Production and Characterization of Biodiesel from Brebra (*Millettia ferruginea*) Seed Oil

Out line of presentation

- INTRODUCTION
- OBJECTIVE
- MATERIALS AND METHODS
- RESULTS AND DISCUSSION
- CONCLUSION AND RECOMMENDATIONS

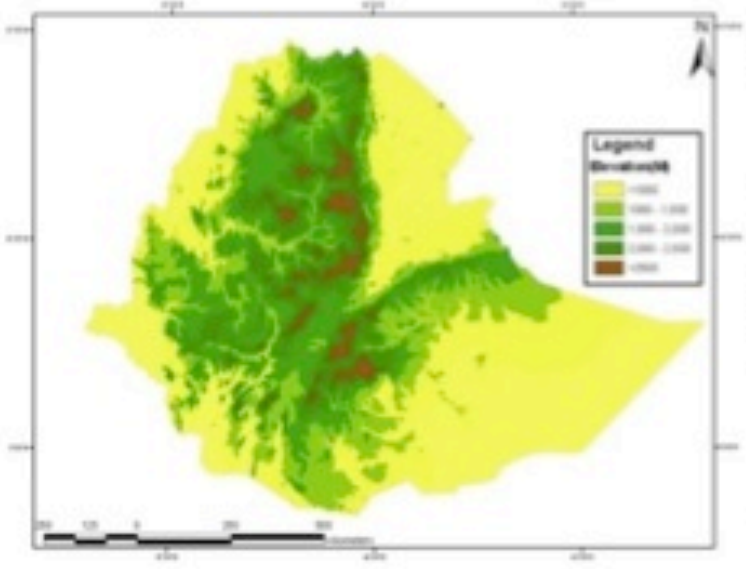
I. INTRODUCTION

- **Energy sources in Ethiopia**
- Main energy source
  - Biomass (95%)
- Population increase
- Implication
  - Rapid deforestation
  - Environmental degradation

  If we produce bio-fuel from bio-resorurces or bio-products, it will contribute a lot for conservation of biodiversity

- Bio-resource technology (Giibitz, *et al.*, 1999)
- Among the different groups of plants that human beings exploit for a variety of application
- In Ethiopia there are some endemic legume plants
- Of those potential plants, Brebra (*M. ferruginea*) is the main focused endemic legume plant

The natural habitat of *M. ferruginea* (Berhanu and Amare, 2013) American Journal of Research Communication



ferruginae is found in North, 1,000 - 2,500 m

While subspecies *darassana* is distributed in Southern, 1,600 - 2,500 m

The hybrid of the two subspecies (Thulin, 1983)

All subspecies (Thulin, 1989)

Continued…

- **Current uses**
  - Dominant coffee shade tree (22.3%) (Muleta, 2007)
  - Wood is widely used as fire fuel and house construction
  - Flowers serve as feed for bees
  - Leaves, shoots and flowers are used as animal feed
- It is well known agro-forestry tree (Thulin, 1983; Abraham Loha, *et al*., 2008)
- It improves soil fertility of land and intercropping is common (Legesse Negash, 1995)

Figure 1: Maize growing under and nearby *M. ferruginea* trees (Wonegeba kebele)

Continued…

- The use of seed is only limited
  - As fish poison and insecticide
  - chiggers and scabies (Stein, 1973)

  - The toxic compound (rotenone)
  - A respiratory inhibitor, toxic to cold blooded animals

- Despite these significant benefits of the plant under this investigation

  - the plant seed oil and other bioproducts are not explored

Continued…

- So, it is possible to produce biodiesel from oil for different purposes
  - To this effect, this study was conducted with the following objective

