



Comparison of Soil Physico-Chemical Properties and Phytochemicals in *Melissa officinalis* L. Grown in non-Cultivated and Cultivated Area of Dibrugarh, Assam

Junali Chetia

Department of Botany, Silapathar College, Silapathar, Dhemaji-787 059, India
E-mail: junali.chetia@yahoo.com

Abstract: The aim of the present study to record the differences in soil physico-chemical properties of the two study area and also to record the difference in antioxidant, antimicrobial activities of different parts of *Melissa officinalis* L. Total phenol and flavonoid content, antioxidant and antimicrobial activities of the plants recorded differences. In most of the cases the plant samples collected from non-cultivated area (DUC) recorded more phenol and flavonoid content and antioxidant and antimicrobial activities than the samples collected from cultivated area (KG). The higher NPK and organic matter content in DUC might be the reason of the presence of more phytochemicals in the plant grown in the respective area. Sample collected from DUC (non-cultivated area) show more activity than KG (cultivated area). The phytochemicals present in plants from DUC is more than the phytochemicals present in the plant cultivated from the KG. The Total phenol and flavonoid content was also higher in plants collected from DUC. Similarly, plants collected from DUC recorded more antioxidant activity against both DPPH and ABTS. The significant differences in antimicrobial activities were also observed in plants collected from DUC.

Keywords: Physico-chemical, Antioxidant, Antimicrobial, Phytochemicals, NPK

Phytochemicals present in plants are mainly responsible for the medicinal properties of the plants. The soil condition and other environmental conditions influenced the medicinal properties of a plant. Synthesis and accumulation of phytochemicals in plants depends on the species, age of the species, climatic factor of the area and season of sample collection (Ezeabara and Egwuoba 2016). The different levels of phytochemicals in plants depend on the method of extraction, age of the plant, location and season of collection of the plant samples. *Melissa officinalis* L. is commonly known as lemon balm. The plant had properties like, anti-bacterial, anti-viral, antifungal and antioxidant, sedative, spasmolytic, anti-inflammatory, mnemonic improvement, reduce excitability, anxiety, stress, gastrointestinal disorders, sleep disturbance, treat fevers and colds, indigestion, hyperthyroidism, depression, mild insomnia, epilepsy, headaches, tooth-aches and treat Alzheimer's disease (Lang and Buchbauer 2012, Chaiyana and Okonogi 2012, Astani et al 2012, Aprotosoie et al 2013, Bounihi et al 2013, Pirbalouti et al 2014). The domestication of medicinal plants needs the knowledge of natural habitats, soil physico-chemical properties and nutrient levels. The present study was an attempt to study the properties of a non-cultivated and cultivated area of *Melissa officinalis* L. The study also includes the comparative analysis of phytochemical, antioxidant and antimicrobial activities of different solvent extracts of different parts of the plant collected.

MATERIAL AND METHODS

Sample collection: Soil and plant samples were collected from the study area during 2017, from two areas of Dibrugarh District, one non-cultivated area (Dibrugarh University Campus, DUC) and another cultivated area (Khanikar Gaon, KG). DUC is considered as wild habitat of the plant and samples from KG is considered as domestic habitat of the plant. Immediately after collection soil samples were air dried at room temperature, sieved and analyzed for different soil parameters.

Plant samples were also collected at their full bloomed stage along with the soil samples. The herbarium specimen of the species was also prepared and deposited in the Department of Life Sciences, Dibrugarh University. From each area, different plant parts (young leaves, mature leaves, inflorescence and stem) were collected separately and cleaned properly and washed under running water to remove dust and other debris. The materials were air dried at room temperature. The stems were sliced before allowed to dry. After few days, the materials were wrapped with brown paper and allow sundry for complete dryness (less than 1-2% moisture content). The materials were grounded to fine powder using mortar and pestle. The fine powder was kept in air tight bottles for further analysis.

Preparation of extracts: Extracts were prepared in five solvents viz- water, methanol, ethanol, acetone and petroleum ether by cold maceration methods and are known

as cold extracts. The extracts were kept in air tight glass bottles at 5°C for further analysis. Hot petroleum ether extract was also prepared using Soxhlet extractor and antimicrobial activity of the extract was done to observe the difference in activities of both cold and hot extract. The dried extracts were dissolved in DMSO (dimethyl sulfoxide) to obtain sample solution at 1mg/ml of concentration. Aqueous extracts were dissolved in distilled water at 1mgml⁻¹ of concentration.

