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Original Research

Ethnomedical survey and safety evaluation of traditional eye medicines used in Misungwi district, Tanzania

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ABSTRACT

Aim: This study aimed at documenting products used as traditional eye medicine (TEM) in Misungwi district, Tanzania, and performing preliminary analysis on safety status. **Methodology:** Ethnomedical study was conducted in Misungwi district. Information was sourced by face-to-face interview with traditional healers, traditional medicine vendors, and knowledgeable people guided by a well-structured questionnaire. Safety was evaluated by determining pH using pH meter and mineral analysis using the Delta, Portable X-ray fluorescence equipment, and qualitative chemical tests. **Results:** A total of 23 TEM products were recorded from botanical (79%) and zoological (21%) sources including animal excreta. Liquid preparation ranked highest among dosage forms. Safety evaluation showed that only one product possessed the pH value of 7.4 as recommended for topical ophthalmic medicines. Fourteen minerals were detected and quantified in three samples; some of these minerals are known for their negative effects to the eyes, of medical interest is strontium used for the management of benign eye tumors. Information providers were unaware of health risks associated with the use of TEM. **Conclusion:** This study has revealed the common use of TEM in Misungwi district. The majority of the products are from the botanical source. Although literature provides supporting data for the application to some of the recorded TEM, safety evaluation by pH and mineral analysis in this study have indicated possible ophthalmological medical problems that could result from using such products. Extensive scientific studies including animal experiments and identification of bioactive compounds are essential to develop safe TEMs.

KEY WORDS: Ethnomedical, minerals, Misungwi district, safety, pH, Tanzania, traditional eye medicine

INTRODUCTION

Eyes are delicate sensory organs that make our everyday life comfortable. When it comes to treatment, eyes require special attention to avoid medical problems including partial or total vision disability. In Tanzania, modern health-care services are inadequate and majority of population especially in rural areas still rely on traditional methods including the use of traditional eye medicine (TEM). Various studies have associated TEM with medical eye problems based on diagnosis made by medical practitioners/ophthalmologist, for example, a study conducted in Tanzania showed that 25% of corneal ulcers resulted from the use of TEM [1] and a similar research conducted in Malawi showed 33% of patients with corneal disease in rural Malawi reported to have used TEM [2]. In the same country, 72% of the interviewee on self-treatment used TEM with no clear borders between biomedical and herbal medicines, i.e., used side by side [3].

TEM products are obtained from botanical, zoological, and mineral sources, and routes of administration are local application to eyelids, eye drops, instillation into the conjunctival sac, or taken orally [4]. Negative consequences of using TEM could be minor such as temporary irritation and pain or major such as permanent loss of vision if not timely and well managed. Examples include the following; *Calotropis procera* latex reported to cause significant ocular morbidity, including painless corneal edema, permanent endothelial cell loss with morphological alteration after intracorneal penetration ending up with keratitis [5-7] and a case study reported in Nigeria where man suffered from ocular discomfort and eventually blindness after applying the raw cassava extract [8]. Recently, Tanzanian plants and other products used for the treatments of eyes diseases/conditions were published in a systematic manner to enable quick search for further scientific research to prove their efficacy and safety [9].

Tanzania is among the African countries rich in natural resources that provide medicinal substances for traditional health care. Misungwi district mainly populated by the Sukuma tribe is a place where traditional medicine is still valued, especially among old people and the rural population. It involves the use of all sorts of natural resources and supernatural powers. It is one of the seven districts of Mwanza region in Northern part of Tanzania situated in the savannah grassland about 156 km from the Serengeti national park at 02°51′S 033°05′E. Its map

is given in Figure 1. The area has tropical climate, the summers have a good amount of rainfall, while the winters have very little. According to the Köppen-Geiger climate classification, the temperature averages 23°C and the rainfall averages 901 mm [10].

This study aimed on documentation of TEM products in Misungwi district as a way conservation of such knowledge and performing preliminary safety evaluation by pH determination and mineral analysis. Our findings are expected to stimulate researches on TEM products on various aspects to enable preparation of standardized TEM product/identify useful bioactive compounds for the development of ophthalmic products.

