

Proceeding of the Scientific Review Workshop on the Ongoing Efficacy and Safety Investigations of Traditionally Used Medicinal Plants in Ethiopia



Editors:

Ashenif Tadele (B pharm, MSc), EPHI
Asfaw Debela, PhD, EPHI

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Acronyms

AAU	Addis Ababa University
ACTs	Artemisinin-Based Combination Therapies
ANOVA	Analysis Of Variance
DM	Diabetes Mellitus
EPHI	Ethiopian Public Health Institute
EOC	Essential Oil Content
FMHACA	Food, Medicine and Health Care Administration and Control
HPLC	High Performance Liquid Chromatography
KAP	Knowledge, Attitude, Practice
MIC	Minimum Inhibitory Concentration
NCD	Non Communicable Disease
OR	Odds Ratio
MoST	Ministry of Science and Technology
SOP	School Of Pharmacy
SPM	Strategic Planning Management
SPSS	Statistical Package for Social Sciences
TLC	Thin Layer Chromatography
TMMRD	Traditional and Modern Medicine Research Directorate
TMP	Traditional medicinal plants
WGARC	Wondo Genet Agricultural Research Center
WHO	World Health Organization

Key note address

Eshetu Lema (PhD), Head SERO, Ethiopian Public Health Institute

Dear Invited guests, ladies and gentlemen!

First of all I would like to welcome you all to this workshop. It is indeed a great honour and pleasure to be present in this scientific reviewing workshop on the evaluation of the ongoing investigations on the efficacy and safety of traditional medicinal plants against diseases of public health importance and setting future directions for the development of plant based medicines against these diseases through collaborative efforts.

Dear Colleagues and Participants,

As you all know malaria, diarrheal diseases and Parasitic helminthic infections that have public health importance causing serious health problems particularly in rural community of Ethiopia that need a joint effort in the prevention aspects as well as searching for safe and effective traditional remedies to address the problems. Hides, skins as well as live animals are among the major commodities that are used to earn foreign currency in Ethiopia. Ectoparasitic skin diseases of domestic ruminants that are caused by lice, sheep keds, ticks and mange mites are among the major diseases that result in serious economic loss to smallholder farmers, the tanning industries and the country at large. Hence searching for effective, safe, environmentally sound, affordable and accessible plant based medicine against animal ectoparasites has paramount importance to increase livestock productivity for farmers as well as to increase the quality of skins and hides thereby enhance the foreign currency of the country, Coming to the coming two days deliberation, participants are expected to review what has been accomplished so far and set clear direction for the coming years activities with the involvement of all expertise in the field of agricultural, health and biomedical sciences belong to Ethiopian Public Health Institute (EPHI), Wondo Genet Agricultural Research Center (WGARC) and School of Veterinary Medicine (FVM), AAU and collaborating universities in order to scrutinize the ongoing planned activities as a means to search for safe and effective potential plant based drugs against diseases of public health importance through multidisciplinary approach.

I would like to acknowledge the efforts of your contribution in the investigational efforts to scientifically validate traditional remedies and develop plant based medicines.

Wishing you a fruitful deliberations

I thank you all.

Workshop objectives:

This scientific review workshop was organized by the Directorate of Traditional and Modern Medicine Research in Ambo from 15 to 17 November 2013 with the following major objectives:

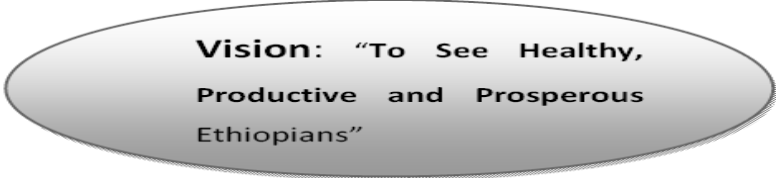
- To review the ongoing research activities on the efficacy, safety and quality evaluation of medicinal plants on diseases of public health importance,
- To create a platform in order to evaluate accomplishments and plan the way forward.

Expected Outcome

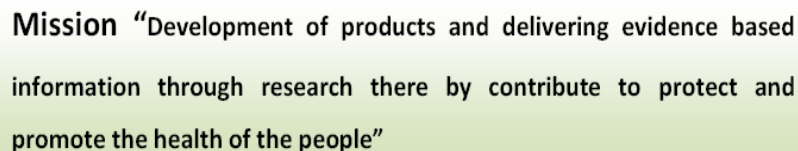
- Evaluating the available experimental data of the ongoing studies and share experiences among participants,
- To strengthen the collaboration among partners,
- Setting future directions for the ongoing studies of projects,
- Publication of summary of deliveries as well as recommendations forwarded in the form of proceedings.

Research themes of TMMD on traditional medicine

- Ethno-medicine /Ethno-botany,
- Community based KAP studies,
- Taxonomic studies on medicinal plants,
- Conservation of medicinal plants (*in situ* and *ex situ*)
- Phytochemistry, efficacy, safety, quality and formulation (dosage development) studies,
- Collaborative research with traditional healers,
- Clinical studies following ethical clearance.



**Vision: "To See Healthy,
Productive and Prosperous
Ethiopians"**



Mission "Development of products and delivering evidence based information through research there by contribute to protect and promote the health of the people"

Collaborating Institutes/organizations

- Research directorates within EPHI
- Wondo Genet Agricultural Research Centre,
- AAU
 - School of veterinary medicine,
 - College of Natural and computational Sciences, Faculty of Life Science
 - College of Health Sciences, School of Medicine, Department of Anatomy and Histology,
 - Department of Pharmacology,
 - Department of Biochemistry and Department of Physiology
 - School of Pharmacy, Department of Pharmaceutical technology and social pharmacy,
 - Department of pharmacognosy and pharmaceutical chemistry
- All African Leprosy Rehabilitation Training Centre (ALERT)
- Institute of Biodiversity and Conservation
- Wondo Genet Forestry College and Natural Resources, Hawasa University
- Adami Tulu Pesticide processing S.C
- Ethiopian Pharmaceutical Manufacturing S.C (EPHARM)

Executive Summary

This scientific review workshop was organized by the Directorate of Traditional and Modern Medicine Research in Ambo from 15 to 17 November 2013. The meeting was convened to evaluate the overall progress on the investigational efforts of traditionally claimed medicinal plants for diseases of public health importance. Experimental evidences that were generated on the safety, efficacy and quality of traditionally used medicinal plants in the last two and half years of the SPM period were presented and discussed by the workshop participants. In this workshop a total of 16 scientific papers were presented and the experimental data were critically evaluated and action points were proposed as the way forward for the remaining SPM period as a means to develop plant based products. The workshop participants pointed out that the TMMRD/EPHI should strengthen the research collaboration on the ongoing in-depth pre-clinical studies on the safety, efficacy and quality of plant based remedies for subsequent community based and clinical trials in order to maximize their benefits. Participants appreciate the efforts of TMMRD/EPHI for the organization of such a forum for updating of evidence based information on traditional medicine and getting constructive ideas for next steps including strengthening collaboration, creating partnership and networking among stakeholders.

The major points of emphasis from the workshop recommendations:

1. The workshop participants appreciated the investigational efforts and achievements of the TMMRD despite its limited manpower, serious shortage of supplies and laboratory facilities.
2. The TMMRD should be more focused on those Medicinal plants and diseases of public health importance for the generation and compilation of evidence based information requirements.
 - On prioritizing and complete the experimental evidence on medicinal plants for animal exo-parasite, skin microbial infestation (dermatological), hypertension and diabetes particularly on *Moringa* and *Thymus Sp.* (local name *Tosigne*) and others which look more promising and commercially feasible.
 - On generating evidence based information on targeted for the registration requirements of FMHACA for category one and two and if possible for the third categories of medicinal plants.
 - On quality issues particularly *Moringa stenopetala* leaves should be well addressed as this plant is highly used by the community for medicinal purpose even exported abroad to safe guard the public health from adulterated plant based products including *Thymus Sp.* (local name *Tosigne*) and *Hebiscus Sp.* (local name *Kerkede*).
3. The need to create linkage with manufacturers of pharmaceuticals, insecticides and pesticides for the research collaboration in the product development. Close collaboration with the regulatory authority, FMHACA was also stated as a means to strengthen and get the necessary support for the ongoing efforts on the scientific validation, product development as well as to fulfill the requirements for the registration of plant base remedies.
4. Participants also stressed on the need to strictly follow official protocols of develop standard operational procedures for investigational efforts of efficacy, safety, quality and other required experimental parameters.
5. The Directorate should also consider the commercial feasibility of the ongoing works through involvement of expertise for the economical analysis of plant based products.

Abstract No 1

Development of plant based medicines against major Ectoparasites of ruminants:

Accomplishments and future directions

Getachew Addis, PhD, Ethiopian Public Health Institute

Background: In developing countries like Ethiopia, livestock are lifeline for the rural communities; important as food source, income generating through export. Ectoparasites affects the ruminants which causes reduction in production & productivity through direct effect such as sucking and chewing, and under extreme cases fatality and indirect effects such as disease transmission such as bacterial, fungal, rickettsial, viral and protozoan diseases, exposing to opportunistic infections. Ectoparasites causes economic losses through lowering meat production, drop in quality or complete rejection of tannery products. Ectoparasites are of Zoonotic importance and have higher prevalence due to the inaccessibility & toxicity of modern drugs. Searching alternative medicine against ectoparasites of ruminants is of the current concern. The objective of this project was the Development of effective and safe plant based acaricide(s) against four ectoparasites (mange mites, ticks, lice and sheep ked) of small ruminants.