Qualitative phytochemical analysis: Qualitative analysis for detection of tannins, phlobatannins, flavonoids, saponins, alkaloids, cardiac glycosides, terpenoids, steroids, anthraquinone, free anthraquinone, carotenoids and reducing sugar were performed using standard laboratory methods (Trease and Evans 2002, Edeoga et al 2005, Egwaikhide and Gimba 2007, Chitravadivu 2009, Majaw and Moirangthem 2009, Aja et al 2010, De et al 2010, Ajayiet al 2011 and Ajiboye et al 2013)

Determination of total phenol content (TPC): Total phenol content (TPC) of the sample extract was estimated following the method described by Malik and Singh (1980).

Determination of total flavonoid content (TFC): The Aluminium chloride method was used for determination of total flavonoid content of the sample extracts (Mervat and Hanan 2009)

Determination of antioxidant activity assay of the sample extract: DPPH radical scavenging activity was determined by the method of Stanojevic et al (2009).

Determination of antioxidant activity assay of the sample extracts: The ABTS assay was carried out following the method of Re et al (1999).

Antimicrobial activity assay of the sample extracts: Antimicrobial activity of the bacterial strains was carried out by agar well diffusion method using 6mm borer (Nair et al 2005).

Test organisms: Gram positive and gram negative bacterial strains and fungal strains are used in this experiment to observe the antimicrobial activity of the sample extracts.

- Gram positive bacterial strains- *Bacillus subtilis*(MTCC 441), *Bacillus cereus* (MTCC 8750), *Staphylococcus aureus* (MTCC 3160), *Staphylococcus epidermis* (MTCC 3615) and *Proteus vulgaris* (MTCC 744).
- Gram negative bacterial strains- *Escherichia coli* (MTCC 443), *Enterococcus faecalis* (MTCC 439).
- Fungal strains- *Candida albicans*(MTCC 3017) and *Penicillium chrysogenum* (MTCC 947).

Determination of soil physico-chemical properties: Soil physico-chemical properties were determined (Goel and Trivedy (1992).

Table 1. Qualitative phytochemical analysis of different parts of *Melissa officinalis* L. collected from two (DUC and KG) areas

Sample	Areas	Tannins	Phlobatannins	Flavonoids	Terpenoids	Steroids	Glycosides	Cardiac Glycosides	Saponins	Anthraquinones	Free Anthraquinones	Carotenoids	Alkaloids	Reducing Sugar	Phenols
Young leaf	DUC	-	-	+	+	-	+	+	+	+	-	+	-	+	+
	KG	-	-	+	-	-	+	+	+	-	-	+	-	+	+
Mature leaf	DUC	+	-	+	+	-	+	+	+	+	-	+	-	+	+
	KG	+	-	+	-	-	+	+	+	-	-	+	-	+	+
Inflorescence	DUC	+	-	+	+	-	+	+	+	+	-	+	-	+	+
	KG	+	-	+	-	-	+	+	+	-	-	+	-	+	+
Stem	DUC	+	-	+	+	-	+	+	+	+	-	+	-	+	+
	KG	-	-	+	-	-	+	+	+	-	-	+	-	+	+

(+) present, (-) absent

Table 4. Antimicrobial activity study of the sample extracts of different parts of *Melissa officinalis* L. collected from DUC and KG