II. Objective

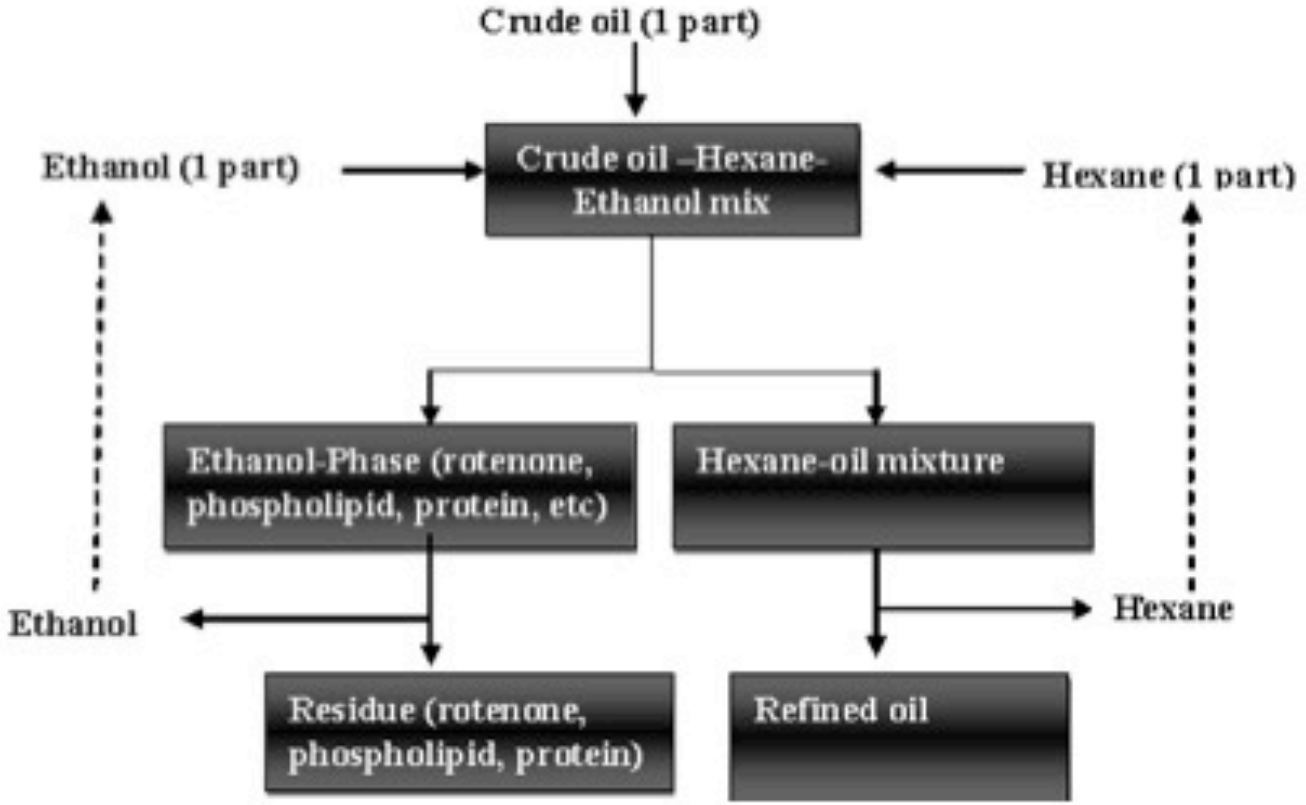
- The objective of this study is to investigate the production of biodiesel from brebra seed oil through the application of biotechnological techniques

III. MATERIALS AND METHODS

- Crude oil was extracted from brebra seed using two methods
  - Mechanical press and solvent extraction

Refining of brebra…

The overall refining process (*Berhanu Andualem and Amare Gessesse, 2012*)



Fatty acid analysis

- The fatty acid profile of oil was determined by GC using standard methods

Biodiesel production

- All the experiments were carried out under the same reaction conditions

- The reaction mixture
  - 100 g brebra oil
  - 22.6 g methanol
  - and 1 g KOH
  - Methanol to oil molar ratio was 6:1

Biodiesel…

- The glycerol phase was removed and the FAME purified (Alamu *et al.*, 2007)
- Analytical TLC (Rachmaniah *et al.*, 2002)
- **The physico-chemical properties** of the biodiesel were determined

Table 1: Standard test methods used to characterize FAME

Properties	Method
Specific gravity	ASTM D1298
Density	ASTM D1298
Distillation	ASTM D 86
Flash point	ASTM D 93
Copper strip corrosion	ASTM D849-09
3Hrs at 100^{0}C	ASTM D 130
Cloud point	ASTM D 2500
Pour point	ASTM D 97
Kinematic viscosity at 40^{0}C	ASTM D 445
Cetane number	Kalaysiri, *et al*., 1996
Cetane index	ASTM D 976
Color	ASTM D 1500
Water content	ASTM D 95
Water and sediment	ASTM D 2709
Acid value	ASTM D 974
Iodine value	OMA, 1984 and Lawson, 1985
Ash	ASTM D 482
Heat of combustion	Parri, 1987

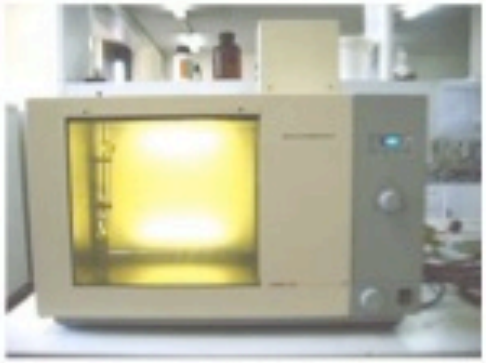
(ASTM standards, 2002)

Device for cold & pour point cold

Flash point apparatus

Distillation apparatus

FFA Determination



Viscometer apparatus

IV: RESULTS AND DISCUSSIONS

- Starting from harvesting to oil extraction

37.50 kg

1312.5-

1500 k

Brebra seed is composed of 48.5% (w/w) of oil

Based on its oil content , brebra seed can be grouped among the high oil containing seeds

Its high oil content and its fatty acid composition makes the oil as an ideal candidate

