Short communication

Variability studies for quality characters in *neikumbalam* accessions

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Abstract

Vaidyakumbalam/medicinal ashgourd or *neikumbalam* is a specific ecotype of ashgourd in Kerala which is highly valued for its medicinal properties. *Neikumbalam* is the principal ingredient used in the preparation of the Ayurvedic medicine "*Kooshmanda rasayanam*" and "*poosanilehyam*", a similar preparation made by the Siddha medicine physicians. In the present study, *neikumbalam* accessions were collected from different locations in Kerala and laid out in field experiment along with vegetable ash gourd variety "Indu". Fully mature fruits were harvested and used for phytochemical screening and biochemical estimation. Phytochemical screening of the fruit extracts revealed the presence of sugars, starch, proteins, amino acids, tannins, phenols, glycosides and flavanoids in both type of ash gourds. The biochemical analysis of mature fruits of *neikumbalam* revealed higher content of total minerals and total free amino acids. The *neikumbalam* type BH 4 recorded significantly highest total free amino acid content and total mineral content than other accessions.

Keywords: Antioxidant property, *Benincasa hispida,* Medicinal ashgourd, *Neikumbalam*, Total free amino acids, Total minerals.

Benincasa hispida is a commonly used cucurbitaceous vegetable which has been used as a food and medicine for thousands of years in the Orient (Mathad et al., 2005). Its fruits contain various minerals and vitamins, having a relatively high level of K and Na, low calorific value and no fat. The fruit also contains 11-70 mg of calcium per100 g and 0.3-0.45 per cent of other minerals (Pandey et al., 2015).

Ash gourd (*Kushmanda*) has been mentioned in 'Charaka Samhita' for its medicinal properties. It has been used in India to treat disorders of the gastrointestinal tract, respiratory tract, urinary tract and *diabetes mellitus* (Asolkar et al., 1992; Sivarajan, 1992). According to Sanskrit texts, *Benincasa hispida* fruit is useful in insanity, epilepsy, constipation, piles, dyspepsia, and other nervous disorders. The fruit of *B. hispida* is used as a diuretic and the seeds have been reported to possess antiangiogenic effects in prostate cells (Nandecha, 2010). Ash gourd, being low in calories, is particularly useful for diabetic and obese people. It is cooling and laxative and thus increases the secretion and discharge of urine. Ash gourd acts as a blood coagulant (Pandey et al., 2015). Fresh juice is given either with sugar or as an adjunct to other medicines for these diseases (Patricha, 1997). A decoction of the fruit is styptic, laxative, diuretic and given to cure internal haemorrhages and diseases of the respiratory tract. For centuries, it has been used in many empirical applications in India for various ailments such as gastro intestinal tract problems like dyspepsia and burning sensation, heart disease, vermifuge, diabetes and urinary disease. The fruit is an important source of water-soluble and hemicellulosic polysaccharides (Mazumder and Lerouge, 2005).

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Vaidyakumbalam/ medicinal ashgourd or *neikumbalam* is a specific ecotype of ashgourd in Kerala which is highly valued for its medicinal properties. The fruits of this ecotype, also used for culinary purposes, are hard pulped and have long keeping quality, with a size slightly larger than a cricket ball (Gopalakrishnan, 1957). *Neikumbalam* is the principal ingredient used in the preparation of the Ayurvedic medicine "*Kooshmanda rasayanam*" and "*poosanilehyam*", a similar preparation made by the Siddha medicine physicians (Subramanian et al., 2013). *Kooshmanda rasayanam* is used as a rejuvenative agent, in epilepsy and in nervous disorders. It is also recommended in Ayurveda for the management of peptic ulcers. It can be stored for several years.

The availability of this special type of ash gourd is scarce at present. The vegetable type ashgourd is often used instead for medicinal preparations. There is an increasing importance for medicinal ash gourd nowadays due to its added health benefits. Scattered attempts are being done at farmers'/ *vaidyas'* level to conserve and cultivate this unique ashgourd. However, not much is known about the morphology and biochemistry of *neikumbalam*. The present study was undertaken in this context with the objective to collect *neikumbalam* (*Benincasa hispida* (Thunb.) Cogn.) types from Kerala and to evaluate the collected types for quality.