Sample	Extracts (mgml ⁻¹)	Areas	Diameter of Zone of Inhibition (mm)										
			Bacterial strains						Fungal strains				
			<i>B. subtilis</i>	<i>B. cereus</i>	<i>S. aureus</i>	<i>S. epidermis</i>	<i>E. coli</i>	<i>E. faecalis</i>	<i>P. vulgaris</i>	<i>C. albicans</i>	<i>P. chrysogenum</i>		
Young leaf	Water extracts	DUC	-	-	-	-	-	-	-	-	-	-	
		KG	-	-	-	-	-	-	-	-	-	-	
	Methanol extract	DUC	11±1.01	8±0	-	-	10±1	10±2	-	-	-	-	
		KG	-	-	-	-	-	8±0.99	-	-	-	-	
	Ethanol extract	DUC	-	-	-	-	-	-	-	-	-	-	
		KG	10±0.1	-	-	-	-	-	-	-	-	-	
	Acetone extract	DUC	10±1	-	10±2	-	10±0	12±1	-	-	-	-	
		KG	-	-	-	10±00	-	-	-	-	-	-	
	Petroleum ether extract	DUC	11±1	-	11±2	-	10±0	14±2	-	-	-	-	
		KG	-	-	-	-	-	-	-	-	-	-	
	Hot petroleum ether extract	DUC	10±1	-	-	-	-	12±0	-	-	-	-	
		KG	12±1	-	-	-	-	-	-	-	-	-	
	Mature leaf	Water extracts	DUC	-	-	-	-	-	-	-	-	-	-
			KG	-	-	-	-	-	-	-	-	-	-
Methanol extract		DUC	8±0	-	-	8±1	-	-	-	-	-	-	
		KG	-	-	-	-	-	-	-	-	-	-	
Ethanol extract		DUC	8±0	8±0	10±2	9±1	8±0	8±1	-	-	-	-	
		KG	-	-	10±1.6	-	-	-	-	-	-	-	
Acetone extract		DUC	-	-	-	-	-	-	-	-	-	-	
		KG	-	-	-	-	-	-	-	-	-	-	
Petroleum ether extract		DUC	-	-	-	-	-	-	-	-	-	-	
		KG	-	-	-	-	-	-	-	-	-	-	
Hot petroleum ether extract		DUC	-	-	12±0	-	-	-	-	-	-	-	
		KG	8±0	-	-	-	-	-	-	-	-	-	
Inflorescenc		Water extracts	DUC	-	-	-	-	-	-	-	-	-	-
			KG	-	-	-	-	-	-	-	-	-	-
	Methanol extract	DUC	10±1	8±0	-	-	-	-	-	-	-	-	
		KG	-	-	-	10±1	-	-	-	-	-	-	
	Ethanol extract	DUC	8±0	10±1	8±0	-	-	8±1	-	-	-	-	
		KG	-	-	-	-	-	-	-	-	-	-	
	Acetone extract	DUC	-	-	12±0	-	-	-	-	-	-	-	
		KG	-	-	-	-	-	-	-	-	-	-	
	Petroleum ether extract	DUC	-	-	-	-	-	8±1	-	-	-	-	
		KG	-	-	-	-	-	-	-	-	-	-	
	Hot petroleum ether extract	DUC	10±0	12±2	-	-	-	-	8±0	-	-	-	
		KG	10±2	-	-	-	-	-	-	-	-	-	
	Stem	Water extracts	DUC	-	-	-	-	-	-	-	-	-	-
			KG	-	-	-	-	-	-	-	-	-	-
Methanol extract		DUC	-	8±1	9±1	-	-	8±0	-	-	-	-	
		KG	-	-	-	-	10±2	-	-	-	-	-	
Ethanol extract		DUC	-	-	-	-	-	-	-	-	-	-	
		KG	-	-	-	-	-	-	-	-	-	-	
Acetone extract		DUC	9±1	8±1	8±1	-	-	-	-	-	-	-	
		KG	-	-	-	-	-	-	-	-	-	-	
Petroleum ether extract		DUC	-	-	-	-	-	-	-	-	-	-	
		KG	-	-	-	-	-	-	-	-	-	-	
Hot petroleum Ether extract		DUC	-	10±0	-	-	-	-	8±0	-	-	-	
		KG	-	-	-	-	10±1	-	-	-	-	-	
Erythromycin (E)15mcg		-	32±2	30±1	28±0	30±0	12±2	48±6	12±2	-	-	-	
Clotrimazole (CC) 10mcg		-	-	-	-	-	-	-	-	11±2	32±0	-	

Diameter of the cork borer=6mm, '-' indicates no inhibition

Table 5. Soil parameters

Sample	Areas	Organic carbon (%)	Organic matter (%)	pH	Moisture (%)	Ash (%)	Total nitrogen content (%)	Phosphorus content (%)	Potassium content (%)
Soil	DUC	2.0±0.02	3.448±0.01	5.85±0.34	4.80±0.01	1.2±0.01	0.005±0.10	0.008±0.002	0.009±0.001
	KG	1.21±0.01	2.08±0.01	6.96±0.10	6.20±0.13	1.11±0.00	0.004±0.01	0.004±0.001	0.008±0.001

RESULTS AND DISCUSSION

Glycosides, saponins, carotenoids and phenols were present in all the parts collected from both the areas, while plantains, steroids, anthraquinone, free anthraquinone and alkaloids are not recorded in all the parts. The number of phytochemicals are more in the sample collected from DUC than KG. Similar kinds of phytochemicals present in the plant were also recorded by some earlier workers (Carvalho et al 2011, Mutalib 2015). The difference in presence and absence of phytochemicals is might be due to the microclimate and soil condition of the study area. Water and methanol extract showed better extraction of phenolic and flavonoid content than ethanol, acetone and petroleum ether extract at 1mg/ml of concentration (Table 2). Good quantity of total flavonoid content was recorded by water extract of various parts. Total phenol and total flavonoid content was higher in leaves than inflorescence and stem. In most of the cases total phenol and total flavonoid content is higher in the sample extracts collected from DUC than KG. The amount of phenol and flavonoid content in the plant recorded by other workers seems more than the present study. The more phenol and flavonoid in the extracts might be due to the soil condition of the study area. The more organic matter present in the soil may cause the more phytochemicals present in the plant. The phytochemicals present in the plant are also depends on the extraction power of various solvents, which may cause the difference in their phenolic and flavonoid content.