METHODOLOGY

Study Design

This study was conducted in two phases as follows:

i. Ethnomedical study comprised oral interview and field work done on alternate days
ii. Safety evaluation included laboratory work.

Ethnomedical Study

Study period, study site, and information providers (IPs)

The study was carried out for 14 days in December 2014 in Misungwi district and covered Mapilinga, Mwaniko, Nange, and Ng'ombe villages plus the Misungwi street in town. Interviewee included traditional healers and knowledgeable people (KP) found at homes in the villages whereas traditional medicine vendors were found at the business center of the town.

Data and material collection

The purpose of the study was explained, and informed consent was obtained from each of the participants. Face-to-face oral interview was conducted in Swahili (national) and Sukuma (local language) to obtain reliable information using a well-structured questionnaire (Annex I), which was translated to Swahili language. The data on TEM including sources, dosage forms, preparation methods, route of administration, knowledge, and awareness of IPs on risks associated with TEM were immediately recorded in the questionnaire. Field work involved collection of plant material for herbaria preparation, pH determination, and mineral analysis. Samples of animal excreta were bought from IPs. Identification/authentication of collected plants using herbaria specimen was done in the Botany Department at the University of Dar Es Salaam where the voucher specimen were deposited.

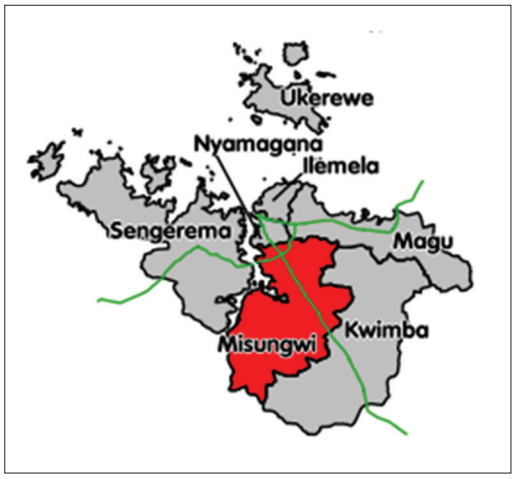

Figure 1: Map of Misungwi district [11]

Quantitative ethnomedical data analysis

The collected information was quantitatively analyzed using an index of relative frequency citation (RFC) as,. $RFC = \frac{FC}{N}$ This index shows the local importance of each product and it is given by the frequency of citation (FC), the number of informants mentioning the use of the product (species) divided by the total number of informants participating in the study (N) [12].

Safety Evaluation

This was performed in February 2015. The pH determination was done in the Laboratory of Pharmacognosy Department at Muhimbili University of Health and Allied Sciences and mineral analysis in the Geology Department at University of Dar es Salaam.

Determination of pH values

Determination of pH values employed the JENWAY 3035 pH meter, made in the UK by Jenway Felsted Dunmow, Essex CM6 3LB. Sample preparation was done according to the method previously described by Maregesi *et al.* [9] with minor modification at room temperature of 25°C. In brief, 1 g of the powdered material of each test sample was weighed in triplicate then macerated with 20 mL distilled water with occasional shaking for the period of 12 h. The filtrates were used for pH determination. The pH of each sample was obtained by taking the average value from triplicate analysis.

Mineral analysis

Mineral analysis was carried out using Portable X-ray fluorescence (XRF) spectrometer, and the application note by Innov-X systems, 2003, was adopted. Each test sample was ground to a very fine powder and then passed through a sieve of 250 μm mesh. About 100 mg of each sample was weighed and then transferred into respective XRF test cups which were then covered tightly with nylon material. The XRF test cups containing samples were then subjected to the analyzer (XRF spectrometer) for spot analysis (Innov-X systems XRF testing guideline). Additional mineral analysis

was done using standard procedures/reagents for qualitative tests of salts.

