Evaluation of Neem (*Azadirachta indica*) Leaf Powder for Assessing Gustatory Function and Comparison with Other Tastants

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Authors' contributions

This work was carried out in collaboration among all authors. Author VV designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors AM, MS, DR and JKM supervised and managed the analyses of the study. Author NJJ managed the literature searches. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

Aims: *Azadirachta indica* (meliacea), popularly known as neem has extreme bitter taste however it has high medicinal properties. The study aimed to use the bitterness property of neem leaf powder to assess the bitter taste along with other tastants and to check reliability of this newly introduced method.
Materials and methods: 60 healthy subjects were recruited in the present study. Bitter, sweet, salt, sour and umami taste solutions are prepared in three different concentrations. Gustatory recognition threshold was recorded against each concentration of five tastants solutions.
Results: A significant difference in the first concentration (.003) of neem between all age groups

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(p=0.026) but no significant difference in the second and third concentrations were observed. Males have higher gustatory recognition threshold in almost all levels of taste parameters. However, medium and higher concentrations of neem have no significant gender wise difference. An acceptable level of reliability was found in the test retest method conducted in a two week interval.
Conclusion: The neem leaf powder solution can be used for bitter taste assessment, is reliable, and can be safely used in the clinical setting.

1. INTRODUCTION

The sensation of taste guides dietary preferences and a healthy gustatory system is crucial to determining the selection and enjoyment of food [1]. To stimulate the appetite, a dish must be tasty and well presented. A balanced diet is a basis for good health and underpins numerous vital functions in the body including the immune defence mechanism, wound healing and child bearing [2]. Assessment of gustatory function is therefore an important, but much neglected, part of the clinical examination. Both olfaction and gustatory function and assessment are highly topical subjects in the scenario of the current pandemic since these functions are affected early in the course of Covid-19 infection [3,4]. Human gustatory function and dysfunction has been far less studied compared to odour perception and olfactory dysfunction. The absence of a rapid and reliable method for evaluating gustatory function may be one reason. Studies have reported early loss of taste presaging further neurological impairment in diseases like Alzheimer's and semantic dementia, Parkinson's disease and chronic renal failure. [5,6,7].

Gustatory function is commonly assessed using edible taste strips, taste tablets, and solution-based taste tests. Sucrose for sugar, sodium chloride for salt, citric acid for sour, quinine hydrochloride for bitter, and monosodium glutamate for umami are five basic tastants often used. Quinine is not easy to procure and neem (*Azadirachta indica* or meliacea), which has a pronounced bitter taste was considered as a potential substitute. Neem is native to the Indian subcontinent and is considered to have a wide variety of medicinal and therapeutic properties that have led to it being dubbed a "panacea for all diseases". The bitter taste derives from the limonoid content in the neem leaf [8,9].

The present study aimed to provide normative data for a solution-based analysis of sweet, salty, sour, umami and bitter tastes in three different concentrations, where the bitter taste was assessed by the solutions prepared from neem leaf powder.

2. MATERIALS AND METHODS

2.1 Subjects and Study Site

The study was conducted at the Department of Physiology, Little Flower Hospital and Research Centre, Angamaly. The investigation involved 60 healthy participants between the age group of 20-65 years who reported a normal smell and taste function. All of the test procedures were explained and informed consent was obtained prior to recruitment. Subjects were instructed not to eat or drink anything other than water, and to abstain from smoking, chewing gum brushing of teeth, and use of mouthwash for one hour before testing.

2.2 Preparation of Standard Taste Solutions

Gustatory function was measured using solutions of five different substances in three concentrations each. Sucrose, sodium chloride, citric acid, monosodium glutamate and neem leaf powder were used as tastants for sweet, salt, sour, umami and bitter respectively. Commercial products were used for the first four tastes; for testing bitter taste, neem leaf powder was prepared locally. Fresh neem leaves were collected from Ernakulam (Dist.) Kerala, then washed and sundried for 3 days until all the moisture was drawn out. These crisp, dehydrated leaves were ground into a fine powder using a blender and stored in an airtight container. Solutions in three different concentrations were prepared using deionized water one hour before the test and stored at room temperature. The concentrations for each taste (Table 1) were chosen based on the pilot study. Solutions were freshly prepared on the day of testing and presented to the subject at room temperature with minimal visual and olfactory distraction.