Table 4: Oil content of some selected oil seeds in comparison with brebra oil

No	Oil seed	Oil content (%)	Reference
1	Brebra	48.5	This study
2	Melon oil seeds	49.5	Ige et al., 1984
3	Pumpkin seed	48.1	Fagbemi and Oshodi, 1991
4	Conophor nut	48.9	Enujiugha, 2003
5	Cashew nut	49.1	Akinhanmi et al., 2008
6	Soybean	23.5	Paul and Southgate, 1980
7	Castor seed	50.0	Massoura et al, 1996
8	Sesame seed	50.0	Massoura et al, 1996
9	Crambe abyssinica seed	45.4	Massoura et al, 1996
10	Groundnut kernel	42.0	Hobbs, 2003
11	Rapeseed	40.0	Agarwal and John, 1996
12	Sunflower	42.0	Hobbs, 2003
13	Linseed	38.0	Hobbs, 2003
14	Safflower	35.0	Hobbs, 2003
15	Millettia obanensis	23.48	Umoren et al., 2005

Table 6: Fatty acid composition of brebra oil (*FAs = Fatty acids)

No	Fatty acids	Carbon number	Quantity (%)
	Saturated FAs*		
1	Palmitic	C16:0	7.2 ± 0.1
2	Stearic	C18:0	2.5 ± 0.1
3	Arachidic	C20:0	0.9 ± 0.1
4	Behenic	C22:0	7.4 ± 0
5	Lignoceric acid	C24:0	2.2 ± 0
	Total		20.2
	Unsaturated FAs*		
6	Oleic	C18:1	30.3 ± 0.2
7	Linoleic	C18:2	44.7 ± 0.2
8	Linolenic	C18:3	1.6 ± 0
9	Eicosenoic	C20:1	2.4 ± 0.1
10	Eicosadienoic	C20.2	0.2 ± 0
11	Eurcic	C22:1	0.6 ± 0
	Total		79.8

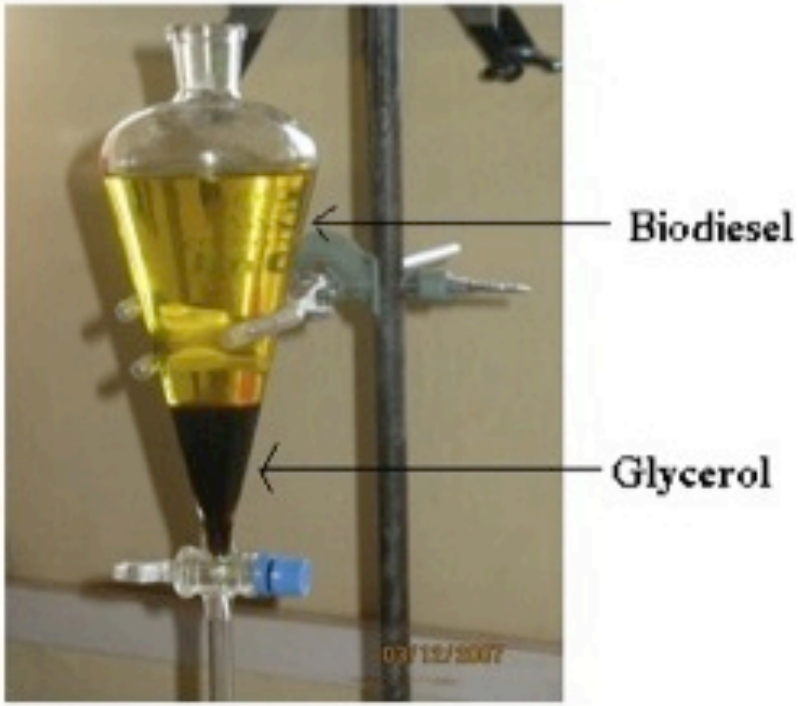
Total polyunsaturated 48.7%

Refining of brebra…

Refinery using ethanol

Equal volumes of crude oil, hexane, and ethanol were mixed and allowed to stand

Refining of brebra…

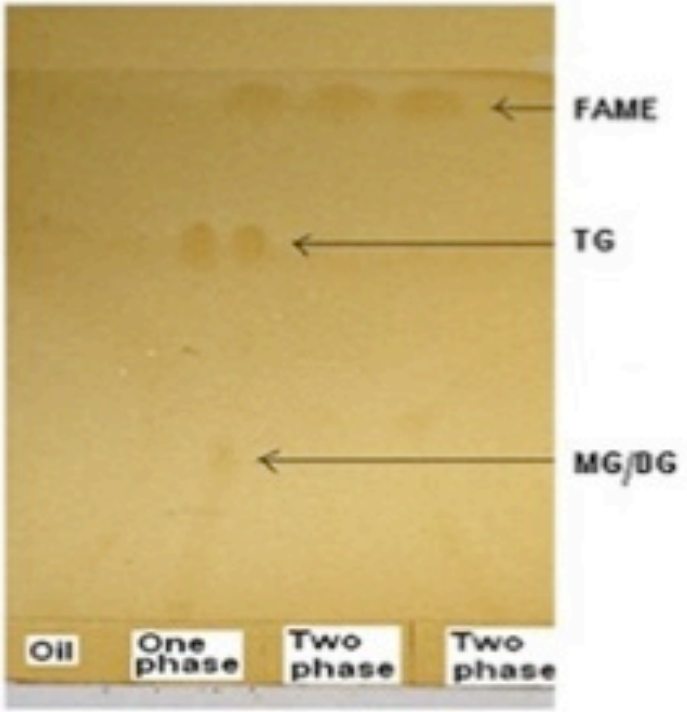


Biodiesel from refined oil was light yellow in colour. Within the standard range (ASTM, 2002)