Methodology: Plant extracts, essential oils and appropriate formulations were developed for the study. Adult mites, sheep keds, lice and ticks were collected from infested animals. The parasites were incubated until nymph is produced and placed in petridishes. The experimental designs were both *in-vivo* and *in-vitro* with positive controls (Diazinon/Ivermectin), Negative controls (Excipients excluding extract). For *In vitro* efficacy study on ectoparasites twelve medicinal plants were tested against Sheep ked and seven medicinal plants against mange mite, ticks & lice. The *in-vitro* efficacy of the extracts and formulations were tested directly on the parasites.

Results: The *C citratus*, *E globules* showed a comparable *in-vitro* accaricidal effect on the sheap ked (*Melophagus Ovinus*) with standard Ivermectin, at 1.565 μ l/ml and 6.25 μ l/ml; respectively. Superior mange mite mortality were observed at 0.3125% *E globules*, 0.15625% *C citratus* than diazinon (0.1%) and Ivermectine(0.01%). *C citratus* (0.625%) and *E globules* (1.25%) of up to 3hrs showed a higher mortality than the diazinon (0.1%) on Lice (*Damalina ovis*). Two formulations: 2.5% *C citratus* with Fixative *Jatropha curcas* oil and 5 % *E globulus* with Fixative *Jatropha curcas* oil show a 100% lethal effect on Lice & sheep ked. The formulations containing *C citratus* & *E globulus* were safe for skin sensitization potential, the community based/simulation efficacy trial was also similar with *in vivo* result.

Conclusions & Recommendation: Formulations prepared from *C citratus* and *E globulus* essential oils are found to be effective and safe acaricides. Ticks are relatively resistant to extracts of the selected medicinal plants. The selected plants used for this study are amenable to large scale production. Product optimization using cheaper & safe emulsifier is recommended. Creating linkage with manufacturing industry, Continue study on use of the plant as a byproducts for forage, compost production and pilot and industrial scale production of the formulated drug(s) is highly recommended.

Goal: Contributing to national development!!!

Abstract No 2

Toxicological study of anti-ectoparasitic formulations comprised of essential oils obtained from *C. citrates* and *E. globules* mixed with *J. curcas* oil on Liver, Kidney and some blood parameters in mice.

Abinet G/Mickael, MSC AAU

Background: Ethiopia's economy is based on agriculture. Livestock was the 2nd major source of foreign currency. Skins from goats and sheep contribute the largest share to export commodities. However in recent years, this rank has been getting down. External parasites cause 35% of sheep skins and 56% of goat skin rejections. Therefore, ectoparasitic infection constitute an important health problem. Lice, keds, mange mites and ticks are the major ectoparasites. Synthetic drugs are available with different grades of success. Rapid development of resistance, high cost and other problems were associated with synthetic drugs. *Cymbopogon citrates* (DC) Stapf Commonly called lemon grass (LomiSar). It is used against common cold, fever and as insect repellent. *E globules* Labill in Ethiopia, it is known as "local name *Nech Bahrzaf*" is an evergreen tree. Its leaf extracts have been used to treat influenza, chest rub, and skin rashes. Eucalyptus oil can act as a natural insect repellent. The formulations comprised of essential oil extracts of *C citratus* and *E. globules* showed anti-ectoparasitic efficacy. So, the present toxicological study is carried out to evaluate any toxic effects of the formulations after oral treatment in mice.

Methods: Volatile oils of *C. citratus* and *E. globules* were obtained from WARC. Different formulations were prepared. Acute Toxicity Study was conducted on adult albino Swiss mice as grouped in to different doses starting with 0.5ml/kg up to 4ml/kg of each formulation while one group was placed as a control group received vehicle. For sub-chronic toxicity study each mice were treated for 12 consecutive weeks in 24 hrs interval by using gavage. At the end of 12th week, blood samples were taken by cardiac puncture. The serum and blood samples were then analyzed in automated analyzer. All animals were sacrificed by cervical dislocation. Then, the liver and kidney were collected for pathological analysis.

Results and discussions: In acute toxicity study: No mortality up to 2.5ml/kg doses of formulations. Signs of toxic effects like debilitation, piloerection and restlessness were observed at and above 2.5ml/kg. Mortality was recorded at and above 3ml/kg doses. No gross changes observed on the liver and kidney. No statistically significant ($p>0.05$) changes in any of the selected hematological parameters and serum biochemical parameters at both doses tested in the sub chronic study. No noticeable changes in the general behavior, and abnormal gross findings on vital organs however, dose-dependent focal alterations were observed in the liver and kidney sections. Mice in all group showed final weight gain.

Conclusion and Recommendations: The formulations do not produce obvious toxic effects in acute and sub chronic treatment. Further toxicological investigation is recommended on: Other vital internal organs of the body and on non- rodent species.

Abstract No 3

The collaborative research efforts of Wondo Genet Agricultural Research Center and TMMRD of EPHI Slides on Animal Ecto-parasites and MAPS

Beemnet Mengesha, MSc, Wondo Genet Agricultural Research Centre

Background: Lemongrass (*Cymbopogon citratus* L. (DC) Stapf) Graminae (Poaceae) is a perennial aromatic and medicinal plant. From the many species of the genus, lemon grass (*Cymbopogon citratus* L. (DC) Stapf) is the one considered economically important for the production of essential oils and aromatic herbs. The name lemongrass is derived from the typical lemon like odour of the EO present in the leaves and pseudostems. Lemongrass is native to South East Asian countries. It is cultivated widely in Thailand, Vietnam, Cambodia, India, Indonesian Islands, Africa, South America, Australia, Europe and North America. It grows best under sunny, warm and humid conditions; however, higher altitudes aggravate infestation of rusts on its economically important parts i.e. all over its expanding leaves. It grows in wider range of soils, but the best yields obtained from well drained sandy to loam. Calcareous and water logged conditions are unsuitable for its cultivation. Lemongrass can be used as carminative, to repel insects and as herbal tea. It has remedial properties and is used to treat: Bronchitis, cold, fever, malaria, hemorrhoids, toothache, baby oil, massage oil, oil for rheumatism, herbal baths, soap and candle making industries, herbal cooking's.

Objective of the study:

Lemon Grass

- Evaluate the performance of agronomic and quality traits under different testing locations
- Evaluate the performances of introduced varieties over the standard check for leaf and oil
- Investigate the influence of chopping size on essential oil content three aromatic grasses
- Determine the response of farm yard manure for further evaluation activities
- Quantify the number of slips per hole and plant spacing required for lemon grass planting

Eucalyptus globulus

- Investigate the effect of ages and site on essential oil content and oil composition of grown under different agro-ecological conditions.

Results:

***Cymbopogon citratus* L. Adaptation trial:** Mean performance of lemon grass for essential oil content and major essential oil compositions tested over six locations of Ethiopia. Leaf yield= 20.15-49.38(t), Essential Oil Yield= 98-225(kg), Essential oil content=0.42-0.53(%), Citral content 74.95-82.68(%). The highest yield observed at Wondo Genet and the smallest observed at Debrezeit than Awada, Hawasa, awash and Melkasa. The result obtained in the present study is comparable to the different reports, it can be said that lemon grass is adaptable to the different parts of Ethiopia and hence it can be cultivated for the production of herbage biomass and essential oils, except in vertisols.

Evaluation of lemon grass varieties for leaf and essential oil yield: The evaluation included three varieties of Lemmon grass introduced from England, West India and Lomisar-I. The trial was conducted at three locations arranged in RCBD with three replications in 2011 and 2012. Over all mean

performance of lemon grass varieties tested at Wondo Genet, Allagae and Hawassa during 2004 and 2005 EC. The LYPHY (kg), EOC, EOYHY 71066.67, 0.41, 286.76; respectively.

Influence of chopping size on essential oil content: The experiment showed that Chopping decreases the essential oil content of lemon grass, palmarosa and citronella grass from 1.06 to 0.86, 0.93 to 0.75, and 1.7 to 1.4, respectively. During chopping, specific oil cells are fragmented upon chopping and EO becomes exposed to the air for early volatilization. Chopping is not advisable for grasses- common mistake in laboratories.

Effect of Farm Yard Manure on Leaf and Essential Oil: As farm yard manure added the total leaf yield (t/ha), and total essential oil (kg) ha increased from 27.6 to 32.8 and 147.11 to 190.1; respectively.

Slips Number and Spacing Determination for planting of lemon grass: The result indicated that, it is possible to use slips number starting from one slip per hole with proper care. A spacing of 60 cm between plants and rows was found good for the cultivation of lemon grass.

Effects of Age and Site on Essential Oil Content and Composition of the Leaves of *Eucalyptus globulus*: Wondo Genet gives the highest essential oil content than Dembecha and Addis Ababa at earlier age of the plant. Leaf oil Composition (%) of *E. globulus* grown under different sites and stand age. 1,8-cineol content was the comparable at wondogenet and Addis Ababa at 7 years of age and other composition addis Ababa gave better yield than wondogenet and Dembecha at different stage of the plant.

The following activities are ongoing Activities that are conducted at WGARC:

- *Varietal Response of Lemon grass (Cymbopogon citratus L.) for agronomic and chemical traits under Different Population Densities*
- *Influence of Harvesting Age on Agronomic and Chemical Traits of Two Lemon grass (Cymbopogon citratus L.) Varieties*
- *Identification of suitable planting time for optimum yield and quality of lemongrass*
- *Response of Lemon grass for supplementary irrigation*



Abstract No 4

Development of Dermatological Formulations of some Herbal Remedies claimed to have Antifungal Activities

Ashenif Tadele, MSc, Marta Alemye, Negero Gemedo, Hirut Lemma, Christina Haile, Asfaw Debella, Getachew Addis, Berhanu Tesfaye, Yehualashet Belete, Abiy Abebe, Bekesho Geleta; Ethiopian Public Health Institute

Background: Skin disease is very common in both developing and developed countries. One-quarter to one-third of the population are suffering from a skin problem. Skin diseases have been of major concern recently due to their association with HIV/AIDS. More than 90% of HIV infected individuals develop skin and mucosal complications at some stage during the disease. In Ethiopia, it is among the most frequent causes of morbidity, the sixth most frequent cause of outpatient visits. The prevalence rate of skin infection is 49.2%, of which fungal and bacterial infection are the most common. Fungal infections account 18.5 - 33%. Treatment of dermatophytosis: azoles and allylamines. Side-effects including hepatotoxicity, neurotoxicity, nephrotoxicity, Skin problems like Stevens-Johnson syndrome; Drug interactions; Increasing resistance -result in treatment failure; The treatment of these infections is prolonged and expensive. Essential oils are rich sources of biologically active compounds and constitute a major source of natural organic compounds: possessing antibacterial, antifungal, antiviral, insecticidal and antioxidant properties, and are used in food preservation, aromatherapy and fragrance industries. Thus the discoveries of essential oil preparations have been the subject of many investigations.