Table 1. concentrations of five standard taste solutions

Standard tastes	Tastants	Mild concentration in gm/dl	Medium concentration in gm/dl	High concentration in gm/dl
Bitter	Neem leaf powder	0.003	0.005	0.007
Salt	Sodium chloride	0.01	0.015	0.02
Sour	Citric Acid	0.005	0.01	0.015
Sweet	Sucrose	0.05	0.1	0.15
Umami	Monosodium Glutamate	0.0015	0.0025	0.005

Solutions were dropped on the tongue, 2cm left to the lingual apex, using a 1ml syringe; subjects were asked to rinse their mouths with water after each stimulus. The following parameters were recorded.

2.3 Gustatory Recognition Threshold

The minimum volume (ml) of the solution, required for the subject to recognize the taste in the mouth for each concentration. For a single taste, three repetitions were conducted at three different concentrations to ensure accuracy. Participants were required to select a taste from the list of 6 options: "sweet," "salty," "sour," "bitter," "undefined taste," and "don't know".

2.4 Gustatory Identification Test

After masking their eyes, 1 ml of the five different taste solutions in the highest concentration was provided to the study participants who were asked to choose from the five choices provided. Each correct response scored as one, while incorrect or no responses scored as zero. The maximum score was five.

2.5 Gustatory Discrimination Test

Ten pairs of gustatory stimuli including five pairs of similar tastes and five pairs of different tastes were used. Each pair was presented in random order to the subjects who were asked to state whether the tastes were same or different. Each correct response was scored as one and the incorrect response as zero with a maximum score of 10.

2.6 Statistical Analysis

IBM SPSS version 20.0 was used to perform the statistical analysis. Age-wise distribution and comparison of gustatory recognition thresholds at different concentrations among different age groups were done using the Kruskal Wallis test. For gender-wise distribution and comparison of the gustatory recognition threshold at different concentrations among males and females, the Mann Whitney U test was used. Friedman test was done for comparison of the gustatory recognition threshold at different concentrations. The $p < .05$ was considered statistically significant. Reliability was assessed by the test-retest method. The Pearson correlation was used for assessing the correlation between two-time points, where $p < 0.001$ was considered as statistically significant.

3. RESULTS

The age of participants ranges from 23to 65 years with a mean of 46.56 ± 13.8. Twenty-nine (48.3%) participants were males and thirty-one (51.6%) participants were females. Age-wise comparison of recognition thresholds scores of various taste parameters in three levels of concentration was compared using the Kruskal Wallis test as the data doesn't follow normality. Age was categorized into three class intervals with an interval size of 15 years. The results have shown a trend of as age increases threshold score also increasing. We have observed a significant difference in the first concentration (.003) of neem between all age groups (p=0.026) but no significant difference in the second and third concentrations. The results are displayed in Table 2.

Gender wise comparison of taste parameters was analysed using the Mann Whitney U test as the data was non-normalised. We have obtained a difference between average scores in males and females in almost all levels of taste parameters. However medium and higher concentrations of neem have no significant difference. It is clear from the results that males have a higher taste recognition threshold score compared to females as depicted in Table 3.

Table 2. Age-wise comparison of taste parameters at different concentration levels

Variable	Level of concentration gm/dl	Gustatory recognition threshold(ml) Age wise distribution			P-Value
		21-35	**36-50**	**51-65**	
Neem	.005	0.02±0	0.021±0.005	0.026±0.009	0.026*
	.007	0.02±0	0.02±0	0.02±0	1.00
	.005	0.02±0	0.02±0	0.02±0	1.00
Sodium chloride	.01	0.029±0.017	0.102±0.083	0.102±0.066	<0.001***
	.015	0.02±0	0.044±0.024	0.052±0.035	<0.001***
	.02	0.02±0	0.02±0	0.02±0	1.00
Sucrose	.05	0.124±0.074	0.144±0.118	0.138±0.076	0.783
	0.1	0.055±0.029	0.061±0.03	0.082±0.052	0.092
	.15	0.02±0	0.026±0.012	0.029±0.018	0.087
Citric acid	.005	0.037±0.02	0.042±0.019	0.051±0.013	0.002**
	.01	0.023±0.01	0.021±0.005	0.052±0.081	<0.001***
	.015	0.02±0	0.02±0	0.034±0.05	0.272
Monosodium glutamate	.0015	0.04±0.033	0.094±0.048	0.184±0.123	<0.001***
	.0025	0.02±0	0.052±0.044	0.118±0.075	<0.001***
	.005	0.02±0	0.029±0.02	0.034±0.027	0.072