This refinery method was efficient in removing all the impurities

Production of Biodiesel

- The reaction progress was monitored using TLC



Production …

- After end of the reaction , 81.6 and 17. 84%

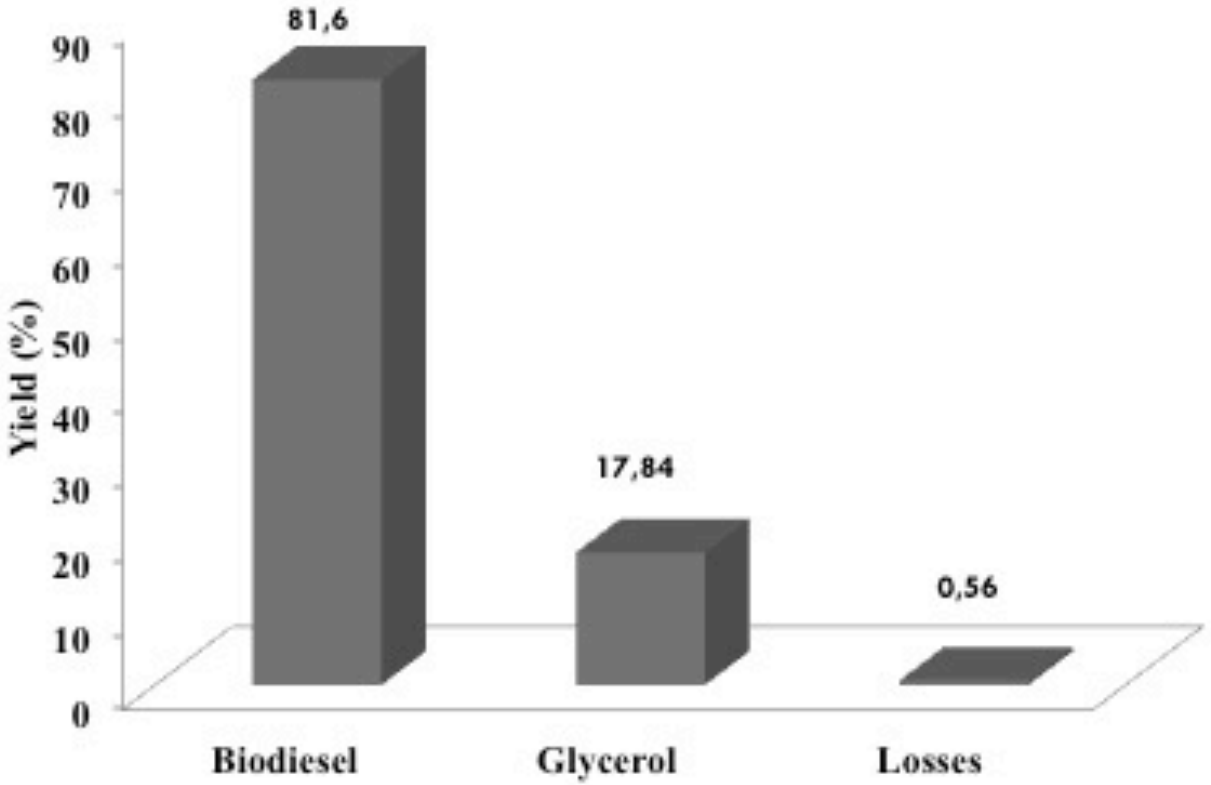
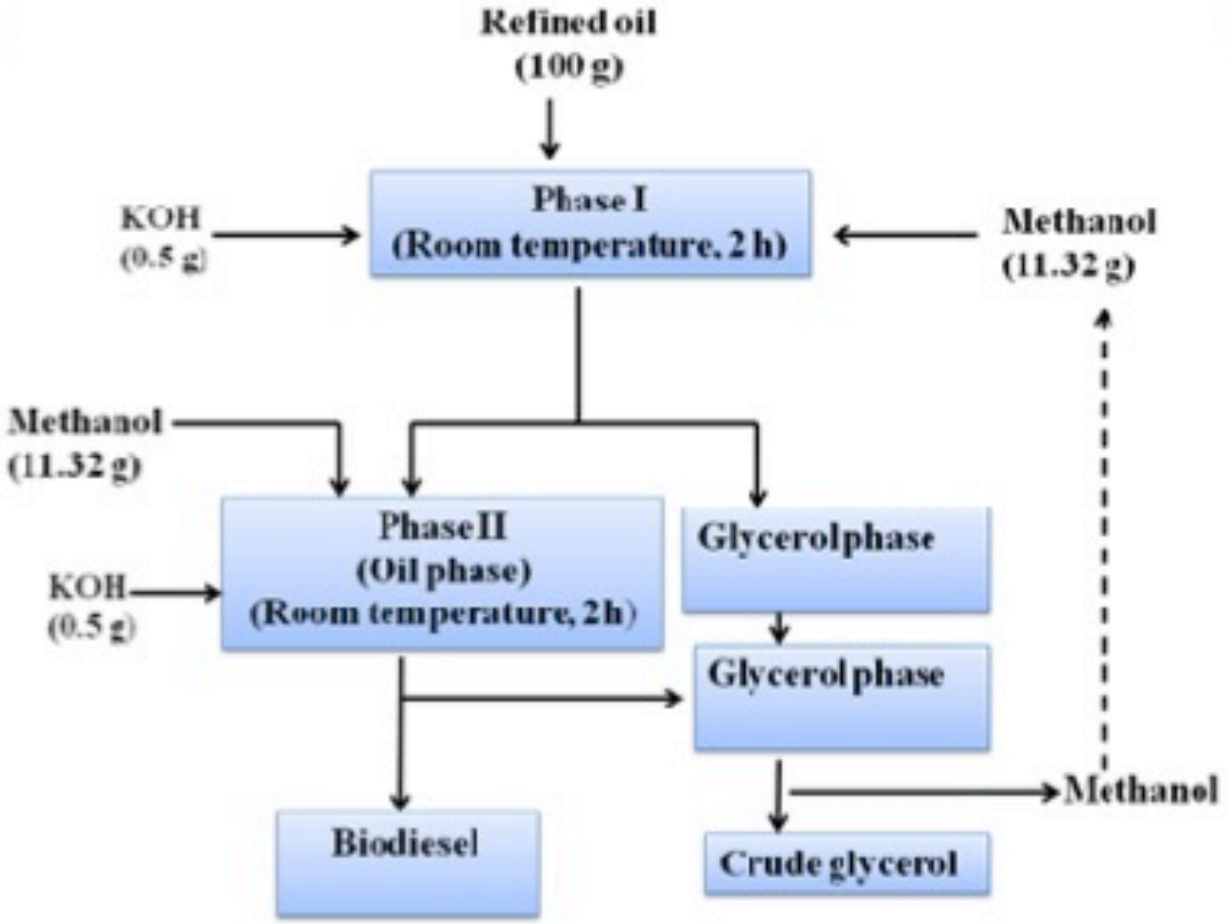