Objective: To develop Safe and Effective Topical Formulations against some fungal strains from medicinal plants.

Methods: the plant materials were collected and their essential oils were collected by using distillation. The Chemical Composition of the oils was analyzed by using GC-MS, GC, and TLC. Antimycotic activity was determined by using both the standard and clinical isolate fungal strains. Acute and Sub chronic toxicity was conducted on mice and rat. Different topical formulations were prepared and evaluated for their organoleptic and physicochemical properties. On the Formulated Products Antifungal activity will be determined by agar well diffusion technique. Skin irritation test - using rabbits, (OECD, 2002, Skin sensitization - using albino guinea-pigs, Acute dermal toxicity test - using mice, Repeated dermal toxicity test - using Albino rats and Shelf Life Determination will be conducted.

Results: Identification of the Chemical constituents of the essential oils were carried out by GC, GC-MS and TLC experiments were generated. No lethality at administered dose range up to 2.5 ml/kg and 3.0 ml/kg for *C Citratus* oil and *T ammi*; respectively. The oils were not produce as toxic effects deferent from the control group on Hematology of and Blood chemistry parameters. The essential oils; *T ammi* and *C citratus* showed antimycotic activity at a concentration of 1% and 0.125%, respectively on *Trychphyton mentagraphytes*, *Trychophyton vercusolium*, *Mycosporium Cannis*, and *Aspirigilus Niger*. On the formulation 1% 1% *C citratus*, and 1% *T. ammi* Macrogol blend ointment shows a higher inhibition zone than the standard clotrimazol and miconazole cream. Pilot study of skin sensitization by using 5% preparation of this ointment, does not produce any skin irritation according to the Magnusson and Kligman Grading Scale.

Conclusion and Recommendation: Both essential oils show a promising antifungal activity. Two formulations show a comparable activity on the common pathogenic fungi. The oils do not produce series mortality up to 4 ml/kg except minor change on liver and kidney. The formulated product does not skin sensitization potential skin up to 5%. Substitution of the synthetic dermatological bases with natural origin such as Shea butter, Aloe vera, Castor oil, *Jatropha curcas* is an ongoing process. There is a need for standardization of the extracts/plant materials and feasibility study of the project.

Abstract No 5

Historical overview on the investigation & responses of PA induced liver disease: *Ageratum conyzoides* & its PA constituents

Asfaw Debella⁷, Dawit Abebe¹, Teshale Seboxa², Getachew Abebe³, Berhanu Gebremedhin⁴, Israel Tareke⁵, Daddi Jima¹, Richard Luce⁶, Ahmed Bedru⁷, Afework Mulugeta⁸, Solomon G/Sellassie⁸, Adamu Addissie², Tsehynesh Meselle⁹ and Amha Kebede¹

¹Ethiopian Public Health Institute, Addis Ababa, Ethiopia, ²College of Medical Sciences, Addis Ababa University, ³Food And Agriculture Organization of the United Nations, Addis Ababa, Ethiopia, ⁴Ministry of Agriculture, Animal and Plant Health Regulatory Directorate, ⁵World Health Organization of the United Nations, Addis Ababa, Ethiopia, ⁶Center for Disease Control and Prevention, Addis Ababa, Ethiopia ⁷AHRI, Addis Ababa, Ethiopia, ⁸Mekelle University, ⁹African Society for Laboratory medicine, Addis Ababa, Ethiopia,

National Planning and Coordination Committee for the investigation of unidentified liver disease in NW & Central zones of Tigray (NPCC)

Background: Unidentified Liver Disease Investigational partners have been conducting a multidisciplinary investigation since 2005 to unravel the puzzle for the etiology of the ULD in some woredas of northwestern and central zones of Tigray. The investigations conducted were public health (clinical & epidemiological), livestock, Plants/weeds and environmental and anthropological studies

Objectives: The major objectives were to determine the root cause of the problem as well as alleviate human and animal suffering and design intervention strategy.

Method: The investigation and intervention involved a multidisciplinary team approach in addressing the relation between crop-animal-human linkages through the food chain, the environment and to rule out other possible causative factors. Hence the study had six major parts: Public health (clinical & epidemiological), livestock, plants and weeds, environmental factors investigation and intervention, anthropological studies and economic impact assessment to develop rehabilitation strategy as well as communication and public health awareness. A dedicated multidisciplinary team was placed in Shire town to coordinate and carry out the investigation and interventional activities in all affected *Woredas* of North-Western and Central Zones of Tigray depending on the available data and standard operational protocol (SOP) requirements. This team was working very closely with the national planning and coordinating committee (NPCC) through the respective working groups established for each discipline. Laboratory investigations that can not be carried out at national level were carried out in collaboration with international partners

Results: All available evidences led to the identification of an environmental hepatotoxin, pyrrolizidine alkaloid (PA) that is produced by a highly invasive local weedy plant species referred as *Ageratum conyzoides* (locally in Tigrigna language is known as Hagay Fetew) as the etiologic agent of Unidentified Liver Disease (ULD). PA toxicity in ULD patients results from ingestion of cereal grains or other agricultural products contaminated by the seeds of the PA-producing plants. Furthermore, humans also ingest the PA through consumption of animal products that forage on the pyrrolizidine alkaloid containing plant. Hence, the disease is more accurately labeled as Hepatic Veno Occlusive Disease (Hepatic VOD).

Conclusion and Recommendation: After years of investigational efforts more emphasis is now placed on prevention activities in order to reduce morbidity and mortality by preventing exposure to pyrrolizidine alkaloids. Interventions include: weeding to zero tolerance, improving soil fertility, selected herbicide use, environmental rehabilitation, availability of pasture or grazing fields free from *A. conyzoides* and other noxious weeds. In addition to this the effective supportive medical treatment, along with nutritional and psychological support, that is in place should be also maintained; strengthening the surveillance to monitor disease epidemiology and impact of prevention activities is also critical, and the potential inclusion of hepatic-VOD as a reportable disease in regional surveillance. The study and the response was an exemplary innovative model for “One Health” approach to improve the lives of humans, animals, plant and the environment. Such multidisciplinary collaborative effort experiences could be expanded to tackle any other public health problems as well as emerging pandemic threats.



National Planning and Coordinating Committee Members
(NPCC members after national award by Ministry of Science and Technology for outstanding achievement on problem solving research)

Abstract No 6

Investigation on Chemical Profiles, Efficacy and Safety of Some Promising Medicinal Plants against Common Diarrhea causing Bacterial Pathogens

Negero Gameda, MSc, Asfaw Debella, Mesfin Tefera, Yehualashet Belete, Rejea, Bekesho Geleta, Yared Debebe, Hirut Lemma, Abiy Abebe, Temesgen Memberu and Mekonnen Debebe; Ethiopian Public Health Institute

Background: Intestinal infection is the most common cause of diarrhoea. Diarrhoea kills 2,195 children every day - more than AIDS, malaria, and measles combined. In Ethiopia, various studies reported that diarrhoeal disease as the major causes of infant and child mortality and morbidity. So far, diarrhoea is routinely treated with antibiotics. However, antibiotics are ineffective against many pathogens, due to antimicrobial drug resistance. Investigation of these traditionally claimed anti diarrhoeal plants could give alternative antimicrobial agent. So far many plant species have been screened for substances with therapeutic activity against diarrhoea causing pathogens. Out of the investigated plants, the crude extracts of: *Albizzia gummifera*, *Syzygeum guineense*, showed good antimicrobial activities against a diarrhoea causing bacterial pathogens. Plants have complex chemical composition; the use of TLC autography (Bio-autography) bioassay allows the detection of bioactive components in crude plants which show activity against pathogens. The objective of this work was further evaluation of 2 to 3 most promising medicinal plants and identify the active antimicrobial constituent(s) that are active against common bacterial culprits involved in the aetiology of diarrhoeal disease

Methods: Following botanical identification, hydroalcoholic (Methanol 80% /Ethanol (70%)) extract of the air-dried plants were prepared. These extracts were evaluated for their ant diarrhoeal properties on Clinical isolates and standard microorganisms. Acute toxicity and sub-chronic toxicity study was conducted by using rats. All the measurements were replicated three times. Antibacterial zone of inhibition diameter, data were entered into excel spreadsheet and are presented as mean \pm SD. For the significance of treatment and control; data were first tested for normality and then ANOVA using statistical software (Minitab 16.0, England). Significant differences between mean values were determined using Tukey's multiple range tests following one-way ANOVA and P values < 0.05 were considered as significant.