The study was carried out in the Department of Plantation Crops and Spices at College of Horticulture, Vellanikkara, during September 2016 to January 2017. *Neikumbalam* accessions were collected from different locations in Southern, Northern and Central Kerala. These were grown in a field experiment along with the ash gourd variety 'Indu', which was used as check. Fully mature fruits were harvested and used for studying various biochemical characters of the fruit.

The samples were initially screened for presence of various phytochemicals. The presence of steroids, tannins, starch, proteins, amino acids, sugars, phenols, glycosides, flavonoids and saponins were tested by the procedure described by Harborne (1999) and Kokate (2000). Samples were dried and powdered after which they were soaked in ethanol in 1:1 ratio. The filtrate obtained from this was used for phytochemical analysis.

The biochemical tests carried out in the fully mature fruit samples are tabulated in Table 1.

The antioxidant activity of the ethanolic extract of dried fruit samples was also studied. The free radical scavenging activity of these sample extracts based on the scavenging activity of the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical was determined by the method described by Braca et al. (2001). The percentage of DPPH quenched was calculated as follows:

$$\frac{\text{OD of blank} - \text{OD of sample} \times 100}{\text{OD of blank}}$$

Where, OD: Optical density of the sample

A graph was plotted using these values, with concentration of solution on x axis and percentage of DPPH quenched on y axis. The IC 50 value was

Table 1. Biochemical tests carried out in *neikumbalam* fruits

S. No.	Biochemical parameter	Method of assay	Reference
1	pH	pH meter	-
2	Titrable acidity	Titration against Sodium hydroxide	-
3	Total minerals	Decarbonization and ignition in muffle furnace	-
4	Total free amino acids	Ninhydrin method	Sadasivam and Manickam (1992)
5	Protein	Lowry's method	Sadasivam and Manickam (1992)
6	Total sugars	Phenol sulphuric acid method	Nielson (2010)
7	Starch	Anthrone reagent method	Hedge and Hofreiter (1962)
8	Total phenols	Folin Ciocalteau method	Sadasivam and Manickam (1992)
9	Tannins	Folin Denis method	Schandrel (1970)

then calculated by the equation so obtained, i.e. y= ax + b.

The analysis of variance was carried out for each character separately as per method suggested by Panse and Sukhatme (1967). Significance of differences among the accessions was tested.

The initial screening for phytochemicals of *Benincasa hispida* fruit extracts indicated the presence of tannins, starch, proteins, amino acids, sugars, phenols and cycloglycosides both in the *neikumbalam* as well as the check variety. Steroids and saponins were not detected in the fruit extracts. The results of phytochemical screening have been presented in Table 2.

Table 2. Phytochemicals present in ash gourd

Sl. No.	Phytochemicals	*Neikumbalam*	Indu
1	Steroids	Absent	Absent
2	Tannins	Present	Present
3	Starch	Present	Present
4	Proteins	Present	Present
5	Amino acids	Present	Present
6	Sugars	Present	Present
7	Phenols	Present	Present
8	Glycosides	Present	Present
9	Flavanoids	Present	Present
10	Saponins	Absent	Absent

The data on the biochemical characters of fully mature fruits is furnished in Table 3 and Table 4.

pH of fruit juice ranged from 5.03 to 5.8. Highest pH was recorded in the *neikumbalam* accession BH 7. pH in the check variety 'Indu' was 5.5. The lowest pH of 5.03 was recorded in the *neikumbalam* type BH 12.

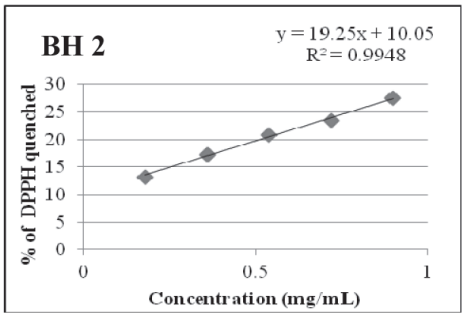
Titrable acidity among *neikumbalam* fruits ranged from 0.23 % to 0.62 %. Acidity in fruits of the check variety 'Indu' was less than all *neikumbalam* fruits. Fruits of BH12 had the highest acidity of 0.62 %.