RESULTS

Ethnomedical Study

Eighteen IPs participated in the study whose demographic data are presented in Table 1. Number of males and KP were higher than females and others groups, respectively. We afforded to record twenty-three TEM products and their methods of preparation, dosage forms, administration routes, FC, and RFC as summarized in Column A of Table 2. Frequency of mention of treated disease/conditions is presented in Figure 2. All IPs were neither aware of any health risks associated with the use of TEM nor making follow-ups on patient progress. In case of treatment failure, patients could opt to report back to get an alternative medicine or seek treatment from other sources including modern medicine.

Safety Evaluation

All tested products were alkaline in nature with the pH values ranging from 7.3 to 10.0 as shown in Table 3. Fourteen minerals detected/quantified in three TEM products are given in Table 4. Hyena excreta ranked highest for the total number of detected minerals.

Table 1: Demographic data of information providers

Demographic data	*N* (%)
A. Gender	
Sex	
Female	7 (39)
Male	11 (61)
B. Source of information	
Groups of information providers	
Traditional medicine vendors	2 (11)
Traditional healers	5 (28)
Knowledgeable people	11 (61)
C. Age	Adults above 30 years
D. Education level	Primary school

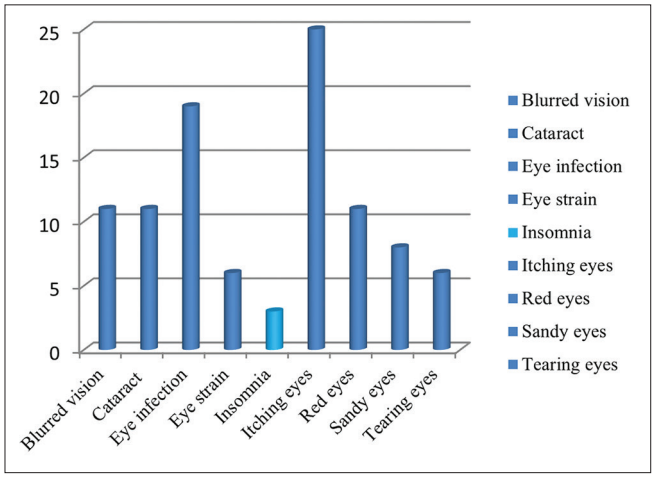

Figure 2: % Frequency of mention of the treated eye disease/condition

DISCUSSION

Ethnomedical Study

The majority of the recorded TEM products (79%) are obtained from plants with half of them prepared from leaves. Animal products comprised of honey (4%) and animal waste (17%). The suitability of the later for use as eye medicines is doubted since microbial contamination with pathogenic microbes is most likely as evidenced by lizard droppings [9]. The majority of reported products are liquid preparations applied as eye drops (91%) and matching with topical ophthalmic solution most commonly used and accepted dosage forms in modern medicine [46]. Most of the recorded TEM products were mono-component except for the following: (i) *Ficus glumosa*, *Ficus natalensis*, and *Nicotiana tabacum*; (ii) lizard and hyena excreta; and (iii) lemon juice and salt – a containing common and easily available substances.

Aloe vera leaf juice (latex) was the most mentioned product with the RFC of 0.61 followed by *Euphorbia hirta* and *Vernonia amygdalina* each with RFC of 0.43. About 61% of the recorded TEM products had previously been reported in Tanzania or other parts of the world for treatment of the same or different eye problem(s). This is a good indication on reliability of the information obtained from IPs. Previous data including those of related species are summarized in Column B of Table 2.

Itching, sand, and tearing eyes are common symptoms of allergic conjunctivitis [45]. In this context, allergic conjunctivitis is the most common treated eye disease/condition constituting 39% of all cases. It is normal that common diseases in a particular society tend to have more attention and various medicines. Allergic conjunctivitis is likely to be the most common eye problem as it relates well to the climate of Misungwi district and especially during the dry season with blowing winds that carry various dust particles capable of causing some allergies.