Results: A total of 6 medicinal plants have been assessed for their antibacterial activity against diarrhea causing bacteria. *Albizzia gummifera* seed methanol extract have showed good antibacterial activity against *E. coli* (10 mm – inhibition zone diameter), *S. aureus* (12- inhibition zone diameter), (*S. pyogen* 15- inhibition zone diameter) and *S. pneumonia* (14- inhibition zone diameter) bacteria; when compared to ciprofloxacin with 22, 20 20 21 mm, respectively. The highest antibacterial activity was shown by *Albizzia gummifera* against diarrhea causing bacteria (*E. coli* MIC = 0.5mg/ml, *Shigella boydii* MIC = 1mg/ml, *Shigella spp* MIC = 0.5 mg/ml and *Salmonella spp* MIC = 0.5mg/ml) and general infection causing bacteria (*S. pneumonia*, *S. pyogen* and *S. aureus* with MIC of 0.25 mg/ml). Another remarkable activity was excreted by methanol extract of *Syzygium guineense* with MIC range of 0.25 -2 mg/ml. Bioautographic evaluation of methanol extract of *A. gummifera* showed high number antibacterial constituents. In acute toxicity study the methanol extract of *A. gummifera* did not show any mortality up to a dose of 3000 mg/kg. Mortality was observed at a dose of 4000mg/kg *A. gummifera* seed extract. Animals have shown no sign of toxicity up to 3000mg/kg such as CNS effect (excitement, ataxia, and sleep), altered feeding and vomiting (4000 mg/kg). And no gross necropsy (on liver and kidneys) was evident in the sub chronic test.

Conclusion and recommendations: The study indicates that *A. gummifera* showed excellent bioactivity. It could be the best botanical alternative for the treatment of diarrhoeal diseases. Bioautography assay of *S. guineense* and other promising plants and Isolation and identification of bioactive components of *A. gummifera* and a comparative study of the most regenerative part of the plant like leaves and flowers are also recommended.

Abstract No 7

Anti-promastigote and haemolytic Activity of selected Ethiopian Traditional Medicinal Plants Used for Treatment of Leishmaniasis

Kidist Zealiyas MSc, Traditional and Modern Medicine Research Directorate. Ethiopian Public Health Institute.

Background: Leishmaniasis is a disease caused by protozoan parasite of the genus *Leishmania*. It is transmitted by female sand fly. Globally, more than 12 million people infected 350 million people at risk developing the disease and about 2 million new cases occur annually. The clinical manifestations of leishmaniasis are known to three forms: cutaneous leishmaniasis (CL), visceral leishmaniasis or Kala-azar (VL) and mucocutaneous leishmaniasis (MCL). In Ethiopia, cutaneous leishmaniasis is endemic in highland of the country and it is caused mainly by *L. aethiops* and occasionally by *L. tropica* and *L. major*. Visceral leishmaniasis is mainly found in the arid and semi-arid area and it is caused by *L. donovani*. The chemotherapy of leishmaniasis is unsatisfactory because of their limited efficacy, significant toxicity and increasing drug resistance. Therefore, there is a need for the developments of herbal medicines which have safer and affordable therapeutic agents.

Objective: To screen traditional medicinal plants for anti-promastigote activity against human *Leishmania* parasites in vitro.

Materials and methods: Based on ethno medicinal information, *Verbascum sinaiticum*, *Albizia gummifera*, *Balanites aegyptica*, *Millettia ferruginea*, *Moringa stenopetala* were collected and 70% ethanol and water crude extracts were prepared. Reference strains of *Leishmania* parasites *L. donovani* (MHOM/50/68/15), *L. chagasi* (MCAN/BR/84/CO9/0), *L. major* (MHOM/IR/72/NAD/MS), *L. tropica* (IROS/NA/80/HD3) and Clinical isolate of *L. aethiops* were used in this study. The Red Blood Cell lysis and Anti-promastigote activity of the crude extract and fractionate were tested. The different extracts of the plants were dissolved by 1% DMSO. Amphotericin B was used as a standard reference in this study. Absorbance of the supernatant were recorded at 540 nm using ELISA reader and compared to positive control for Red Blood Cell lysis activity of the extracts and standard drug. Optical density of each plate measured using ELISA reader fluorometrically and percent growth inhibition of treated samples compared to control for measuring the Anti-promastigote activity test extract and reference drug. Graphpad Prism version 6 (Graphpad Software, Inc. USA) was used for statistical analysis of IC₅₀ and LC₅₀ values at 95% CI.

Results: *A. gummifera* showed anti-promastigote effect comparable to the standard Amphotericin B drug. The crude ethanol extract of *A. gummifera* (seed) have effects on the growth of *Leishmania* promastigote with IC₅₀ (µg/ml) of 0.78, 0.94, 8.65, 0.35, 9.21 on *L. tropical*, *L. major*, *L. donovani*, *L. changes*, and *L. aethiops* respectively. The IC₅₀ (µg/ml) values of the aqueous partition of *A. gummifera* had 1.11, 1.11, 1.11, 1.41 and 2.83 on *L. tropical*, *L. major*, *L. donovani*, *L. changes*, and *L. aethiops* respectively. The n-butanol partition of the same plant also showed very high anti-promastigote effect with IC₅₀ (µg/ml) 0.22, 0.18, 0.28, 0.18, 0.27 on *L. tropical*, *L. major*, *L. donovani*, *L. changes*, and *L. aethiops* respectively. Ethanol extracts of *B. aegyptica* (leaf) and *M. ferruginea*. IC₅₀ values of 17.91 and 1.01µg/ml against *L. aethiops*. However, *B. aegyptica* (fruit) and *M. stenopetala* lack of anti-leishmanial effect.

Conclusion and recommendations: *A. gummifera* could potentially be developed in to a broad spectrum anti-leishmanial drug. The n- butanol and aqueous residue showed an excellent activity against all *Leishmanian* species. *B. aegyptica* (leaf) and *M. ferruginea* (seed) extracts showed very high activity against *L. aethiops*. It is that In-vivo studies must be carried out on *A. gummifera* crude extracts/ n-butanol and aqueous residue. Most active fractions of *A. gummifera* (n-butanol and aqueous) must be further isolated

Abstract No 8

Overview on the studies of some traditionally used medicinal plants against parasitic helminthic infestations

Yared Debebe, MSc, Ethiopian Public Health Institute

Background: Parasitic infections are important public health issue particularly in developing world in which Social and economic deprivation, Poor hygienic condition, Warm climates are the major challenges. Globally, 3.5 billion people are affected by intestinal parasites. In Ethiopia more than half million visits of OPD was due to Intestinal parasitic infections. Taeniasis is among the most prevalent IPIs in Ethiopia. Conventional drugs used praziquantel and niclosamide. In addition Traditional herbal remedies such as *Embelia schimperi* (Enkoko), *Maesa lanceolata* (Kelewa), *Myrsine Africana* (Kechemo) have been used in treating tapeworm infestations for ages. However, there is a lack of scientific based evidences regarding efficacy studies, safety studies, phytochemical analysis and appropriate dosage form formulations those plants despite their frequent use as anthelmintics.

Objective: To generate scientific evidence on efficacy, safety, phytochemistry and other relevant preclinical information there by develop Anthelmintic herbal drug.

Materials and methods: *E. schimperi*, *M. lanceolata*, *M. Africana* fruits were collected from their natural habitat (Jimma, Menagesha, Bahirdar, Gonder). Voucher specimen were collected, identified and deposited in the herbarium of TMMRD of EPHI. The 80% EtOH crude extract and different fractionate were prepared. The Phytochemical screening via chemical tests and TLC and Chemical characterization using UV-spectrophotometry, HPLC, NMR were conducted. The Anthelmintic activity evaluation was conducted using *In vivo* by infecting albino mice with *H. nana* and *In vitro* by eggs of Hookworm & strongyloides spp from human stool and allowed to hatch into larvae. The assay methodology was set-up in a 96- well micro titer plates. Acute & Sub chronic toxicity study was conducted on Swiss albino mice. Data analysis was carried out using SPSS version 16. The mean difference between the treatment and control group was compared using one way ANOVA and Post-hoc analysis. P-values less than 0.05 were considered significant.

Results: the crude extracts of both *E. schimperi* *M. Africana* contains tannins, cardiac glycosides, anthraquinones, free quinones, Polyphenols. The Safety study indicated that the two plants have LD50 above 5,000mg/kg. The Sub chronic toxicity study indicated that no statistically significant observed in the Changes in body weight, hematological & biochemical parameters in treated & control groups. No changes in histology of the kidney tissues at 1200mg/kg and no abnormalities observed on the Liver & kidney tissues of mice at 400mg/kg. *In vivo* Cestocidal activity of 80% EtOH crude extract of *E. schimperi* showed that

100% parasite clearance at 2gm/kg, while embelin isolated from *E. schimperi* showed 85.3% parasite clearance at 750mg/kg. The crude extract of *E. schimperi* showed that 90, 69, 33 and 25 % mortality of Hook worm spp and 93,80, 64, 23 % mortality of strongyloid spp at 400,200, 100, and 50mg/ml, respectively. 25mg/ml 80 % ethanol fraction (67%), 25mg/ml dichloromethane fraction (67%) showed better percent mortality of hook worm spp than 20 mg/ml of standard albendazole drug (60%). The n-butanol, aqueous and pet-ether fracton have less or no mortality effects on hook worm spp.

Conclusion & recommendations: Fruit extracts of *E. schimperi* and *M. africana* have indeed an anthelmintic activity. *E. schimperi* has wide range of safety margin & may be relatively safe for oral medication for human use if further investigation is carried out. Further experimentation is mandatory for *In vitro* assay on taenia spp and toxicity in non-rodent animals.

Abstract No 9

Characterization, Elixir Formulation and Evaluation of Ammonia-Treated and Defatted Hydroalcoholic Extract of Fruits of *Embelia schimperi* Vatke

Endale Seifu, MSc, AAU

Background: *Embelia schimperi* Vatke belongs to *Myrsinaceae* family. It is locally known as (Enkoko(Amh.). Traditionally the fruit is used for the treatment of tape worm infestations and it is believed to eliminate adult stage of the human tapeworm. Pharmacological *in-vitro* studies indicated that a single oral dose of 1g/kg showed a 100 % hookworm and at 400 mg/ml showed 92% of strongyloides killing activity. The plant has wide of safety margin, LD₅₀ above 10,000mg/kg. Therefore studying the suitable dosage form is required for the better use of the plant for the management of helminthic infestations by the society.