Table 13 shows the physicochemical characteristics of brebra biodesel

Fuel Property	Unit	Standard test method (ASTM D6751)	Brebra biodiesel	Specification/ Standard	
				USA	EU
Calorie or energy content	MJ/Kg	-	37.632	35.00	**
Kinematic viscosity, at 40°C	mm^2/s	D445	5.063	1.9-6.0	3.5-5.0
Specific gravity at 60°F	kg/l	D1298	0.8842	0.88	0.860-0.900
Density at 15°C	g/ml^3	D1298	0.8867	**	0.86-0.90
Density at 2^0 C	g/ml^3	D1298	0.8884	**	0.86-0.90
Water content	v/v	D95	0.04	≤ 0.03	**
Water and sediment,	V/V %	D2709	0.04	≤ 0.05max	0.03
Boiling point	°C	D86	305	315 to 350	Max 360
Flash point	°C	D93	145.8	≥ 120	≥ 130
Cloud point	°C	D2500	26	-3 to 12	**
Pour point	°C	D97	21	-15 to 10	**
Cetane number	-	D613	52	≥ 47	≥ 51
Cetane index	-	D976	46.8	≥ 45	≥ 45
Iodine value	$gI_2/100$ g	-	104.48	-	≤ 120
pH value	-	-	8.9	9.0	9.0
Color	-	D1500	1.5	≤ 3	**
Copper strip corrosion 3H at 100^0 C	-	D130	1b	≤ 3b	Class 1
Acid number	mg KOH/g	D664	0.69	0.80 max	0.50
Ash	wt%	D482	0.2	0.02	**

Flow chart of biodiesel production



Biodiesel …

The cost of raw materials is often the biggest obstacle

For example, if edible vegetable oils are used 70-85% of the cost is used to purchase raw material (Noordam and Withers, 1996)

If biodiesel is produced from non-edible vegetable oils with one or more byproducts (Dorado and Lóperz, 2006)

Biodiesel …

- Production of biodiesel from brebra can help
  - Avoid land competition with crops
  - Reduce the amount of currency expenditure for petroleum
  - Address environmental problems
  - It can provide and improve the quality of multiple energy services

- It also opens market for producers and also creates job opportunity

V: CONCLUSIONS and RECOMMENDATION

1. Conclusions

- The refined oil would have important application for the production of biodiesel
- Co-products, such as glycerol, phospholipids, protein and rotenone are expected to lower the production cost
- The DF left after oil extraction, which is rich in content of protein, could have enormous applications

Conclusions

- Seed gives multiple benefits in terms of generation of
  - income for farmers
  - create job opportunity
  - and thereby contributes a lot for economic development of the country

2. Recommendation

- **Main recommendations**
- 1.The agronomy of the plant should be studied
- 2. The biology of the plant should be studied
  - genetics and its genetic variation
- 3. There is a need to scale up the whole process
  - First pilot scale operation is significant to evaluate the consistency of the production at industrial level
- 4.To avoid irreversible loss of this species we have to develop mechanisms to preserve and use the plant at national level