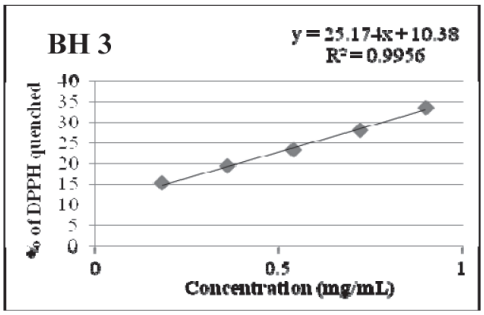
Table 3. Biochemical characters of fruits of *neikumbalam* accessions and vegetable ashgourd

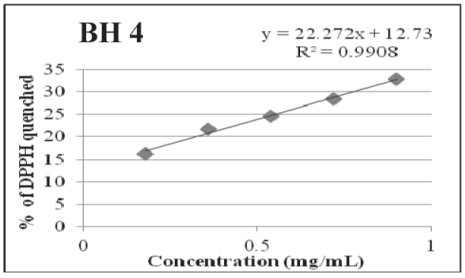
Accession No.	pH	Titrable acidity (%)	Total minerals (mg/100g)	Protein (mg/100g)	Starch (%)	Total sugars (g/100g)
BH 2	5.31^{f}	0.45^{b}	364.00^{d}	199.50^{h}	0.67^{a}	1.54^{b}
BH 3	5.76^{ab}	0.38^{cd}	322.00^{f}	305.50^{c}	0.59^{b}	1.24^{d}
BH 4	5.69^{b}	0.43^{bc}	415.00^{a}	230.50^{f}	0.02^{f}	0.87^{g}
BH 6	5.61^{c}	0.57^{a}	394.00^{b}	198.50^{h}	0.73^{a}	0.75^{h}
BH 7	5.80^{a}	0.42^{bc}	329.00^{f}	311.00^{c}	0.38^{c}	0.72^{i}
BH 8	5.18^{g}	0.33^{d}	363.00^{d}	250.50^{d}	0.18^{e}	0.93^{f}
BH 9	5.40^{e}	0.38^{cd}	347.00^{e}	210.00^{g}	0.55^{b}	1.44^{c}
BH 11	5.12^{g}	0.23^{e}	361.00^{d}	343.00^{b}	0.26^{d}	1.11^{e}
BH 12	5.03^{h}	0.62^{a}	380.00^{c}	242.00^{e}	0.25^{d}	0.87^{g}
Mean	5.43	0.42	363.89	254.50	0.40	1.05
Check	5.50^{d}	0.18^{e}	347.00^{e}	440.00^{a}	0.23^{de}	1.66^{a}
CD (5%)	0.07	0.06	12.06	8.47	0.06	0.02

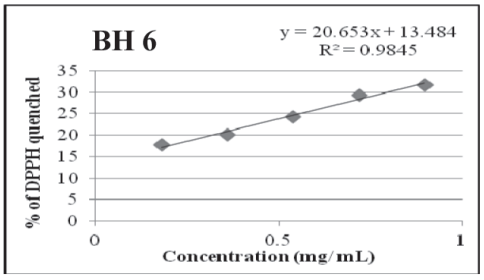
Table 4. Biochemical characters of fruits of *neikumbalam* accessions

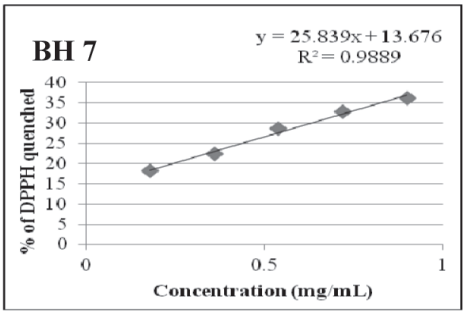
Accession No.	Total free amino acids (percentage equivalent of leucine)	Total phenols (mg catechol equivalent per 100 g sample)	Tannins (mg tannic acid equivalent per 100 g sample)
BH 2	0.91^{c}	104.00^{c}	688.00^{e}
BH 3	0.72^{de}	88.00^{e}	640.00^{f}
BH 4	1.09^{a}	72.00^{h}	610.00^{g}
BH 6	0.54^{g}	80.00^{fg}	960.00^{a}
BH 7	0.54^{g}	84.00^{ef}	574.00^{h}
BH 8	0.72^{de}	136.00^{a}	914.00^{c}
BH 9	0.74^{d}	96.00^{d}	898.00^{d}
BH 11	0.70^{e}	76.00^{gh}	944.00^{b}
BH 12	0.94^{b}	86.00^{ef}	610.00^{g}
Mean	0.77	91.33	759.78
Check	0.67^{f}	112.00^{b}	920.00^{c}
CD (5%)	2.52	7.96	11.66