Objective: To develop Safe and Effective Anthelmintic herbal remedy from traditionally used *Embelia schimperi*.

Methods: the dried fruits of *E. schimperi* were collected from the local market (Bahir Dar, Gondar) botanical identified and voucher specimen were deposited at herbarium of EPH I. The traditional dose of the extract was prepared by extracting 8.23g of the powdered plant material as a single dose using 6% Ethanol. For elixir preparation the crude extract was treated with ammonia and then defatted and filtered. Quantification and characterization of embelin was conducted using HPLC. The stability of the elixir of *E. schimperi* was conducted at ambient (25°C/60 RH and accelerated stability conditions 40°C/75 RH) and then the general physical screenings (such as clarity, pH, assay and microbial load) was conducted on the elixir samples.

Results: The solubility, moisture content and total ash content of the crude extract was 6.03±0.50g/100ml, 8.2±1.5%, 7.17 ±0.89%, respectively. The concentration of embelin in the traditional dose (0.44mg/ml) and final extract (0.15mg/ml) was 13±0.024µg/ml and 20±0.015 µg /ml, respectively. The absorbance of the elixir samples stored under ambient and accelerated stability conditions at λ_{max} 428nm were 0.51±0.002, 0.5±0.001, 0.5±0.001, 0.5±0.002, and 0.48±0.001, 0.48±0.001, 0.47±0.002, 0.50±0.001 and the pH values were 7.51±0.02, 7.50±0.001, 7.50±0.02, 7.52±0.01 and 7.52±0.03, 7.50±0.02, 7.49±0.03, 7.50±0.02, at 0,1,2 and 3 months of storage, respectively.

Conclusion and recommendations: Water solubility of Embelin can be improved by the addition of a weak base, ammonia. RP-HPLC can be used to determine the dose of the plant material in the crude extract. The elixir formulation of the plant material preserved its stability over three months of storage under ambient and accelerated storage conditions. The safety and efficacy of the formulation will be ascertained before conducting clinical trials. The effect of other stability factors, such as light, should be studied. Other dosage forms should also be considered.

Abstract No 10

Overview on the studies of some traditionally used medicinal plants against malaria and insect repellent activity

Temsgen Menberu, BSc, Ethiopian Public Health Institute

Background: Malaria is currently the leading global health problem, especially in the poor nations of Africa and Asia. Globally, 3.3 billion people at risk, an estimated 219 million cases, and 660,000 deaths mostly children under 5 years of age in sub-saharan Africa. In Ethiopia, approximately 4-5 million cases of malaria are reported annually and it is prevalent in 75% of the country, putting over 50 million people at risk. An epidemic which traditionally occurs every 5-8 years is a hallmark of malaria. The deadliest form of malaria, *P. falciparum* occurs with combined infection with *p.vivax*. The malaria control program such as vector control interventions, preventive therapies and treatment strategies are challenged by emerging parasite resistance to the treatment and insecticides. This leads for the urgent need for new, safe and effective drug for the treatment of malaria.

Objective: Developing safe and effective anti-malarial herbal drug from traditionally claimed locally available medicinal plants.

Methods and materials: Various plant materials were collected from different parts of the country. Voucher specimen were collected, identified and deposited in the herbarium of TMMRD of EPHI. The aqueous, hydro-alcoholic and different fractionate were prepared for subsequent study. Phytochemical studies of the extracts were conducted. *In-vivo* efficacy test in animal models (4 day-suppressive, curative and prophylactic test were conducted according to the standard methods in addition to the *In-vitro* efficacy test in laboratory cell culture plates. The acute and chronic toxicity protocols were conducted on animal model according to the OECD guidelines. The root of *G stenophylla* and *Cordia sinensi* were tested for analgesic and antipyretic activity in Swiss albino mice.

Results: A total of 19 plants have been screened for their anti-malarial activity. Among them *Cissampelos mucronata*, *Asparagus africanus*, *Gnidia stenophylla* shows a promising activity. The root aqueous extract of *G stenophylla* showed a 55 % inhibition in *P. berghei*, the hydro-alcoholic extract of the roots of *A africanus* 55.5% inhibition in *P. berghei* and 52.7% inhibition in *P. berghei* was obtained from the aqueous extract of the leaf of *V bipontini*. The analgesic and antipyretic activity of *G stenophylla* and *Cordia sinensi* was in progress. The mosquito Repellent test showed that *Cympobogo nardose* and *Clinia spp* have 4 hour and 3 hour complete protection time at 10% conc. The experiment is on progress. The acute and sub chronic test showed that extracts have no significant toxic effect on the animals at their effective dose level.

Conclusion and recommendation: The research findings conducted so far show the possibility of finding a promising anti-malarial drug from plant in the future, if a rigorous search is made and the capacity of the lab, and the researchers working there maintained at best performing state. The ethno-botanical survey and evaluation of additional medicinal plants should be consolidated. The search for traditional medicinal plants in ethno botanic survey is currently undergoing to cover various cultures and traditions throughout the country to bring additional candidates for scientific evaluation.

Abstract No 11

Standardization of the Roots of *Gnidia stenophylla* Gilg

Mebruka Mohammed, MSc, AAU

Background: *Gnidia stenophylla* Gilg belongs to the family Thymelaeaceae. The family contains 50 genera and 600 species. It is a small perennial shrub; it has slender stems, needle like leaves with yellow/white flower. The plant locally known as Kataricha (Oromigna) and used in folk remedies for Malaria, Syphilis, Wound, cancer etc.... the In-vivo study showed that at 400 mg/kg per day 87.8% paracetamol inhibition. The plant also showed LD50 greater than 6000mg/kg. Hence there is a need for the standardization of the plant material before conducting further clinical study.

Objective: To set pharmacopoeial standard/monograph of *G. stenophylla* root

Methods: The fresh roots/ off aerial portion the plant was collected from Gongoma (557 km SE of Addis). The root was washed and dried under shade then ground to powder. Then macroscopic (color, taste, odor and test), microscopic, physicochemical (Moisture content (%), Ash Values (mg/g), Swelling Index ml/g), Tannin content, Extractive values, Foaming Index), phytochemical were conducted. The purity/Residue Analysis such as heavy metal, pesticide and radio chemical residue analysis were conducted by using AAS (Shimadzu, AA-6800), GC/ECD (Agilent 7890A) and Rados survey meter (RDS, 30).

Results: The powder is khaki, dark brown color, highly bitter taste, dusty, pungent, irritating and unpleasant odor internally smooth and 10-15cm long and 1-3cm in width. The physicochemical analysis indicated that the powder had Moisture content -6.689 (\pm 0.053) %, total ash- 40.800 (\pm 1.076) mg/g, Acid insoluble ash- 8.000 (\pm 0.150)mg/g, Water soluble ash- 7.550 (\pm 0.433)mg/g, Swelling index- 7.600 (\pm 0.854) ml/g, Foaming Index- 100.012 (\pm 1.987), Tannin content- 401.910 (\pm 5.523) mg/100g, the highest Methanol soluble extractive value -15.266 (\pm 0.524)% and the lowest Hexane soluble Extractive value-0.7272 (\pm 0.238)%. The extract of the plant showed g-BHC 0.0005, p,p-DDT 0.0013 and p,p-DDE0.0011 μ g/g which very much lower than the WHO limit 0.6, 1 and 1(μ g/g, respectively. However the Heavy metal residue is much greater than the WHO limit, Cd- 0.9, Co-5.7, Ni-8.4, Cr-7.5, Pb-17.2, Cu-205.4 μ g/g while the WHO limit is <0.3, <3, <6,<2, <10 and <150 μ g/g, respectively.; and contamination of Radiochemical residue is 0.110+0.012 (mSv/h) while the WHO limit negligible amount.

Conclusion and Recommendations: The finding showed that there is Purity/Trace/residue contamination of *Gnidia stenophylla* Gilg plant. This is an indication of the needs of standardization of other medicinal plants, strengthen regulatory process in addition to assuring the safety, efficacy and promote effective use. The source of pesticide and heavy metal such as As and Hg should be assessed. Bioassay guided isolation is mandatory for further standardization of the plant materials. It should be encouraged that standardization of other medicinal plants of Ethiopia and issue a policy to regulate quality of medicinal plants and their products.

Abstract No 12

Overview on the studies of some traditionally used medicinal plants against Diabetes mellitus

Berhanu Tesfaye, MSc, Ethiopian Public Health Institute

Background: Diabetic Mellitus (DM) - a systemic metabolic disease characterized by hyperglycemia and hyperlipidemia. It is Resulting from reduced insulin secretion, insulin action, or both. Long-term hyperglycemia leads to micro & macrovascular complications. Global prevalence of diabetes estimated to increase from 8.3 %(366M) in 2011 to 9.9 % (522M) by 2030. Almost 80% of diabetes deaths occurred in low and middle-income countries. Diabetic cases in Ethiopia in the year 2000 were estimated 800,000 and are expected to increase to 1.8 million by 2030. The therapeutic agents for these are long live that leads to patient compliance problem and have disreputable side effects in addition to unaffordability. There is a great need for the development of therapeutic agents from medicinal plants. Some research finding showed that the crude aqueous extract and its solvent fractions of the leaves *M. stenopetala* demonstrated hypoglycemic and antihyperglycemic activity. The hydro alcoholic extract of the leaves *J. schimperiana* demonstrated bronchodilatory, anti-inflammatory. Therefore, the aim of this study is in-depth evaluation of the pharmacological activity of these plants.

Objective:-To evaluate pharmacological activity using different models.

