Original research article **JOHS: Journal of Optometry and Health Sciences, Vol. 1 No. 1, 2021**

Tear Film Production and Stabilizing Potentials of *Dennettia tripetala*

Augustine U. Akujobi [1], ThankGod C. Awuzie [2]

[1] Department of Optometry, Imo State University, Owerri, Nigeria
[2] Ladkem Eye Hospital, Lagos, Nigeria.
Email: au.akujobi@imsu.edu.ng
Phone: +2348033727967

Abstract
Dennettia tripetala (DT) is an ethno-medicinal plant whose seeds are consumed for nutritional and medicinal purposes. Based on this, the tear film production and stabilizing potentials of *Dennettia tripetala* were investigated. A single dose of 750mg/kg body weight of the seed extract was orally administered on 50 healthy adults aged 18-35 years (26.4±1.98). Schirmer I test (SIt) and Tear-break-up time (TBUT) scores were recorded at 30 minutes, 60 minutes and 90 minutes post-ingestion using calibrated Whatman No. 41 Schirmer strips and cobalt blue filters of the slit lamp biomicroscope respectively. Data were analyzed with Z-statistics at 95% confidence interval using the Decision Analyst STATS^{TM} version 2.0 Software. Across the age groups, the mean of means baseline SIt score was 15.4±1.34mm. At 30 minutes and 60 minutes post-ingestion, the mean of means SIt scores significantly ($p<0.05$) increased to 19.2±1.68mm and 25.8±3.97mm respectively compared with the baseline score, while at 90 minutes post-ingestion the mean SIt score significantly ($p<0.05$) increased to 16.0±0.88mm when compared with the baseline score. The age distribution of mean TBUT scores indicates a baseline mean TBUT score of 15.0±2.31 seconds across the age groups. At 30 minutes and 60 minutes post-ingestion, the mean TBUT scores significantly ($p<0.05$) increased to 19.7±2.12 seconds and 24.3±2.58 seconds respectively compared with the baseline score. At 90 minutes post-ingestion, there was a reduction of the mean TBUT score to 19.2±1.71seconds, although it was significantly higher ($p<0.05$) than the baseline mean TBUT score. Males recorded significantly ($p<0.05$) lower mean SIt and TBUT scores than females. On the overall, the seed extracts of *Dennettia tripetala* proved effective in potentiating tear film production and stability; hence, can provide a natural remedy to keratoconjunctivitis sicca (dry eye syndrome).
Keywords: Tear film; Production; Stability; Potential; *Dennettia tripetala.*

Introduction

Dennettia tripetala (DT) is an ethno-medicinal plant which belongs to the family of *Annonacea*. It has been reported that various morphological parts of the plant serve as stimulants and are rich in other phyto-nutrients[1,2] such as protein, carbohydrates, vitamins and oil [3]. Chromatography-mass spectrometry identified about 25 compounds in the n-hexane fraction of the seed extract, including linoleic acid, ethyl ester, caryophyllene, 3-carene, phenyl ethyl alcohol, and cubebene [4]. Phytochemical screening of the ethanolic extract revealed the presence of tannins, alkaloids,

steroids, flavonoids, cardiac glycosides, saponins, and terpenoids [4]. These compounds provide the scientific basis for the use of DT in traditional medicine; hence, Saponins, tannins, and flavonoids are reported to be effective against diabetes and also possess antimicrobial and anti-inflammatory properties [5, 6], while alpha-linoleic acid reduces the risk of cardiovascular disease and prostate cancer in men [7, 8]. Dry fruits of DT consist of carbohydrates, trace elements, minerals, water-soluble vitamins, protein, fiber, ash, lipids and moisture[2, 9]. The moisture component increases with ripening and the amounts of phytochemicals, including phenols, saponins, tannins, flavonoids, and alkaloids also increase [9].