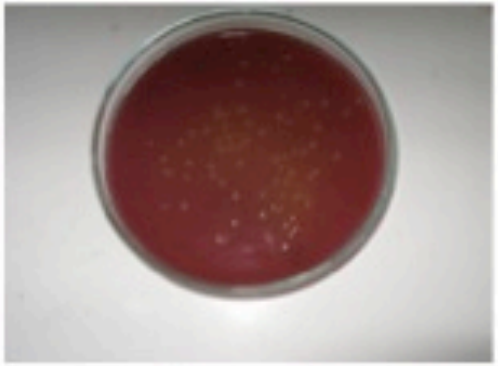
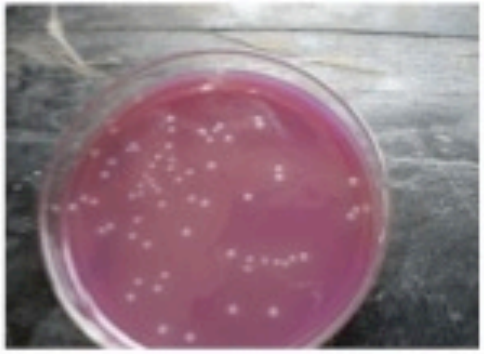
- *Publication from this presentation*
- *1. Berhanu Andualem and Amare Gessesse, 2012.* **Methods for Refining of Brebra *(Millettia ferruginea)* Oil for the Production of Biodiesel.** World Applied Sciences Journal, 17 (3): 407-413.
- **2.** Berhanu Andualem and Amare Gessesse, 2012. **Production and Characterization of Biodiesel from Brebra (*M. ferruginea*) Seed Oil. Biotecnology, 11(4): 217-224.**
- **3.** Berhanu Andualem and Amare Gessesse
- **Assessment of Brebra *(Millettia Ferruginea) Geographical Distribution, Economical* Importance and its Current Threat. American Journal of Research Communication www.usa-journals.com**

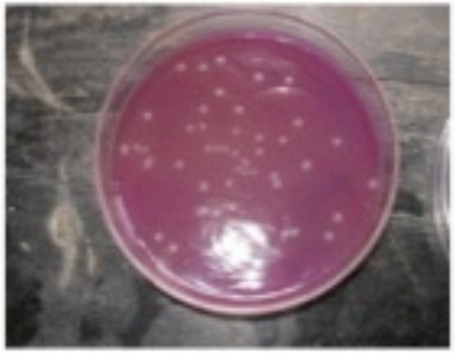
- **Thank You !!**

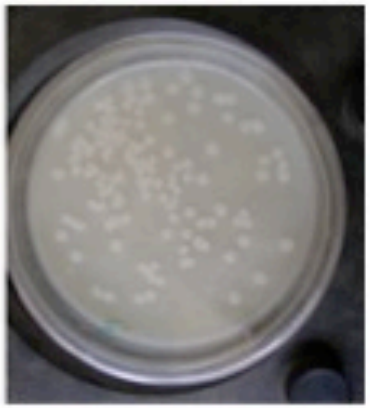
Annex 1: Preparation of brebra defatted flour agar at pH 6.5.

Annex 2: Colony of Shigella dysenterae on differently treated defatted brebra seed flour agar and commercial peptone agar. A = from cooked DBFA, B = Non-cooked DBFA and C = Commercial peptone agar

FIG



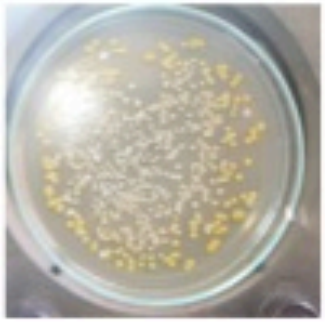


Table 3: Proximate chemical composition of brebra seed (g/100 g dry matter)

Component	Mean ± SD (%)	
Crude oil	48.5 ± 0.99	
Crude protein	29.7 ± 0.23	
Crude fiber	2.41 ± 0.12	
Ash	3.24 ± 0.0	
Moisture	4.24 ± 0.04	
Carbohydrate (by difference)*	11.9 ± 0.2	
Rotenone	0.701 ± 0.02	
Dry matter	95.8 ± 0.07	
Organic matter	92.52 ± 0.03	
Nitrogen free extract (NFE)**	11.91 ± 0.2	
Energy (Kcal/g)***	602.94 ± 0.01	

All tests were performed in triplicates and mean values are taken

*Carbohydrate = 100- (H_2O + Ash +CP + EE)

** NFE = 100 - (H_2O + CP + CF + EE + Ash)

***Energy (kcal) = 4 (g protein +g carbohydrate) + 9 (g lipid)

Table 8: Total amino acid composition of brebra seed (g/100 g)