Chemical constituents of TEM products render therapeutic effects through various biological/pharmacological activities such as antimicrobial, anti-inflammatory, analgesic, and wound healing. For example, (i) honey is used against eye infection and eye itching, the former can be related to the antimicrobial activity of honey in aerobic conditions brought about by the osmotic effect of its sugar contents, hydrogen peroxide produced by the action of the enzyme glucose oxidase in diluted honey and phytochemicals found in the nectar including flavonoids and aromatic acids [47]; (ii) management of wounds resulting from conjunctivitis/irritation may be linked with the wound healing activity of *E. hirta* whose probable mechanism of action is promotion of collagen biosynthesis [24]; and (iii) curative effect of *Cocos nucifera* shell charcoal could be due to adsorption of foreign substances causing red eyes or minerals dissolved in the aqueous solution.

An interesting observation from this study is the use of related species for treatment of a particular eye disease/condition as reported for *Crotalaria* and *Ficus* species. Based Based on

Table 2: TEMs used in Misungwi district

A. Current ethno medical data TEMs from botanical sourcex						B. Data from previous studies
Plant and family names, voucher specimen no	Vernacular name	Plant part, preparation and route of administration	Eye disease/ condition	Frequency of citation	Relative frequency of citation	TEM uses, biological/ pharmacological/phytochemicals and related species
Aloe vera (L.) Burm. f. Aloaceae JM2014 (1)	Magaka (Sukuma) Makaka (Kerewe/Jita)	One drop of leaf juice used as eye drop twice a day	Cataract eye discharge, itching eyes	14	0.61	The *A. vera* gel is useful for dry skin conditions, especially for treatment of eczema around the eyes. It had shown antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*[13] as well as anti-inflammatory and antiseptic properties [14]
Cassia siamea (Lam.) Irwin et Barneby. Caesalpiniaceae JM2014 (2)	Mchongoma (Sukuma)	Steam from boiled leaves is allowed to enter the eye	Sandy eyes, itching eyes	6	0.26	*C. siamea* leaf juice is used to treat eye stye, conjunctivitis, and other minor eye problems [15]. In addition, a formulation prepared from leaves is used for treatment of bacterial/fungal eye infection [16]
Citrus limon (L.) Burm.f. Rutaceae JM2014 (3)	Limao (Swahili)	Salted lemon juice used as eye drops. 1 drop every morning until recovery	Cataract	6	0.26	*C. limon* juice had shown antibacterial activity against *S. aureus, Escherichia coli,* and *Klebsiella pneumoniae* among others [17]
Cocos nucifera L. Arecaceae JM2014 (4)	Makala (Sukuma) Mkaa (Swahili)	Very fine powder of the shell charcoal is directly used or dissolved in water to obtain the filtrate which is used as eye drops three times a day	Red eye	2	0.09	*C. nucifera* shell charcoal was recently, reported to treat eye problems among many other ailments [18]
Crotalaria laburnifolia L. Papilionaceae JM2014 (5)	Lupi (Sukuma)	Fresh leaf juice is used as eye drops (2 drops) three times a day	Itching eyes	4	0.17	Related species including *Crotalaria retusa* is used against eye infection [9], *Crotalaria pallida* and *Crotalaria assamica*
Crotalaria rogersii Baker f. Papilionaceae JM2014 (6)	Matulo (Sukuma)	Fresh leaf juice is used as eye drops. 2 drops three times a day	Itching eyes	5	0.22	possess anti-inflammatory effect due to flavonoids and pterocarpanoid [19] and *Crotalaria pusilla* have both analgesic and anti-inflammatory properties [20]
Datura stramonium L. Solanaceae JM2014 (7)	Malongelonge (Sukuma)	One tablespoon of dried seed powder is soaked in a full glass of hot water for at least 6 hours and taken orally at night only	Insomnia	7	0.30	*D. stramonium* seeds are used to induce sleep and treatment of insomnia. Alkaloids are present in varying concentrations in different organs of *Datura* plant, e.g., scopolamine and atropine classified as anticholinergics [21,22]
Euphorbia hirta L. Euphorbiaceae JM2014 (8)	Kashono (Sukuma)	Fresh latex is applied as eye drops three times a day	Itching eyes	10	0.43	*E. hirta* latex and leaf juice are used to treat eye problems including sore eyes, conjunctivitis, and eyelid stye [23,24]. Extracts of aerial parts exhibited anti-inflammatory activity [25] and antimicrobial activity against *P. aeruginosa*, *S. aureus* and others [26]
Ficus glumosa Delile Moraceae JM2014 (9)	Ngumo (Sukuma)	Fresh stem fibers mixed with fresh tobacco leaves and fresh bark of *F. natalensis* are macerated overnight. The filtrate is used as eye drops twice a day	Blurred vision	4	0.17	*F. natalensis* is used against cattle blindness, conjunctivitis and related ocular infections [27], eye tumor and cataract [9]