Methods and materials: The leaves of *M stenopetala* was collected from Gammo Gofa zone, identified and authenticated, dried under shade and crushed to powder for extraction. Aqueous, hydro-alcoholic and solvent fractions were prepared for subsequent laboratory experiments. Mice were injected intrapertonea diabetogenic agent, alloxan monohydrate dissolved in distilled water. The diabetic mice were randomly allocated to different dose of the extract, fractionate and standard drugs. The blood glucose level each mouse were checked at 60,120,180 and 240minutes interval. Acute toxicity study was conducted by using mice according to OECD guideline.

Results: *M Stenopetala* showed blood glucose and lipid lowering activity in solvent and chromatographic fractions. The n-butanol fraction has low toxicity profile with wide safety margin (LD50 >5000mg/kg). The n-butanol fraction of the extract showed a significant lowering of blood glucose and anti-hyperglycemic effect in alloxan induced diabetic mice in a dose dependent manner through time intervals. The

Conclusions and recommendations: Active principles in n-butanol fraction have role in the management of hyperglycemia on chronic administration. Further studies are needed to determine the mechanism(s) of action and conducting sub-chronic toxicity test is mandatory.

Abstract No 13

Phytochemical Analysis and In-vitro Enzyme Inhibitory Activity of Hydro-alcoholic extract of *Moringa stenopetala* Leaves

Alemayehu Toma, MSc, AAU

Background: Diabetes mellitus (DM) is systemic metabolic disorder characterized by hyperglycemia. Long-term hyperglycemia leads to micro & macrovascular complications which in turn decreased quality of life and increased morbidity and mortality. It could occur alone but more often co-exists with other systemic diseases such as hypertension, dyslipidaemia, ischaemic heart disease, renal diseases, Glycation, hypercholesterolemia and hyper-triglyceridemia are common complications of diabetes mellitus in addition to hyperglycemia. Currently, no drug in the market that is devoid of any adverse effect. This leads to an increasing search of affordable, accessible and safe anti-diabetic drugs from medicinal plants. Globally, more than 1000 plant species are being used as folk medicine for diabetes. Up to 30% of patients with DM use complementary medicine. One of the commonly used medicinal plant in folk medicine is used for anti-hyperglycemic activity is *Moringa stenopetala* leaves.

Objective: To evaluate contents of phytochemicals and enzyme inhibitory activities of *Moringa stenopetala* leaves

Methods and materials: The leaves of *M stenopetala* was collected from Gammo Gofa zone, identified and authenticated (voucher number AL-001), dried under shade and crushed to powder for extraction. The powdered leaves (1.2 Kg) were extracted by percolation using 70 % (V/V) ethanol. The extract was dried by evaporating it using rotary vaporizers under reduced pressure at a temperature of 40-45°C. The phytochemical contents (total flavanoid content, total polyphenolic content and condensed tannin content) of *M stenopetala* leaves were determined by standard established methods. The percent inhibition of hydro-alcoholic extract on alpha amylase, alpha glucosidase, pancreatic lipase and cholesterol esterase activities were evaluated by using well established methods. The results were expressed as milligram equivalent of standard/gram dry weight of extract.

$$\% \text{ Inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Results: The phytochemical contents of the hydro alcoholic extract of *M stenopetala* showed that the total flavanoid content, total polyphenolic content and condensed tannin content was 71.73±2.48, 79.81±2.85 and 8.82± 0.77mg/ml per gram of *M stenopetala* leaves extract, respectively. The enzyme inhibitory activities of *M stenopetala* leaves increases as the dose increased. 5mg/ml of the hydro-alcoholic extract showed 6%, 85%,48%,50% and 50% inhibition on alpha amylase, sucrose, maltase, pancreatic lipase and cholesterol esterase activities.

Conclusion and Recommendation: High content of phytochemicals especially total polyphenolic and total flavanoids may contribute to pleiotropic effects of *M stenopetala* leaves that support use of the plant for different metabolic disorders. Antihyperglycemic activity of *M stenopetala* may be associated with inhibition of alpha glucosidase and its antihyperlipidemic activity could be due to inhibition of lipase and cholesterol esterase enzymes. *In-vitro* Antiglycation activity of hydroalcoholic extract of *M stenopetala* leaves on fructose induced Bovine Serum Albumin glycation is on progress. Further illustration on mechanism(s) of the *M stenopetala* leaves on insulin secretion and plasma lipid inhibition on animal models.

Abstract No 14

Overview of the evaluation and development of drugs from claimed medicinal plants for the treatment of Hypertension.

Bekesho Geleta, Amelework Eshetu, Yohannes Ayele, Selamu Kebamo, Wondim Melkam, Yewalashet Belete; Ethiopian Public Health Institute

Background: Non-communicable diseases caused two thirds of all deaths globally; about 36million deaths in 2008. Nearly 80% of deaths are occurred in low-and middle-income countries. Cardiovascular disease is leading cause mortality in none communicable diseases. It contributes 17million deaths (48% of NCD deaths) a year. According to WHO estimate, Hypertension is responsible for 45% of deaths due to heart disease, and 51% of deaths due to stroke. Hypertension affects around one billion people worldwide and 10% of the global disease burden. In Ethiopia, hypertension causes and 12th top killing diseases. Several modern antihypertensive medications are available thus far, but expensive, full of side effects and unpalatable due to polypharmacy. Various herbal preparations have been used and claimed to have benefit for hypertension in the folk medicine such as *S guineense*, *T serrulatus*, *T schimperi*, *M stenopetala*.

Objective: To evaluate and develop drugs from claimed medicinal plants for the treatment of Hypertension.

Methods and materials: The plants used in this study were collected from wild and identified and authenticated, dried under shade and crushed to powder for extraction. Aqueous, hydro-alcoholic and solvent fractions were prepared for subsequent laboratory experiments. The diuretic and antihypertensive activities were conducted using rat model by metabolic cage and Blood pressure Analyzer (Model 179 IITC INC USA), respectively. Rats were randomly allocated to the treatment group, the control and reference group. After administration of the sample, a rat was placed on a metabolic cage. The urine will be collected in measuring cylinder up to 5hrs after dosing and compared with standard diuretic agents like Furosemide (10mg/kg body weight). The saluretic potential of the different extract was measured based on Na^+ , K^+ , Cl^- , Na^+/K^+ . Vasorelaxant effects of the different extracts were conducted on guinea pigs thoracic aorta by reversing the vasoconstriction activity of KCl using Organ bath and Polygraph. Results were expressed as means \pm standard errors of means.

Results: The crude extract of *S. guineense* showed lowering the SBP, MABP and DBP at 50,100,150 mg/kg oral doses on the rat model on each three consecutive date. The same extract also showed an increase in vaso-relaxant effect to 43.8% by cumulative addition of the extracts to a dose of 70 mg/ml. The n-butanol fraction of *S guineense* at the dose of 500 and 1000 mg/kg possessed the same diuretic potential and comparable electrolyte excretion(Na^+ and K^+) to that of hydrochlorothiazide(10mg/kg). The saluretic index of the hydro alcoholic extract (500 mg/kg) and essential oil(1ml/kg) of *T serulatus* is higher on of k^+ , but less on Na^+ than the of hydrochlorothiazide(10mg/kg).

Diuretic activity of different fractions of *M stenopetala* in swiss albino mice showed that 50mg/kg of 70% ethanol extract (1.33), 50mg/kg of the n-butanol fraction (1.44), and 150 mg/kg of the aqueous fraction (1.17) showed more diuretic activity than Furosemide (10mg/kg) (1). Diuretic effect of the tea simulation of fine and coarse powder of the *T serulatus*, *M stenopetala* and *T schimberi* showed better diuretic activity than the standard drug Furosemide (10 mg/kg).

Conclusions and Recommendation: The study validates the claimed antihypertensive uses of these medicinal plants in folk medicine. Both plants showed a promising efficacy study on the antihypertensive activity. The acute toxicity study showed the plants are safe. The herbal tea preparation requires standardization of the tea preparation. Further efficacy and safety study (chronic toxicity test) is mandatory by using different models and techniques. A detailed study is needed to establish the mechanism of action and duration of action of both plants.

Abstract No 15

Evaluation of diuretic and antihypertensive activity of *Thymus schimperi*

Hussein Haji, MSc, AAU

Hypertension is a major risk factor for several cardiovascular diseases such as atherosclerosis, Heart failure, renal insufficiency, cardiovascular diseases and stroke. Hypertension affects approximately 1 billion people globally and 7.1 million deaths annually. According to the latest estimation of WHO, deaths in Ethiopia reached 9,743 or 1.19% of total deaths. There are various antihypertensive drugs for the management of hypertension. These drugs are effective for 40-60% and combinations drugs are used, this leads to increase the cost of treatment and side effects. Therefore newer antihypertensive agents are required needed to to expand therapeutic options, to increase the treatment efficacy and to enhance and to enhance patient adherence. This effort looks to explore alternative therapies particularly from herbal sources. *Thymus schimperi* locally known as "tossign" is a perennial herb endemic to the Ethiopian highlands between 2200-4000 m above sea level. It has many traditional medicinal applications such as gonorrhea, respiratory problems, rheumatism, urinary retention and hypertension.

Objective: To evaluate diuretic and antihypertensive activity of *Thymus schimperi*

Methods and Material: Fresh plant leaves were collected from Chilalo Mountain, identified and a voucher number (HH001) was given and deposited. The plant material was dried under shade and crushed to powder for aqueous extraction. The Essential oil was prepared by hydro-distillation in a Clevenger-type apparatus for 3 h, at a temperature of 70°C. Hypertension was induced on male albino rats by using 10 % table sugar and 2% salt. The experimental animals were warmed to about 32–35.4 °C. The blood pressure was measured from the tail of rats using non-invasive BP monitoring apparatus (Model 179, IITC Inc, USA). The SBP and MABP were read from the pulse tracings. The DBP was calculated from SBP and MBP using the equation: $DBP = (3MBP - SBP)/2$. Results were expressed as means \pm standard errors of means. Data analysis was carried out using SPSS version 16. The mean difference between the treatment and control group was compared using one way ANOVA and post hock comparisons were made by Dunnet's and Tuckey's HDS. P-values less than 0.05 were considered significant. The oral acute toxicity study was conducted on wistar female rats (160-200 g).