*Kruskal Wallis test, p<0.05 considered as statistically significant. *-p<.05, **-p< .01, ***-p<.001.*

Table 3. Gender wise comparison of taste assessment parameters at different concentration levels

Variable	Level of concentration gm/dl	Gustatory recognition threshold (ml)		P-Value
		Male	**Female**	
Neem	.003	0.024±0.008	0.022±0.006	0.305
	.005	0.02±0	0.02±0	1.00
	.007	0.02±0	0.02±0	1.00
Sodium chloride	.01	0.085±0.05	0.081±0.085	0.080
	.015	0.05±0.035	0.034±0.022	0.005**
	.02	0.02±0	0.02±0	1.00
Sucrose	.05	0.129±0.071	0.142±0.102	0.707
	0.1	0.076±0.051	0.063±0.032	0.374
	.15	0.028±0.019	0.023±0.007	0.408
Citric acid	.005	0.046±0.017	0.044±0.018	0.265
	.01	0.049±0.08	0.023±0.009	0.007**
	.015	0.034±0.049	0.02±0	0.119
Monosodium glutamate	.0015	0.168±0.121	0.078±0.069	<0.001***
	.0025	0.102±0.077	0.047±0.05	0.004**
	.005	0.037±0.029	0.022±0.006	0.015*

*Mann Whitney U test, p<0.05 considered as statistically significant. *-p<.05, **-p< .01, ***-p<.001*

Within-group comparison of taste assessment parameters at different concentration levels was analysed by Friedman test. Comparison of recognition scores at various thresholds levels is found to be highly statistically significant in all concentration of taste parameters. The trend observed is, higher the concentration the average recognition threshold score will be less (Table 4). Descriptive data of gustatory parameters were displayed in Fig. 1.

3.1 Reliability Test

The reliability test was carried out among 40 subjects using the test-retest method. The correlation (R-value) of all 5 items at 3 concentrations shows an acceptable level of internal consistency. The Karl Pearson correlation was used for assessing the correlation between two-time points in a two week time interval. All tastants variables in three different concentrations had either r value closer

to 1 or 1, which shows an acceptable level of reliability. The correlation was significant (p<0.001) as shown in Table 5.

4. DISCUSSION

The present study aimed at testing taste using different concentrations of standard tastant solutions and developing a bitter taste assessment solution using neem leaf powder. The analysis confirmed that gustatory recognition scores decreased as concentration increases

which in line with the findings of other studies [10].

Other studies have reported that ageing will affect the gustatory function in an inverse relationship [11-14]. A general trend was observed across all age groups that the gustatory recognition threshold increases with age, except for higher concentrations of sodium chloride and medium and higher concentration of neem.

Table 4. Within-group comparison of taste assessment parameters at different concentration levels

Variables		Mean±SD (ml)	X^2 Statistic	p-value
Neem	0.003	0.023±0.007	16.000	<0.001
	0.005	0.02		
	0.007	0.02		
Sodium Chloride	0.01	0.083±0.07	84.228	<0.001
	0.015	0.041±0.029		
	0.02	0.02		
Sucrose	0.05	0.136±0.089	104.702	<0.001
	0.1	0.069±0.042		
	0.15	0.026±0.014		
Citric Acid	0.005	0.045±0.017	75.887	<0.001
	0.01	0.035±0.055		
	0.015	0.026±0.033		
Monosodium Glutamate	0.0015	0.119±0.106	84.587	<0.001
	0.0025	0.072±0.069		
	0.005	0.029±0.021		