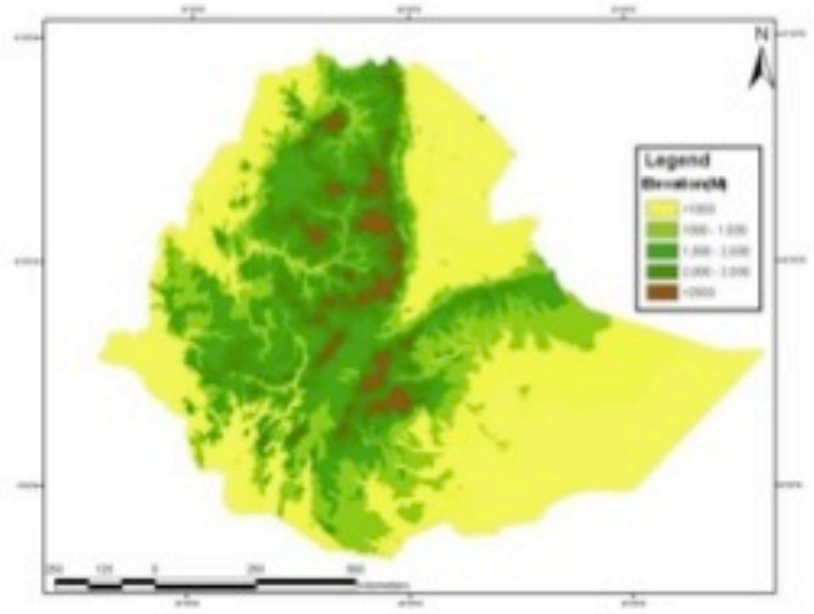
Amino Acids	Brebra seed	Soybean seed
Essential amino acids		
Isoleucine	1.63	1.971
Leucine	2.98	3.309
Lysine	1.79	2.706
Methionine	0.12	0.547
Phenylalanine	1.75	2.122
Threonine	0.97	1.766
Histidine	0.70	1.097
Non-essential amino acids		
Alanine	1.33	1.915
Ornithine	0.02	-
Asparagine	1.60	-
Aspartate	6.43	5.112
Cysteine	0.09	-
Glutamate 230+170)	18.62	7.874
Glycine	1.10	1.880
Proline	1.20	2.379
Serine	1.21	2.357
Tyrosine	0.52	1.539
Total free NEAA	0.037	-
Total free EAA	0.4	-
Total essential amino acids (E)	11.88	13.52
Total nonessential amino acids N)	32.12	23.10
E/N	0.37	0.59
Total AAS	**44.00**	**36.62**

Table 9: Mineral composition of defatted brebra seed flour

Mineral	mg/100 g			
	Brebra seed	*Conophor nut	**Cashew nut Kerrel	***Ripened beans
Magnesium (Mg)	112.38 ± 0.1	57.37± 2.53	19.3 ± 0.1	28.7 ± 2.8
Calcium (Ca)	61.55 ± 0.01	42.06 ± 2.01	21.5 ± 0.0	140.0 ± 7.8
Sodium (Na)	93.26 ± 0.1	-	8.2 ± 0.2	60.8 ± 5.0
Zinc(Zn)	2.0 ± 0.2	6.84 ± 0.02	0.8 ± 0.1	10.7 ± 0.7
Iron (Fe)	27.81 ± 0	1.55 ± 0.08	0.6 ± 0.1	1.2 ± 0.1
Potassium (K)	281 ± 0.1	-	27.5 ± 0.4	1327.0 ± 2.3
Manganese (Mn)	25.5 ± 0.2	-	-	2.02 ± 0.1
Copper (Cu)	17.39 ± 0.1	1.56 ± 0.05	-	0.34 ± 0.1
Phosphorus(P)	1062.1±0.3	465.95	14.0 ± 0.2	214 ± 14.1

*Enujiugha, 2003, ** Akinhanmi *et al*., 2008, *** Bhagya *et al*., 2006

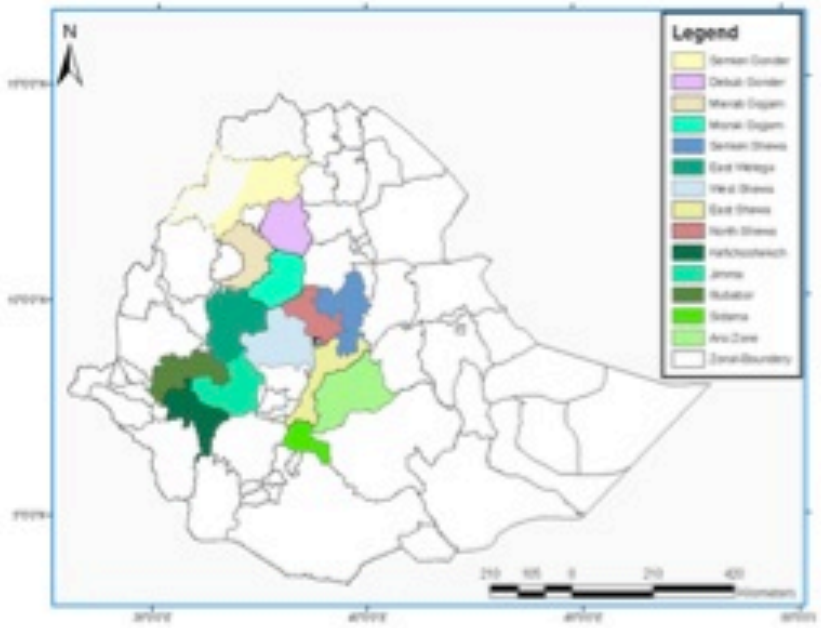
Geographical distribution



The natural habitat of *M. ferruginea*

Geographical distribution

- The current distribution of *M. ferruginea*



- The main threats of plant
  - Agriculture, Fire fuel
  - And house construction