The tear film, also known as pre-corneal tear film is the fluid covering the anterior cornea surface. It consists of three layers viz; superficial oily or lipid layer, middle aqueous layer and innermost mucoid layer [10]. According to a study [11], the lipid layer is the outermost layer of the tear film, secreted by the meibomian glands and is responsible for retarding the evaporation of tears. It also serves as a lubricant which allows the lids slide through the globe without friction. The aqueous layer is the middle layer of the tear film which is secreted by the main lacrimal gland and the accessory lacrimal glands and makes up 90% of the total tear film density. It incorporates water soluble components of the tear film including salts, glucose, enzymes and proteins and functions to maintain normal tear quantity on the globe. The mucoid is the innermost layer of the tear film which makes the cornea surface more hydrophilic and consists of mucin secreted by the goblet cells and gland of Manz. Mucin regularizes the distribution of tears over the ocular surface by lowering the surface tension of the cornea and forming an adsorbed layer of hydrated mucus, which is weakly bound to the corneal surface and thus converts the corneal surface from a hydrophobic to a hydrophilic surface [10].

Tear film can be assessed qualitatively (stability) and quantitatively (production). Tear-break-up time (TBUT) is a qualitative assessment of the pre-corneal tear film which measures the rate of evaporation of the tear film. Tear-break-up time is the time interval in seconds between the last blink and the appearance of the first dry spot on the cornea under a topical 0.5% fluorescein dye [12 , 13]. To measure TBUT, fluorescein dye is instilled into the patient's tear film and the tear film is observed under a broad beam of cobalt blue filter of a slit lamp biomicroscope. TBUT under 10 seconds is considered abnormal [14].

Schirmer-1 test assesses the tear film quantitatively and determines whether the eye produces enough tears to keep it moist. The evaluation involves the use of a piece of Whatman No. 41 filter paper, 5 mm wide and 35 mm long, partially folded at the 5 mm point from one end. The folded end is placed on the lateral 1/3 of the lower tarsal conjunctiva and the patient is asked to fix his eye on any object slightly above the direct line of gaze for 5 minutes. A wetting length of less than 10mm denotes a hyposecretion of both basic and reflex secretors suggestive of keratoconjunctivitis sicca (dry eye syndrome), 10-30 mm implies a pseudoepiphora and >30mm indicates that the patient may have pseudoephiphora, hypersecretion, or a normal secretion [11] .

Dysfunctional tear film is responsible for keratoconjunctivitis sicca (KCS) and its associated ocular complications leading to blindness and decreased quality of life. The treatment of KCS includes the administration of antibiotics and application of certain invasive procedures. Some of the antibiotics have been reported to elicit adverse side effects during prolonged clinical use. Therefore, the development of a safer treatment regimen has become expedient. Based on this, the tear film production and stabilizing potentials of *Dennettia tripetala* seed extract were investigated.

Methodology

Dennettia tripetala fruits were procured from a local market, washed and authenticated by a Botanist in the Department of Plant Science and Biotechnology, Imo State University Owerri, Nigeria. The seeds were extracted and air-dried under normal room temperature and subsequently triturated using mortar and pestle. The specimen were weighed to 750mg using a standard weighing scale and wrapped in sterile packaging nylons.

The study protocols complied with the Helsinki Declaration on Human Experiment. Ethical consents and approval were obtained from the participants and appropriate authority respectively. Consenting participants were clerked; external and internal ocular examinations using a pen torch and direct ophthalmoscope were performed to rule out external and internal ocular pathologies respectively. Fifty (50) participants who satisfied the inclusion criteria were recruited into the study on consecutive basis and examined for baseline SIt and TBUT scores

pre-intervention. The body weights of the participants were measured using standard weighing scale.

Each subject was comfortably seated and the Schirmer strip inserted in the middle and outer 1/3rd of the lower fornix of the right eye. The subject was instructed to continue blinking normally or keep the eyes closed. At 5 minutes post-insertion, the strip was removed and the SIt score measured by the length of the wetted portion of the strip in millimeters.

The right eye of the subject was stained with 2 drops of 0.5% fluorescein dye and the subject was seated behind the slit lamp biomicroscope (SLB). The cobalt blue light of the SLB was beamed on the cornea, and the subject instructed to suspend blinks. The cornea was observed through the SLB microscope for the appearance of a dry spot. The TBUT was recorded as the time interval in seconds between the last blink and the first appearance of a dry spot on the cornea using a stop watch.