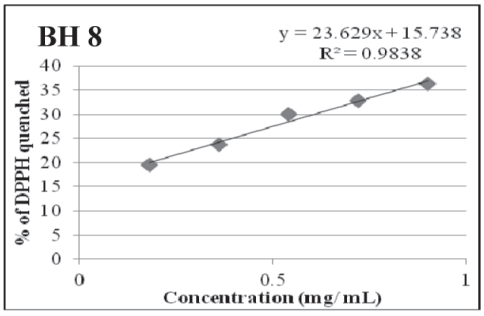


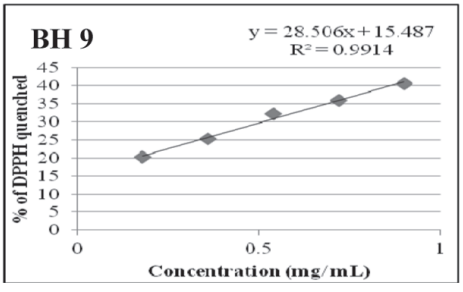


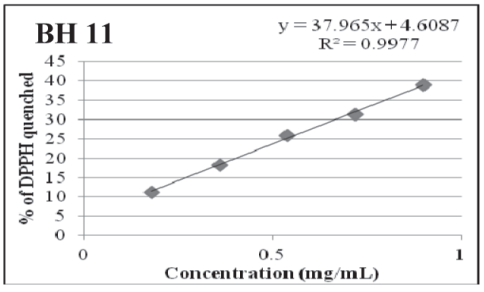


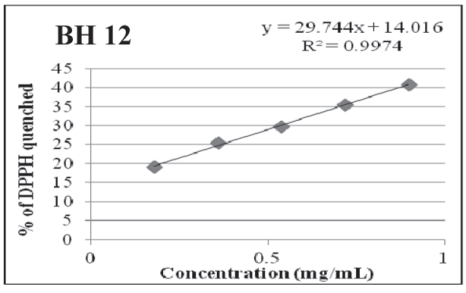












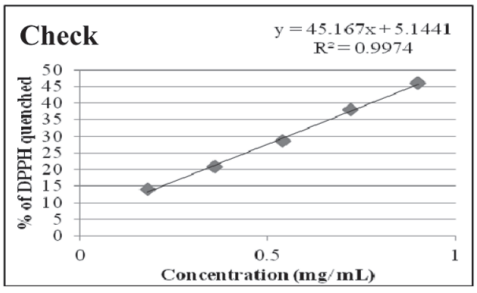


Figure 1. Antioxidant activity of neikumbalam accessions and vegetable ashgourd

It was observed that the mean value of mineral content in fruits of *neikumbalam* accessions was higher than mineral content in fruits of 'Indu'. Total mineral content in fruits of 'Indu' was 347 mg/ 100g and the accession BH 4 had the highest total mineral content of 415 mg/ 100g.

In an earlier study, Mishra et al. (2016) had reported the presence of minerals like calcium, potassium and zinc in ash gourd fruits. Pandey et al. (2015) observed the presence of mineral elements with high level of K and low level of Na in ash gourd fruits. Dobre (2013) had reported that the amount of total ash in ash gourd fruit is within the range of 0.3 to 0.5 % of the fruit pulp.

The mean value for total free amino acid content in *neikumbalam* fruits (0.77 percentage equivalent of leucine) was higher than amino acid content in fruits of 'Indu', the check variety. The accession BH 4 had the highest total free amino acid content (1.09 percentage equivalent of leucine).