From the general characteristics of brebra such as

- From ecological point of view,
- Its chemical diversity

- Brebra is more superior and sustainable for
  - biodiesel, protein and other bio-

DF Protein

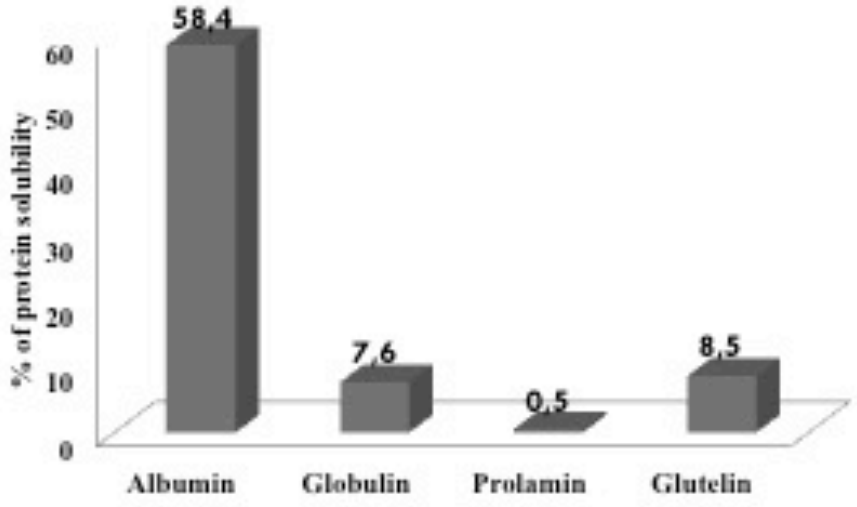
	Sample	
	Defatted brebra flour %	Defatted soybean flour %
Protein content	48.5 ± 0.20	44.3 ± 0.04
Dispensability index	58.4 ± 0.10	58.2 ± 0.3
Protein concentrate	74.5 ± 0.01	70 ± 0.02
Protein nitrogen	98.3 ± 0.00	ND
Non-protein nitrogen	1.7 ± 0.00	ND

- The protein concentrate of DF (74.5±0.01%) is better (Lusas and Rhee, 1995; Bhatty and histison, 1984)

PI

Protein Fractionation …

- About 75% of brebra seed protein was fractionated



- High amount of albumins (58.4%) can substitute the albumins found in egg, blood and milk

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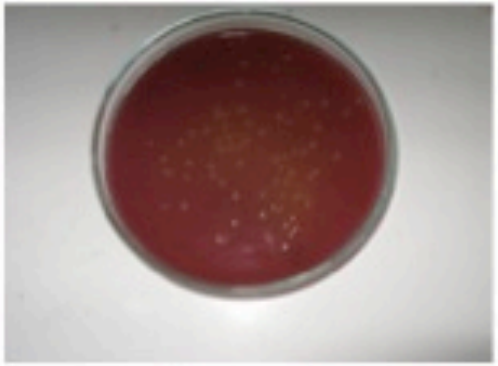
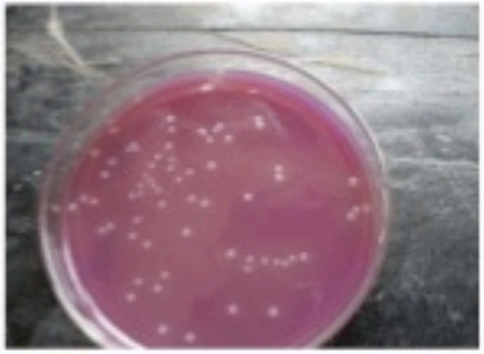


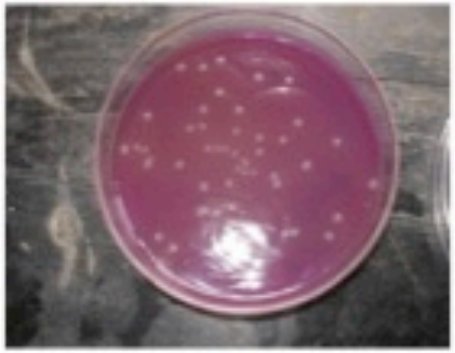

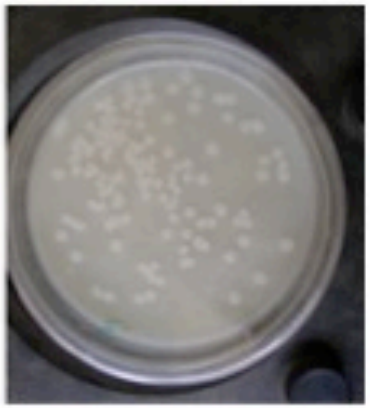

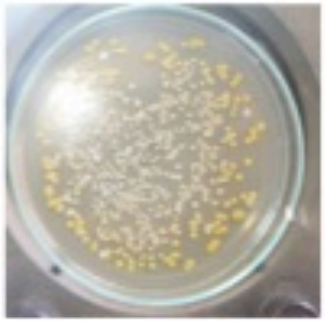

Thank You !!