Results: The aqueous extracts of leaves of *T. schimperi* showed a dose dependent and significant diuretic activity in normal rats at a dose of greater than or equal to 250 mg/kg ($p < 0.05$), however the diuretic activity of its essential oil is not statistically significant. The aqueous extract and essential oil showed a significant difference than the control group (normal saline) on electrolyte excretion at a dose of 750 and 1000 mg/kg of Na^+ , K^+ , and Cl^- . The essential oil also showed a significant difference from the control group on K^+ and Cl^- at the dose of 1ml and 5ml/kg. The aqueous extract has anti hypertension effect against the SBP at higher dose. The aqueous extract of *T. schimperi* showed a significant change on body weight and LDL at a dose of 250 and 500 mg/kg, respectively compared to control groups (only tap water). The plant does not show any clinical signs of toxicity and any behavioral change up to dose of 5 g/Kg.

Conclusions and Recommendation: The study indicated that *T schimperi* possesses strong dose independent diuretic activity. The aqueous extract has anti hypertension effect against the SBP at higher dose. The finding of the acute toxicity showed the leaf extract was safe. In general study may account, at least in part, for the reported beneficial action for urinary retention and hypertension activity in folk medicine. It is recommended that further studies are could be conducted to determine needed the effects of different fractions on the diuretic and antihypertensive activity on Reno vascular, endocrine, neurogenic, psychogenic hypertension, exact mechanism of action and site (s) of action and to determine the effect.

Abstract No 16

Development of effective water treating agent from *Moringa Stenopetala* seeds

Hirut lemma¹ MSc, Christina Haile¹, Redwon Muzain², Negero Gameda¹, Getachew Addis (Dr.), Mulugeta Guta, Frehiwot Teka, Kirubel Tesfaye, Aynalem Lakew, Kidist Yirsaw, Ethiopian Public Health Institute

Background: *Moringa stenopetala* – A branched tree that grows 6-10m tall. It grows abundantly in south western Ethiopia. It is known by different vernacular names such as shiferaw, Aleko and cabbage tree. *M. stenopetala* is known in folk medicine as having value in treating a wide variety of ailments. Among 14 species of moringa tree, *M. oleifera* is well studied with regard to potential medicinal uses and the identification of compound of potential therapeutic importance, which is native to India.

Objective: To investigate the Antibacterial activity of extracts and Evaluate the efficacy of optimum concentration of *M. stenopetala* for water clarifying activity and to develop appropriate dosage form for water clarifying agent from *Moringa stenopetala* seeds.

Methodology: The seeds of the plant were collected from Arbaminch where it is widely cultivated. A voucher specimen was identified by taxonomists and deposited at TMDRD in EPHI. The seeds were air dried and then powdered for extraction. Powdered seeds were defatted with petroleum ether. Defatted marc was then extracted with 70% ethanol. The aqueous extract was macerated with distilled water, filtered and freeze dried using lyophilizes. The Anti-bacterial activity was determined by using both Agar well diffusion and Agar dilution method. The Optimum concentration of *M. stenopetala* for water clarifying activity was also conducted and the comprehensive physico-chemical and microbiological analysis moringa treated water was studied

Results: Both the petroleum (5, 10, 20, 40%) and ethanol (5, 10, 25, 50 and 100 mg/ml) and aqueous (5, 10, 25, 50 and 100 mg/ml) extract of the plant did not show any zone of inhibition against standard and clinical strains of E coli, Salmonella, and shigella. Serial agar dilution of pet ether (0.5-8%), both ethanol and aqueous extract (0.25-4mg/ml) of the seeds didn't show activity against the above test organisms. More over the contaminated water treated with the optimum concentration of extract (62.5mg/ml) didn't show any effect on physicochemical and microbiological quality of the water

Conclusions and Recommendations: From this study the result of different extracts of *M. stenopetalla* seed didn't show antibacterial activities. The optimized concentration of extract (62.5mg/250ml) didn't have any effect on physicochemical and microbiological analysis of treated water. The aqueous extract of seeds works as a flocculent by binding the bacteria to the solids in water and causing them to sink to the bottom but additional treatment of filtering and boiling of water is needed to render it completely safe to drink. So, there is a need to study other dosage forms.

Abstract No 17

Dosage optimization and standardization of *Moringa stenopetala* seeds powder as water purifying agent

Christina Haile, B. Pharm, Redwan Muzeyin*, Melaku Gizaw and Daniel Abera; Ethiopian Public Health Institute

Background: Safe water is not an option rather it is basic human right. Over a billion people still do not have access to improved water sources. About 3.4 million deaths recorded annually with relation to inadequate supply of safe water. In Ethiopia Only 24% of the rural populations have access to safe drinking water sources. Only 20% is served by utility piped supplies and 37% from protected source like public standpipe and protected boreholes, springs and dug wells. The rest 63% relies on sources that are unimproved source, such as ponds, lakes, rivers and open dug wells. By considering the socio economic states of the country and the severity of the situation there is a need for developing safe effective, standardized and economically sound plant base water clarifying agent.

Objective: To develop water purifying agent from *Moringa stenopetala* seeds.

Methods: Fresh seeds of *M. stenopetala* were collected from Arba Minch (505 km south of Addis Ababa). The seeds were powdered and extracted with 0.1 M NaCl for 2hr. The extractive were filtered and lyophilized after freezing. The dirt water collected from different places were treated by different concentration of moringa extract ranging from 0.2gm to 0.5gm/500 ml. Beshangari 0.6125/500ml and untreated dirt water were used as a control. Turbidity, total dissolved solutes, pH and conductivity were measured.

Result: The water treated with extract showed a higher turbidity, total dissolved solutes, and conductivity than bishangari and control for both tested doses for the first 7 hrs. The pH of the treated water is all the same but it decreases to 7.0 for a concentration of 0.5gm/500ml. Organoleptic properties of the plant extract showed that it has creamy color with 14.5mm length and 31.3mm width, have moldy odor, sweet in low dose and bitter in higher doses and had rough texture and smooth fracture. The phytochemical constituents of the extract contain alkaloids, tannins, saponins, glycosides, proteins and carbohydrates. The moringa seed Water soluble extractives (cold extraction) 5.88%, Water soluble extractives (hot extraction) 7.04%, Total ash 12.25%, Acid insoluble ash Below detection, water soluble ash 7.15%, Moisture content 5.5%, swelling indx 5.1ml, Microbial load All indicator microorganisms were absent.

Conclusion and Recommendation: *M stenopetala* seed is one of the natural products which are safe for its water purifying activities. There is a need for the purification of the active protein coagulant to potentiate its effectiveness, characterization of the marker compounds and designing appropriate dosage forms for stability study, quality control and conducting community trial study for final product development as water clarifying agent.

Abstract No 18

Investigation of the mosquito larvicidal effect activities of some medicinal plants against *Anopheles gambiae*

Asfaw Debella, Yonas Woletaw, Kissi Mudi, Frehiwot Teka, Messay Getachew, Dawit Abebe, Zenebech Adella, Beruk Teka, Samuel Woldekidane, Feyessa Challa, Abel W/Tensi, Asgedech Sirak, Chrisitna Haile, Yehuwalashet Belete, Aynalem Lakew; Ethiopian Public Health Institute

Background: Mosquitoes are most important insects in terms of public health, which transmits vector born diseases that causes millions of deaths every year. *Anopheles* mosquitoes (female): usual definitive hosts and transmitters of plasmodium species. To control the malaria, one approach is controlling the vector. Medicinal plants tried for the control of the vector. Thirty three medicinal plants screened for the anti-larvae activity of the vector mosquito species.

Objective: In-depth evaluation of the efficacy, safety and develop appropriate dosage forms

Methodology: Plant parts were collected from their natural habitat from South and Southwest Ethiopia at altitudinal range of 900 – 1600 m. Identified by a taxonomist & voucher specimen was deposited at the herbarium of TMMD. Hydro alcoholic, aqueous extract and their solvent partition were used for subsequent experiments. Larval susceptibility test, toxicity, histological and toxicity of effluents in marine organisms were conducted on the extracts and formulated products.

Results: Larval efficacy of *Albizia gummifera* and *Milletia ferruginea* 70% ethanol extract in ppm LC₅₀ 50, LC₉₅ 100, and 25 and 50 respectively. Oral administration of 125, 250 mg per Kg body weight does not show any physical changes in the skin, fur, eyes, respiratory system and general behavioral patterns, no significant weight difference between the control and the treated groups. LD₅₀ > 3000 mg/Kg body weight. There were no any pathological findings in the liver and kidneys up to a dose of 125 mg per Kg body weight, however congestion was observed in the liver at the dose of 250 mg per Kg body weight. No toxicity of fish for *A. gummifera* up to 12.50 µg per ml in 20,000 liter aquarium volume until 24 hours, however *M. ferruginea* extract was found to be lethal for fish at concentration of 6.25 µg per ml in 20,000 liter aquarium volume after 8 hours.

Conclusion and recommendation: *A. gummifera* (hydroalcoholic extract) and *M. ferruginea* (aqueous extract) were found to susceptible to *A. gambiae* larvae and safe at the effective dose, shelf life determination of the formulated sample.

General Discussion and the Way Forward

This scientific review workshop was organized to evaluate the overall progress on the investigational efforts of traditionally claimed medicinal plants for diseases of public health importance. Experimental evidences that were generated on the safety, efficacy and quality of traditionally used medicinal plants were presented and discussed by the workshop participants. The experimental data were critically evaluated and action points were proposed as the way forward for facilitation of product development from medicinal plant.