Participants were fed with the appropriate quantity of the specimen based on the standard 750mg/kg body weight and the SIt and TBUT scores recorded at 30 minutes, 60 minutes and 90 minutes post-ingestion using the same techniques. Data were analyzed with Z-statistics at 95% confidence interval using the Decision Analyst STATS^{TM} version 2.0 Software. $P<0.05$ was considered significant.

Results

Table 1: **Age distribution of study participants.**

Age (Years)	Frequency	% Frequency
18 – 20	6	12
21 – 23	11	22
24 – 26	12	24
27 – 29	9	18
30 – 32	7	14
33-35	5	10
Total	**50**	**100**

The mean age of the participants was 26.4±1.98 years. The highest number of subjects was found within the age group of 24-26 years (24%). The least number was found within the 33 – 35 years (5%) group.

Table 2: Age and gender distribution of study participants

Age (Years)	Frequency		% Frequency	
	Males	**Females**	**Males**	**Females**
18 – 20	3	3	13.6	10.7
21 – 23	4	7	18.2	25
24 – 26	5	7	22.7	25
27 – 29	5	4	22.7	14.3
30 – 32	4	3	18.2	10.7
33-35	1	4	4.6	14.3
Total	**22**	**28**	**44**	**56**

44% of the study subjects were males, while 56% were females. Among the males, the highest number of subjects were within the age groups of 24 – 26 years and 27 -29 years (22.7%) each; The least number was found within the 33 – 35 years age groups (4.6%) . The highest number of females were recorded among age groups of 21 – 23 and 24 – 26 years (25%).

Table 3: Table 3 Age distribution of mean Schirmer scores (mm).

Age (Years)	BL(mm)	30min (mm)	60min (mm)	90min (mm)
18 – 20	16.5	19.0	29.8	16.8
21 – 23	16.9	20.5	30.2	17.2
24 – 26	16.2	21.0	27.1	16.1
27 – 29	15.1	20.0	24.5	15.4
30 – 32	13.4	16.6	22.7	14.8
33-35	14.5	17.9	20.3	15.9
Mean of means±SD	15.4±1.34	19.2±1.68	25.8±3.97	16.0±0.88
p-value		**0.00001**	**0.00001**	**0.003167**

Legend: BL= Baseline

The mean SIt baseline score was 15.4mm, 30mins later it was 19.2mm. At 60mins post-ingestion it was 25.8mm, while at 90mins post-ingestion it was 16.0mm. At various time intervals, age co-related significantly and inversely ($p<0.05$) with mean SIt scores and the mean scores were significantly higher than the baseline mean SIt score ($p<0.05$).

Table 4: Age distribution of mean TBUT scores (secs).

Age (Years)	BL(secs)	30min (secs)	60min (secs)	90min (secs)
18 – 20	17.5	21.5	27.7	18.5
21 – 23	16.6	21.2	26.3	17.0
24 – 26	17.2	21.9	25.6	18.0
27 – 29	13.4	18.0	22.1	14.5
30 – 32	13.2	17.0	22.3	14.3
33-35	12.3	18.3	21.6	13.9
Mean of means±SD	**15.0±2.31**	**19.7±2.12**	**24.3±2.58**	**19.2±1.71**
p-value		**0.00001**	**0.00001**	**0.00001**

Legend: BL= Baseline

The mean baseline TBUT score was 15 secs, 30mins later the mean TBUT was 19.7secs. At 60mins after consumption, the mean TBUT result was 24.3secs, while at 90 mins it was 19.2secs. For various time intervals, the mean TBUT scores were significantly higher than the baseline mean TBUT score ($p<0.05$).

