare no conflict of interest with respect to research, authorship and publication.

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