Amino acid composition of different parts of ash gourd fruit (pulp, seed and skin) were studied by Mingyu et al. (1995) and they found that total protein and free amino acids were present in high amounts in seed compared to pulp. In Ayurvedic literature, *kushmanda* is considered as a *medhya* (rejuvenating) drug. The high amount of free amino acids observed in the present study is an indication of the rejuvenating property of *neikumbalam* fruits. The protein content in the *neikumbalam* fruits varied from 198.50 mg/100g to 343 mg/ 100 g and the protein content in the check variety 'Indu' was higher than all *neikumbalam* accessions (440 mg/ 100 g). Among the accessions, protein content was highest in BH 11 (343 mg/ 100 g) and lowest protein content was observed in BH 6 (198.5 mg/ 100 g). Total sugar content in fruits of the check variety 'Indu' (1.66 g/ 100 g) was also higher than fruits of *neikumbalam* accessions. Total sugar content among fruits of *neikumbalam* types was highest in BH 2 (1.54 g/ 100 g) and was least in BH 7 (0.72 g/ 100 g).

The starch content of fruits among the *neikumbalam* accessions showed a wide variation. Starch content in the type BH 6 (0.73 %) was highest. The fruits of the check variety 'Indu' had a starch content of 0.23 %.

Total phenol content in *neikumbalam* fruits varied from 72 to 136 mg catechol equivalent per 100 g of sample. Accession BH 8 had the highest phenol content of 136 mg catechol equivalent per 100 g sample. It was lowest in BH 4 (72 mg catechol equivalent per 100 g sample). Phenol content in 'Indu', the check variety was 112 mg catechol equivalent per 100g sample.

The tannin content among the fruits of *neikumbalam* accessions ranged from 574 mg tannic acid equivalent per 100g sample in BH 7 to 960 mg tannic acid equivalent per 100 g sample in BH 6. Tannin content in fruits of 'Indu' was 920 mg tannic acid equivalent per 100 g.

Phenols and tannins are important phytoconstituents which contribute to the antioxidant property. Badhani et al. (2013) have reported the presence of phenolic compounds and tannins as major constituents of ash gourd fruits.

The continuous formation of free radicals in human body can be controlled naturally by different beneficial compounds known as antioxidants. The percentage inhibition of DPPH by varying concentrations of sample extracts have been graphically represented in Fig. 1. Ascorbic acid had an IC 50 value of 8.4 (reference standard). The IC 50 value among the *neikumbalam* accessions was found to range from 1.20 to 2.08. IC 50 value of the fruit extract of the check variety 'Indu' (0.993) was significantly lower than all other accessions. The accession BH 2 recorded the highest IC 50 value.

According to Hatano et al. (1989), phenolic compounds contribute directly to antioxidative action. High correlation has been observed in antioxidant capacities and phenolic contents. Huang

et al. (2004) and Roy et al. (2007) after conducting *in vitro* and *in vivo* studies on ash gourd fruit also reported that its juice and extract has antioxidant activity especially on human tissues like liver and brain.

This unique type of ash gourd is different from the vegetable type ash gourd not only with respect to its smaller fruit and seed size and lesser fruit weight, but also with respect to quality parameters. Phytochemical screening of the fruit extracts revealed the presence of sugars, starch, proteins, amino acids, tannins, phenols, glycosides and flavanoids in both medicinal and vegetable ash gourds. *Neikumbalam* fruits had higher content of total minerals and total free amino acids than 'Indu' which is an indication of the rejuvenating property of this fruit. Total phenol content in *neikumbalam* fruits varied from 72 to 136 mg catechol equivalent per 100 g sample. Tannin content ranged from 960 to 574 mg tannic acid equivalent per 100 g sample. Phenols and tannins are important phytoconstituents which contribute to the antioxidant property. The *neikumbalam* type BH 4 recorded significantly highest total free amino acid content and total mineral content than other accessions but exhibited a relatively high IC 50 value. The higher amount of the phytochemicals like total free amino acids and total minerals observed in this study might attribute to the medicinal quality of *neikumbalam*.

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