Table 5: Gender distribution of mean Schirmer scores (mm)

Age	BL	30mins	60mins	90mins (males)	BL	30mins	60mins	90 mins (females)
18 – 20	16.3	18.7	31.3	16.6	16.7	19.3	28.3	17.0
21 – 23	16.8	19.3	29.5	16.5	17.0	21.7	32.9	17.9
24 – 26	16.4	20.0	24.6	15.0	16.0	21.9	29.6	17.1
27 – 29	15.8	20.02	24.2	15.8	14.3	19.8	24.8	15.0
30 – 32	13.8	16.5	22.7	15.0	13.0	16.7	23.7	14.6
33 – 35	14.0	17.0	20.0	15.0	15.0	18.8	20.8	16.8
Mean±SD	**15.5±1.29**	**18.6±1.55**	**25.4±4.42**	**15.7±0.76**	**15.3±1.53**	**19.7±1.94**	**26.6±4.48**	**16.4±1.30**
P-value		**0.000968**	**0.088508**	**0.000233**		**0.000968**	**0.088508**	**0.000233**

Legend: mean= Mean of means

For males, the mean baseline SIt score was 15.5mm. At 30mins post-ingestion it was 18.6mm, at 60mins, the mean Schimer test result was 25.4mm, while at 90mins the results was 15.7mm. The mean base line schirmer result for female subjects was 15.3mm; 30mins later the mean Schimer result was 19.7mmm. At 60mins the Schirmer result of 26.6mm was recorded, while at 90mins the Schimer test result was 16.4mm. At all time intervals, females had significantly ($p<0.05$) higher mean SIt scores than males, except at 60 minutes post-ingestion where females had insignificantly ($p>0.05$) higher mean SIt score than males.

Table 6: Gender distribution of mean TBUT scores (secs).

Age(years)	BL	30mins	60mins	90mins(males)	BL	30mins	60mins	90 mins (females)
18 – 20	17.0	21.3	25.7	19.3	17.3	21.7	29.7	17.0
21 – 23	16.0	20.8	25.0	16.0	17.1	21.6	27.6	17.9
24 – 26	19.0.	22.8	27.6	19.8	15.3	21.0	23.6	16.1
27 – 29	12.8	17.6	21.6	14.4	14.0	18.3	22.5	14.5
30 – 32	12.0	15.0	20.8	12.8	14.3	19.0	23.7	15.7
33 – 35	15.0	20.0	22.0	14.0	13.5	20.5	25.3	13.8
Mean±SD	**15.3±2.6**	**19.6±2.8**	**23.8±2.7**	**19.0±1.75**	**15.3±1.6**	**20.4±1.4**	**25.4±2.75**	**15.8±1.53**
P-value		**0.051**	**0.001866**	**0.00001**		**0.051**	**0.001866**	**0.00001**

Legend: mean= Mean of means

For males, the mean base line TBUT score was 15.3secs, 30mins later the mean TBUT was 19.6secs. At 60mins the results obtained was 23.8secs, while at 90 mins, 19.0secs was recorded as mean TBUT. Among the female participants, the mean base line TBUT score was 15.3mins; 30mins later the mean TBUT was 20.4secs. At 60mins, the mean TBUT score was 25.4secs, while the mean TBUT of 15.8secs was obtained at 90mins. At 30 minutes post-ingestion, females had insignificantly ($p>0.051$) higher mean TBUT score than males. Females had a significantly ($p<0.05$) higher mean TBUT scores at 60 minutes post-ingestion than males, while at 90 minutes post-ingestion, males had a significantly ($p<0.05$) higher mean TBUT score than females.

Discussion

The morphological parts of *Dennettia tripetala* have medicinal properties attributed to the presence of phyto-nutrients such as Tanins, alkaloids, steroids, flavonoids, cardiac glycosides saponins and terpens. These phytochemicals have been widely reported to possess antobiotic, antioxidant and anti-inflammatory properties and are responsible for the hypoglycemic, analgelsic, hypolipidemic and hemotoxic effects of the morphological parts[3]. The effects of *D. tripetala* on ocular functions have scarcely been investigated, although it has been found to be effective in reducing the intraocular pressure of normotensive emmetropic humans [15]. *Dennettia tripetala* contains certain phytochemicals similar to the biochemical components of the tear film and therefore; exerts biochemical influences on the integrity and function of the tear film. Based on this, the present study investigated the tear production and stabilizing potentials of *Dinnettia tripetala* on 50 healthy adults.