*Friedman test, p<0.05 considered as statistically significant. *-p<.05, **-p< .01, ***-p<.001*
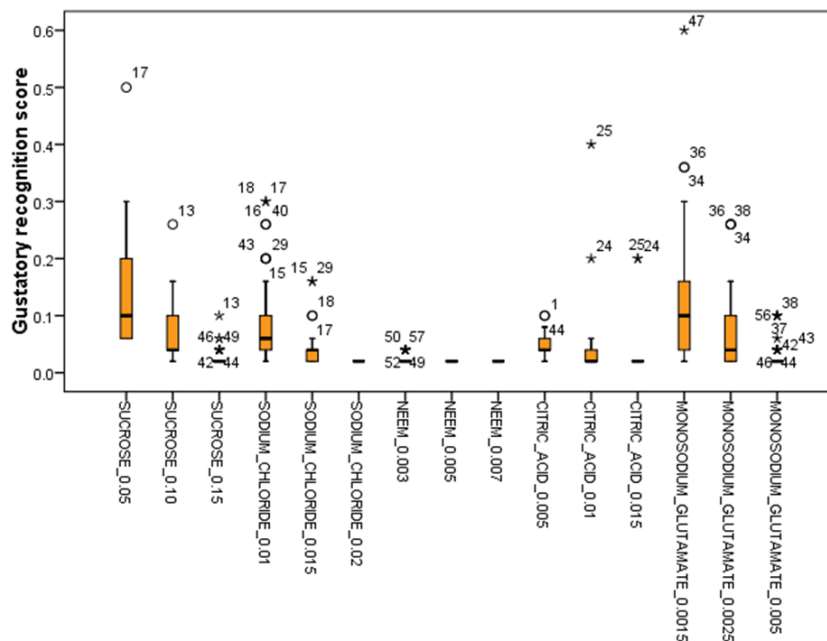
Fig. 1. Box plot of gustatory variables in the study group

Table 5. Reliability assessment based on various taste parameters

Variables	Concentration (gm/dl)	R-value	P-Value
Neem	0.003	0.724	<0.001***
	0.005	NA	<0.001***
	0.007	NA	<0.001***
Sodium Chloride	0.01	0.996	<0.001***
	0.015	1	<0.001***
	0.02	NA	<0.001***
Sucrose	0.05	0.996	<0.001***
	0.1	0.682	<0.001***
	0.15	0.994	<0.001***
Citric Cid	0.005	0.97	<0.001***
	0.01	1	<0.001***
	0.015	1	<0.001***
Monosodium Glutamate	0.0015	0.994	<0.001***
	0.0025	0.997	<0.001***
	0.005	1	<0.001***

Pearson correlation, p <0.001 considered as statistically significant ***-p<.001

Previous studies have stated that compared to men, women had a low gustatory recognition threshold [15-17]. Michon C *et al* detailed that, women have superior sensitivity to all the tastes as they are born with more taste buds compared with men, and taste bud number decreases only after menopause [18]. This was suggested by our data as well, but the difference was statistically significant only in medium concentrations of sodium chloride and citric acid and in all the concentrations of monosodium glutamate. The level of circulating estrogen produces both organizational and activational variance in taste and taste-guided behaviours might be the reason for this dominance in females. Estrogen modulates taste detectability and preference in developed animals [16,19].

We used solutions of neem leaf powder instead of quinine hydrochloride for bitter taste assessment. Neem is proved to have various pharmacological activities like antioxidant, anticancer, anti-inflammatory, wound healing effect, antidiabetic, antibacterial, antiviral, antifungal, antimalarial, anthelminthic, antinephrotoxicity, neuroprotective, immunomodulatory, hepatoprotective, and growth-promoting effect [20-23]. Apart from these functions, neem can be considered as a diagnostic tool for the assessment of bitter taste. Ethanol extract from neem leaves can reduce the urea concentration and has no adverse effect on renal and hepatic function [24,25]. Neem is safe in patients with renal failure, hepatic disorders, and degenerative disorders and can be used to test gustatory function in these conditions.

5. CONCLUSION

The gustatory function was assessed using five taste solutions in different concentrations. Instead of the usual quinine, we used neem leaf powder for bitter taste assessment here. Neem leaf powder is easy to procure and safe to use. Neem, a storehouse of many medicinal properties, can now be used to measure bitter taste. The solutions developed in the present study are reliable and cost-effective and can be safely used to detect gustatory dysfunction in clinical settings.

CONSENT

Informed consent was obtained from all the participants involved in the study.

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee of the Little Flower Hospital and Research Centre (EC/24/2018), Angamaly, Kerala, India.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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