The TMMRD should continue and further strengthen the research collaboration on the ongoing in-depth pre-clinical studies on the safety, efficacy and quality of plant based remedies for subsequent community based and clinical trials in order to maximize their benefits. The collaboration focused on the conservation, propagation and cultivation as well as development of sustainable use of affordable and validated plant based remedies by the public through creating networking and coordination, organizing periodic technical forums for information updates and experience sharing.

The workshop helps the directorate for updating of evidence based information on traditional medicine, getting evaluation and constructive comments on the methodology and generated data, getting constructive ideas on the way forward for next steps including strengthening collaboration, creating partnership and networking among stakeholders.

The major points of emphasis from the workshop recommendations:

1. The workshop participants appreciated the investigational efforts and achievements of the TMMRD despite its limited manpower, serious shortage of supplies and laboratory facilities.
2. The TMMRD should give more focused on those Medicinal plants used for diseases of public health importance for the generation and compilation of evidence based information requirements.
 - 2.1 Giving priority for completing the experimental evidence on medicinal plants for animal exo-parasite, skin microbial infestation (dermatological), hypertension and diabetes particularly on *Moringa* and *Thymus Sp.* (Tosigne) and others which look more promising and commercially feasible.
 - 2.2 On generating evidence based information for the registration requirements of FMHACA for 2nd and if possible for the 3rd categories of medicinal plants. The research effort could be extended step by step targeting to the 4th category.
 - 2.3 On experience sharing from African and other developing countries that have a good experience in the marketing of medicinal plants products.
 - 2.4 The directorate should not be over ambitious. It should be practical when planning taking into consideration the existing manpower and facilities.
 - 2.5 On quality issues particularly *Moringa stenopetala* leaves should be well addressed as this plant is highly used by the community for medicinal purpose even exported abroad to safe guard the public health from adulterated plant based products including *Thymus Sp.* (Tosigne) and *Hebiscus Sp.* (Kerkede).
 - 2.6 Participants also pointed out the scientific evaluation of traditional medicinal plants employed for rabies. The community particularly the rural community are in high health

risks due to their strong attitude towards the use of traditional medicine on rabies in the presence of alternate safe options usage of post exposure prophylaxis.

- 2.7 The directorate also gives attention for the standardization of those selected medicinal plants for further study which helps for strengthen regulatory process in addition to assuring the safety, efficacy and promote effective use. In addition to that there needs to be documentation of the medicinal plants is of paramount importance for the development of National database pharmacopoeia of the country.
3. The need to create linkage with manufacturers of pharmaceuticals, insecticides and pesticides for the research collaboration in the product development. Close collaboration with the regulatory authority, FMHACA was also stated as a means to strengthen and get the necessary support for the ongoing efforts on the scientific validation, product development as well as to fulfill the requirements for the registration of plant base remedies.
 4. Participants also stressed on the need to strictly follow official protocols of develop standard operational procedures (SOP) for investigational efforts of efficacy, safety, quality and other required experimental parameters.
 5. The Directorate should also consider the commercial feasibility of the ongoing works through involvement of expertise for the sustainability, and cost benefit analysis of plant based products.



Closing Remarks

Asfaw Debella, PhD, Director of Traditional and Modern Medicine Research Directorate, Ethiopian Public Health Institute

- **Honorable guests, partners, participants, ladies and gentlemen!**

It is indeed a great honor and pleasure to be present in this Scientific Review Workshop on the ongoing efficacy and safety investigations of traditionally used Medicinal Plants.

Dear Colleagues and Participants,

As you all know research and development activities on traditionally claimed medicinal plants have paramount importance in searching for effective, safe, environmentally sound, affordable and accessible plant based medicines for diseases of public health importance.

Hence this workshop is good platform to evaluate the generated safety and efficacy data, exchange experiences and set directions for strengthening the collaboration in the validation of the efficacy and safety as well as conservation and sustainable use of promising medicinal plants.

Coming too yesterday and today's deliberations, participants has got a good opportunity to be aware of the ongoing different research projects on human and animal use. Furthermore, a clear direction was set on how to strengthen the research activity towards plant based product development. The involvement of expertise's in the field of biomedical sciences and agriculture have paramount importance as a means to search for validated and standardized potential plant based medicines through research outcomes for the benefit of the public.

I appreciate your efforts and the contributions, and we give value for your comments and suggestions and congratulate all for the successful conduct of the Scientific Review on the Ongoing Efficacy and Safety Investigations of Traditionally Used Medicinal Plants Workshop.

I thank you all.

List of Participants

No	Participants	Organization
1	Dr. Eshetu Lemma	SERO, EPHI ,
2	Ato Tadesse Nigatu	HRGSPD, EPHI,
3	Ato Gemechu Tadesse	Parasitology, zoonotic & bacteriology EPHI
4	Ato Elias Asfaw	Plan case team, EPHI
5	Ato Redewan Muzein	Nutrition and Food Science Research, EPHI
6	Ato Abel Woldetensie	Environmental Chemistry EPHI
7	Ato Yonas Woletaw	other parasitic disease research case team, EPHI
8	W/o Alemeesh Hailemariam	Malaria, vector biology & other parasitic disease EPHI
9	Ato Feseha Zeleke	Master of ceremony, EPHI
10	Ato Nigussie Awegechew	EPHI
11	Dr. Abrham Ali	Zoonotic disease research case team EPHI
12	Dr. Fasil Mengistu	Zoonotic disease research case team, EPHI
13	Ato Mesfin Tefera	Polio lab. EPHI
14	Habtamu Tulu	Finance case team, EPHI
15	Mestewat Kebede	Finance case team , EPHI
16	Milion Kebede	Public Relation, EPHI
17	Dr. Asfaw Debella	TMMRD, EPHI
18	Dr. Getachew Addis	TMMRD, EPHI
19	Ato Mulugeta Guta	TMMRD, EPHI
20	Ato Negero Gameda	TMMRD, EHNRI
21	W/o Hymanot Ewunetu	Jimma University
21	W/o Kidist Yersaw	TMMRD, EPHI

22	Ato Kissi Mudie	TMMRD, EPHI
23	Ato Berhanu Assaye	TMMRD, EPHI
24	Ato Samuel W/Kidane	TMMRD, EPHI
25	Ato Yared Debebe	TMMRD, EPHI
26	W/t Christina Haile	TMMRD, EPHI
27	Ato Yehualashet Belete	TMMRD, EPHI
28	Ato Bekesho Geleta	TMMRD, EPHI
29	Ato Abiy Abebe	TMMRD, EPHI
30	W/o Aynalem Lakew	TMMRD, EPHI
31	Ato Birhanu Tesfaye	TMMRD, EPHI
32	Ato Ashenif Tadele	TMMRD, EHNRI
33	W/t Hirut Lemma	TMMRD, EPHI
34	Ato Temsgen Menberu	TMMRD, EPHI I
35	W/o Abebawork Mengstu	TMMRD, EPHI
36	W/o Beletu Abate	TMMRD, EPHI
37	Ato Germew Hailu	TMMRD, EPHI
38	Ato Elias Daneke	TMMRD, EPHI
39	W/o Tigist Beshada	TMMRD, EPHI
40	Ato Messaye Getachew	TMMRD, EPHI
41	Dr. Dinka Ayana	School of Veterinary Medicine, AAU ,
42	Dr. Hagos Ashenafi	Faculty of Veterinary Medicine, AAU ,
43	Dr. Yakob Hailu	Faculty of Veterinary Medicine, AAU ,
44	Dr. Hailu Wondemu	Private formerly Arsi Regional Vetrinary laboraotry
45	Dr. Ahmed Yasine	Wollo University,

46	Dr. Asnake Fekadu	Sodo Regional veterinary Lab,
47	Ato Baemnet Mengesha	WGARC, EIAR
48	W/o Zewdinesh Damtew	WGARC, EIAR
49	W/t Gelila Asaene	WGARC, EIAR
50	W/o Tsehynesh Lemma	ALERT
51	Ato Robel Gessese	ALERT
52	Ato Amedemariam Mache	Adametulu pesticide SC
53	Ato Gemechu Tafa	Adametulu pesticide SC
54	Dr. Fitsum F. Sahle	School of Pharmacy, AAU
55	Dr. Mekbebe Afework	Department of Anatomy & Histology AAU
56	Dr. Kaleab Asres	School of Pharmacy, AAU
57	Dr. Araya Haymte	School of Pharmacy, AAU
58	Dr. Tesfaye Tolosa	Department of physiology AAU
59	Ato Endale Seifu	School of Pharmacy, AAU
60	Ato Getachew Chane	Jimma University
61	Ato AsayeBirhan Getachew	Dire Dawa University
62	Ato Alemayehu Toma	Hawassa University, Graduate student sponsored by TMMRD
63	Ato Abdu Hassen	Dillas University, Graduate student sponsored by TMMRD
64	Ato Abinet G/Michael	Arbaminch University, Graduate student sponsored by TMMRD
65	Ato Selamu Kebamo	Wollega University, Graduate student sponsored by TMMRD
66	W/t Mebruka Mohammed	Addis Ababa University, Graduate student sponsored by TMMRD

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|----|---------------------|---|
| 67 | W/o Kidist Zealiyas | Addis Ababa University, Graduate student sponsored by TMMRD |
| 68 | Ato Mokonnen Debebe | Addis Ababa University, Graduate student sponsored by TMMRD |
| 69 | Ato Hussein Haji | Addis Ababa University, Graduate student sponsored by TMMRD |
| 70 | Ato Nigatu Sima | Addis Ababa University, Graduate student sponsored by TMMRD |



Some of the workshop participants during visiting Adis Ababa University conservation site



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