Across the age groups, the mean of means baseline SIt score was 15.4±1.34mm. At 30 minutes and 60 minutes post-ingestion, the mean of means SIt scores significantly ($p<0.05$) increased to 19.2±1.68mm and 25.8±3.97mm respectively compared to the mean baseline score, while at 90 minutes post-ingestion the mean SIt score significantly ($p<0.05$) increased to 16.0±0.88mm when compared with the baseline. The elevation of mean SIt scores indicates the ability of *D. tripetala* seed extracts to increase the tear production of the lacrimal gland and accessory lacrimal glands; hence, can be useful in the treatment of aqueous deficiency dry eye syndrome. Although the mean SIt score at 90 minutes post-ingestion reversed comparatively to the baseline score, it was still significantly higher ($p<0.05$) than the baseline value. The peak elevation of

mean SIt score was recorded at 60 minutes post-administration among all age groups. This indicates that the peak and therapeutic concentration of the specimen can be sustained by an hourly consumption of not less than 750mg/kg body weight of the seed extract, although the duration of consumption required to sustain the therapeutic dose was not investigated by the present study. In the present study, increasing age co-related significantly and inversely ($p<0.05$) with the distribution of mean SIt scores across various time intervals; corroborating a study [15] which opined that dry eye syndrome is more prevalent in older patients and more marked in women than men.

The age distribution of mean TBUT scores indicates a baseline mean TBUT score of 15.0±2.31 seconds across the age groups. At 30 minutes and 60 minutes post-ingestion, the mean TBUT scores significantly ($p<0.05$) increased to 19.7±2.12 seconds and 24.3±2.58 seconds respectively compared with the baseline score. At 90 minutes post-ingestion, there was a reduction of the mean TBUT score to 19.2±1.71seconds, although it was significantly higher ($p<0.05$) than mean baseline TBUT score. The seed extracts of *D. tripetala* significantly ($p<0.05$) increased mean TBUT scores of the participants across age groups when compared with the baseline mean TBUT scores. The ability of the seed extracts of *D. tripetala* to elevate mean TBUT scores is an indication of the therapeutic relevance of the specimen in the treatment of evaporative dry eye syndrome. The peak concentration and effect of the extract was attained at 60 minutes post-ingestion; hence, an hourly ingestion of 750mg/kg body weight of the specimen can maintain a therapeutic concentration. Since no adverse side effect of the seed extract has been documented and the duration of intake for therapeutic purposes has not been reported, prolonged and repeated intake of the extract for the mitigation of ocular complications associated with evaporative dry

eye syndrome appears explorable. The present study recorded an inverse and significant ($p<0.05$) co-relation between increasing age and the distribution of mean TBUT scores across various time intervals.

The gender consideration of mean SIt scores showed a mean baseline score of 15.5±1.29mm among the male participants. At 30 minutes and 60 minutes post-ingestion, the mean SIt scores significantly (<0.05) increased to 18.6±1.55mm and 25.4±4.42mm respectively. The mean SIt score at 90 minutes post-ingestion was observed to be 15.7±0.76 mm, although it was significantly higher ($p<0.05$) than the baseline score. Among the female participants, the baseline mean SIt score was reported at 15.3±1.53 mm. At 30 minutes and 60 minutes post-ingestion, the mean SIt score significantly increased to 19.7±1.94 mm and 26.6±4.48 mm respectively, while 16.4±1.30mm was recorded at 90 minutes post-ingestion. Although the mean SIt score at 90 minutes post-ingestion was less compared with 30 minutes and 60 minutes, it was significantly ($p<0.05$) higher than the baseline score. Although a study[15] had reported that dry eye syndrome is more marked in females than males, the result of the present study showed that males had significantly ($p<0.05$) lower mean SIt scores across time intervals than females; hence, are more predisposed to aqueous deficiency dry eye syndrome. The discrepancy between the results of the previous and present studies may be related to a previous report[15] that higher prevalence of dry eye syndrome among females is associated with hormonal changes of the aging process. The female participants in the present study were below the age of hormonal changes and exceptionally excluded from the reported hypothesis; hence, recorded higher mean SIt scores than males.