(Contd...)

Table 2: *(Continued)*

A. Current ethno medical data TEMs from botanical sourcex						B. Data from previous studies
Plant and family names, voucher specimen no	Vernacular name	Plant part, preparation and route of administration	Eye disease/ condition	Frequency of citation	Relative frequency of citation	TEM uses, biological/ pharmacological/phytochemicals and related species
Ficus natalensis Hochst Moraceae JM2014 (10)	Numbaga (Sukuma)	Fresh stem fibers, fresh barks of *F. glumosa* and tobacco leaves are macerated in water for overnight. The filtrate is used as eye drops twice a day	Blurred vision, eye infection	6	0.26	
Indigofera colutea Burm.f. Papilionaceae JM2014 (11)	Mburulambuli (Sukuma)	Root bark decoction is used as eye drops (3 drops) three times a day	Sandy eyes, itching eyes	3	0.13	*I. colutea* shoot had shown antibacterial activity against *Bacillus cereus* and *S. aureus* [28]
Jatropha curcas L. Euphorbiaceae JM2014 (12)	Makale (Sukuma)	Two drops of latex used as eye drops three times a day	Eye infection, tearing eyes	3	0.13	*J. curcas* latex is traditionally used to heal wounds and stem bark exhibited antimicrobial activity against *S. aureus, P. aeruginosa,* and *E. coli* [29,30]
Manihot esculenta Crantz Euphorbiaceae JM2014 (13)	Kayeba (Sukuma)	Leaf juice mixed with *Jatropha curcas* juice and python feaces. One drop applied into the eye three times a day	Sandy eyes and eye style	6	0.26	*M. esculenta* leaf juice is used to treat conjunctivitis and sore eyes [31]. It has anti-inflammatory, antimicrobial, and analgesic activity [32,33]
Nicotiana tabacum L. Solanaceae JM2014 (14)	Tumbaku (Swahili)	Fresh leaves mixed with *F. natalensis* and *F. glumosa* roots. The filtrate is used as eye drops twice a day	Blurred vision	7	0.30	The decoction of *N. tabacum* leaves is applied for muscle relaxation and relieving pain. Nicotine in its zinc complex isolated from leaves showed the antibacterial activity against ten different strains of Gram-positive and Gram-negative bacterial strains [34]
Ocimum canum Sims Labiate JM2014 (15)	Manung'u (Sukuma)	Fresh leaf juice is used as eye drops. 2 drops three times a day	Itching eyes	4	0.17	*O. canum* shoot was reported to treat conjunctivitis and possess antibacterial and antifungal activities against *S. aureus* and *Aspergillus* species. It contains essential oil rich in linalool along with several other compounds [35,36]
Solanum incanum L. Solanaceae JM2014 (16)	Matura (Sukuma)	Sun dried leaf powder is mixed with cooking oil. The paste obtained is topically rubbed on inflamed eye veins	Tearing eyes	5	0.22	*S. incanum* leaf juice is used against eye disease [37]
Vernonia amygdalina Delile Compositae JM2014 (17)	Mbarizi (Haya, Ha)	Liquid oozing from plant leaves at night is used to wash the eyes (with infection) prior to the application of other eye medicine	Eyes infection	10	0.43	*V. amygdalina* leaf is used to treat cataract [9], possess antimicrobial activity against *S. aureusa* and *P. aeruginosa* [38,39], drug resistant bacteria viruses and have anti-inflammatory activity. It contains alkaloids, tannins, saponins, phenolics, glycosides, and phlobatannins that may account for the therapeutic effects [40,41]
Vitex mombassae Vatke Verbenaceae JM2014 (18)	Nsungwi (Sukuma)	Two drops of the fruit juice is used as eye drops three times a day	Red eyes	5	0.22	The related species *Vitex doniana* is used for treatment of eye disease and possess anti-inflammatory activity [42]

(Contd...)