The gender distribution of mean TBUT scores recorded a baseline mean TBUT score of 15.3±2.6 and 15.3±1.6 seconds among the male and female participants respectively. Among the male participants, there was a significant ($p<0.05$) and progressive increase in the mean TBUT scores from 30 minutes to 90 minutes post-intervention compared with the baseline score. Among the females, the mean TBUT scores significantly ($p<0.05$) increased from 20.4±1.4 seconds at 30 minutes to 25.4±2.75 seconds at 60 minutes post-intervention. At 90 minutes post-intervention, there was a reduction in the mean TBUT score (15.8±1.53secs), although it was significantly higher ($p<0.05$) than the baseline score. In both males and females, the peak mean TBUT score was attained at 60 minutes post-administration. At 30 and 60 minutes post-ingestion, females recorded significantly ($p<0.05$) higher mean TBUT scores than the males contrary to the postulation of a previous study[15], while at 90 minutes post-ingestion, the mean TBUT score decreased to 15.8±1.53 seconds, but remained significantly ($p<0.05$) higher than the baseline mean score.

Conclusion

The seed extracts of *Dennettia tripetala* proved effective in increasing tear film production and stability and can provide a natural remedy to keratoconjunctivitis sicca (dry eye syndrome).

Recommendation

The determination of the duration of consumption and fractionation of the seed extract of *Dennettia tripetala* for identification and isolation of the active phyto-nutrient(s) specific to tear film integrity are needful in therapeutic application. Further studies are therefore recommended in these areas.

Declaration of conflict of interest

The authors declare that there is no conflict of interest.

References

1. Egharevba H, Idah AE. Major Compounds from the Essential Oil of the Fruit and Comparative Phytochemical Studies of the Fruits and Leaves of *Dennettia tripetala* in North Central Nigeria. International Journal of Pharmacognosy and Phytochemical Research 2015; 7(6):1262-1266 ISSN: 0975-4873.

2. Okwu D, Morah F (2004). Mineral and Nutritive Value of *Dennettia tripetala* Fruits. Fruits 2004; 59: 437–442.

3. Iseghohi SO. A Review of the Uses and Medicinal Properties of *Dennettia tripetala* (Pepperfruit). Medical sciences 2015; 3(4):104–111.

4. Elekwa I, Okereke SC, Chukwudomo CS. Phytochemical Screening and GC-MS Analysis of the Essential Oil of *Dennettia tripetala* (Pepperfruit) seeds. ABSU J. Environ. Sci. Tech 2011 ;1:93–98.

5. Reihemann K, Obertreis B, Teucher T. (1999). Plant Extract from Stingy Nettle (Urticadioca) and Anti-rheumatic Remedy, Inhibits the Pro-inflammatory Transcription Factor NF-κB. FEEBs Lett. 1999; 442: 89–94.

6. Sparg S, Light M, Stadan J. Biological Activities and Distribution of Plant Saponins. J. Entopharmacol. 2004; 94: 219–243.

7. William E. Importance of Ω-3 Fatty Acids in Health and Diseases. Am. J. Clin. Nutr. 2000; 71 : 171–175.

8. Koralek D, Peters U, Andriole G. A Prospective Study of Dietary α-Linoleic Acid and the Risk of Prostate Cancer. Cancer Causes Control 2006; 17: 783–791.

9. Ihemeje A, Ojinnaka MC, Obi KC, Ekwe CC. (2013) Biochemical Evaluation of Pepper Fruit (*Dennettia tripetala*) and its Use as Substitute for Ginger in Zobo Drink Production. Academic Research International 2013; 4(6): 513-521.

10. Khurana A, Khurana A, Khurana B. Comprehensive ophthalmology 2015; 4th ed. jaypee, p.364.

11. Jones LT. The Lacrimal Secretory System and Its Treatment. Indian J Ophthalmol 1966;14:191-6.

12. Holly FJ, Lemp MA. Tear Physiology and Dry Eyes. Survey of Ophthalmology 1977; 22 (2): 69-87.

13. Lemp MA, Hamil JR. Factors Affecting Tear Film Break-up in Normal Eyes. Acta Ophthalmol. 1973; 89:103-5.

14.Vislisel J. Tear Break-up Time(TBUT). https://webeye.ophth.uiowa.edu/eyeforum/atlas/pages/TBUT/index.htm# 2015.

15. Guillon M, Maissa C. Tear Film Evaporator—Effect of Age and Gender. Contact Lens Anterior Eye 2010; 33(4): 171-175.