Table 2: *(Continued)*

TEMs from animal source					
Product name and source Vernacular name	Preparation and route of administration	Eye disease/condition	Frequency of citation	Relative frequency of citation	TEM uses
Honey bees Bhuki (Sukuma)	2-3 drops of raw honey is applied as eye drops twice a day	Eye infection and itching eyes	7	0.30	Honey is used since ancient times in treatment of various diseases including wounds and prevention of corneal scarring due to measles [43,44]. It is a common eye medicine for minor trauma, redness, pain, itching, crusting, and vision sharpening in Pakistan [45]
Hyena feaces Hyaenidae Mashi-Gambiti (Sukuma)	Equal parts of the powdered of lizard (white portion) and hyena feaces are macerated. The filtrate is used as eye drops twice a day	Eye strain and red eyes 6	6	0.26	None
White part of lizard feaces Lacertilia Mashi-Gakuli (Sukuma)				0.26	*Python feaces*, lizard droppings, and snail shells have been reported for cataract treatment [9]
Sea snail shells molluscs Shilungu (Sukuma)	Powdered shell is soaked in in water for at least six hours. The filtrate is used as eye drops twice a day	Blurred vision, eye infection, cataract and itching eyes	5	0.22	
Python faeces Python Mashi-Ganogwasato (Sukuma)	Powder is soaked in hot water. The filtrate is applied as eye drops three times a day	Cataract	4	0.17	

TEM: Traditional eye medicines

Table 3: pH of traditional eye medicine products

Plant/product	pH
V. amygdalina leaf extract	7.6
Python excreta micella	7.4
Hyena excreta micella	8.1
Sea snail shell micella	8.6
Lizard droppings (white portion) micella	7.3
Charcoal/water suspension	10.0
Distilled water	6.9
Prepared 0.9% NaCl	7.3

V. amygdalina: Vernonia amygdalina

chemotaxonomy, i.e., related species contain same/related phytochemicals thus likely give the same therapeutic effects as reported by the IPs. Another observation was that the concept of eye diseases was not clear among few IPs who regarded insomnia as an eye problem simply because it involves closure of the eyes by reporting *Datura stramonium* to treat this condition which is in accordance with the known use of the plant [22]. In addition, IPs were able to specify different eye diseases/conditions as compared to those from previous studies, who gave generalized information.

The lack of awareness and follow-up of the patient is a drawback on this particular traditional medical service. The fact that treatment the failure oblige patients to the seek treatment from modern medical services is in agreement with observation made in studies conducted in Tanzania and Malawi [1,2] and elsewhere. The unfortunate part is that the harm could have reached an irreversible stage by the time patients consult the medical practitioner/ophthalmologist due to switching from one or more TEM until desperation.

Safety Evaluation

Among the six analyzed samples, the python excreta micella was the only TEM with the recommended pH of ophthalmic products of 7.4 which is the same as that of the lacrimal fluid due to isotonicity importance. However, pH values of 7-9 are tolerated by the eye without marked irritation. Acidic and too alkaline products are corrosive to the eye [48]. Regarding the pH of *V. amygdalina* leaf extract, it was alkaline (pH of 7.6) compared to the acidic pH of 5.6 in our previous work [9]; at this point, no definitive comment can be made but just to speculate the causes such as unspecified age of leaves collected from different locations.

Table 4: Mineral contents of the excreta and sea snail shells

Mineral	Hyena excreta (%)	Python excreta (%)	Sea shells (%)
Ca	21.794±0.19	42.886±0.24	0.28±0.02
K	1.385±0.07	ND	6.291±0.18
P	8.723±1.68	ND	ND
S	1.10±0.23 (ppm)	ND (ppm)	ND (ppm)
Ti	489±30	ND	ND
Mn	100±9	31±8	9±0.3
Fe	2728±86	375±43	ND
Zn	217±12	21/7	ND
Rb	36±4	60±5	26.5±1.6
Sr	569±21	1239±45	20.2±1.6
Zr	179±10	ND	ND
As	ND	ND	13.4±1.8
Cl	ND	4425±8.32	1130±10.4

Qualitative analysis showed the presence of CO_3. *ND=Not detected

With regard to minerals, some metals causes toxicity by their action on the retina and optic nerves and are implicated in structural and physiological damage in the mammalian eye [49]. Some of the negative health effects of the detected metals and salts include vision impairment by manganese [50], chemical burn of eyes and skin by rubidium hydroxide formed from the reaction between rubidium and skin moisture [51], redness, pain and inflammation by calcium carbonate [52], skin and eye irritation by zinc salts particularly the carbonate [53], granuloma caused by zirconium and arsenic being carcinogenic [49]. Thus, frequent use of TEM containing these minerals could lead to medical eye problems. On the other hand, strontium has medical application for management of benign tumors of the eye [54].

This study and literature data show the role of TEM in various communities despite reported harmful consequences of some TEM products [5-9] and lack of evident scientific support to justify their uses. However, the use of TEM is inevitable due inadequate modern health services, thus a need to educate the public about health risks associated with TEM through the media and other means. In parallel, scientists should focus their research to produce standardized, safe TEM products and/or identify bioactive compounds for the development of modern medicine.

CONCLUSION

The findings of this study showed that majority of the recorded TEM products (79%) are botanical products while animal products (21%) comprised of honey and animal wastes. Some of the recorded products have previously been reported in Tanzania/other countries, suggesting that the information given by IPs is reliable but this not a guarantee for efficacy and safety. IPs and the public as a whole need to be informed on risks associated with the use of TEM. Results from safety evaluation, though preliminary, necessitate a very comprehensive study to identify safe TEM products as well as getting evidence for public declaration of unsafe products. Animal experiments to establish the safety status of some botanical TEM products are in progress.

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ANNEX

Annex I: English version - questionnaire for ethnomedical study on traditional eye diseases/conditions7

1. Source of information:
   i. Name of traditional healer/herbalist/ others ……
   ii. Age and education level: …….
   iii. Sex: ……..
   iv. Date: ……..…….
   v. Address: ……..……
   vi. Level of education: ……..
   vii. Tribe: ……
2. Do you treat any eye diseases/condition? Yes/No: …….
3. If yes, what are they? .....…..……...
4. Which of the mentioned diseases do you treat and have obtained positive results?
   (i) …..……
   (ii) …..……
   (iii) ……...
5. Which plant(s) and plant part(s) do you use? ……
6. Do you use a one or a mixture of plants in treating your patients? ……
7. Besides plant material(s) do you use other products like minerals or animal products? Yes/No: ……..
8. If yes what are they? (Vernacular names: ……
9. Do you have any specific time/season for collection and storage conditions for you plant material? ……
10. How to you prepare your medicine? (e.g., soaking/boiling in water, powdering, juice, latex, etc.) ……
11. How do you administer your medicine to your patients (e.g., orally, topical application, eye drop, etc.) ……..
12. What amount/quantity of medicine do you administer to your patient at one time? ……..
13. How many times per day is the medicine to be taken? ……
14. With regard to the amount of medicine given, do the age/weight matter? Yes/No: ……...
15. If yes, how do you determine the amount to be given to your patients? ……
16. How long does the patient use the medicine? ……
17. Are you aware of any side effect such as blindness that can be caused the medicine you supply to the patients? ……
18. Do make any follow-up of your patient to see if they fully recovered? …….....
19. Are you willing to show me the plant(s) so that we can carry out some scientific research to confirm their efficacy? Yes/No ……
20. If no, what reasons do you have for that? If yes, what are your future expectations from scientific finding ……
    ……