Extracts from leaves recorded higher antioxidant activity against DPPH and ABTS, at 500µl of sample at a concentration of 1mg/ml (Table 3). The extracts of the plant collected from DUC recorded more antioxidant activity than the extracts collected from leaves recorded more antibacterial inhibition than the inflorescence and stem (Table 4). Petroleum ether extract of young leaves collected from DUC was recorded highest (14 mm) inhibition against *E. faecalis* than the other extracts of the plant at 1mgml⁻¹ of concentration, while the petroleum ether extract from KG did not recorded any inhibition against the tested bacteria. All the sample extracts collected from both the areas did not recorded antifungal inhibition against *C. albicans* and *P. chrysogenum* water extract. Antimicrobial activity of the plant was recorded by other workers (Mutalib 2015, Jalal et al 2015). The difference

in antimicrobial activities in different solvent extracts collected from different areas might be due to the phytochemicals responsible for the antimicrobial properties of the plant. Cold extraction in water may be the reason of inactiveness of the water extracts against the tested bacterial strains.

The soil from DUC recorded good physico-chemical properties than the soil collected from KG. Study recorded high soil pH in KG (6.96%) than DUC (5.85%). Sharma et al (2013), Abad (2014) and Maqbool et al (2017) also reported that pH of cultivated land more than the forest land. This difference is might be due to the acidic nature of litter in forest area. The soil moisture of cultivated land from KG (6.20) is higher than the forest area DUC (4.80%), which might be due to the fine texture of the soil and water supply into the land during cultivation. Wang et al (2012) from China reported that high moisture content in corn cultivated area than other places. Maqbool et al (2017) recorded that soil moisture is higher in agricultural land than the forest land which is due to the soil texture, water supply during cultivation.

Organic carbon content of non-cultivated land (2.002%) was higher than the cultivated land (1.21%), which might be due to the higher biomass production in that area. Yitbarek et al (2013), Yihenew et al (2015) and Maqbool et al (2017) reported higher organic carbon content in soil from forest area than agricultural lands.

The percentage of NPK in the cultivated area is lower than the NPK of the non-cultivated area. The good physico-chemical properties of the non-cultivated soil may cause the more phytochemicals in the plant grown in that area. Stanton-Geddes et al (2012) reported the interaction of soil habitat and plant in that area. The positive effect of environmental factors on the bio-synthesis and accumulation of phytochemicals in various plant was studied by various workers (Ibrahim et al 2013, Roux et al 2017, Shaaban et al 2018, Goldo 2019).

CONCLUSION

Sample collected from DUC (the non-cultivated area) show more activity than KG (cultivated area). The phytochemicals present in plants collected from Dibrugarh University campus soil was more than the phytochemicals present in the plant cultivated from the Khanikar Gaon. The

total phenol and flavonoid content was also higher in plants collected from Dibrugarh University campus. Similarly, plants collected from Dibrugarh University campus recorded more antioxidant activity against both DPPH and ABTS. Significant antimicrobial activities were also recorded by the plants collected from Dibrugarh University campus.

REFERENCES

- Abad JRS, Khosravi H and Alamdarlou ES 2014. Assessment the effects of land use changes on soil physicochemical properties in Jafarabad of Golestan province, Iran. *Bulletin of Environment, Pharmacology and Life Sciences* **3**(3): 296-300.
- Aja PM, Okaka ANA, Onu PN, Ibiam U and Urako AJ 2010. Phytochemical composition of *Talinum triangulare* (water leaf) Leaves. *Pakistan Journal of Nutrition* **9**(6): 527-530.
- Ajayi IA, Ajibade O and Oderinde RA 2011. Preliminary phytochemical analysis of some plant seeds. *Research Journal of Chemical Sciences* **1**(3): 58-62.
- Ajiboye BO, Ibukun EO, Edobog G, Ojo AO and Onikanni SA 2013. Qualitative and quantitative analysis of phytochemicals in *Seneciobiafrae* leaf. *International Journal of inventions in Pharmaceutical Sciences* **1**(5): 428-432.
- Apotsoaie AC, Raileanu E, Trifan A and Cioanca O 2013. The polyphenolic content of common lamiaceae species available as herbal tea products in Romanian pharmacies. *Revista Medico-Chirurgical a Societati de Medici si Naturalist din Iasi* **117**(1): 233-237.
- Astani A, Reichling J and Schnitzler P 2012. *Melissa officinalis* extract inhibits attachment of herpes simplex virus in vitro. *Chemotherapy* **58**(1): 70-77.
- Bounihi A, Hajjaj G, Alnamer R, Cherrah Y and Yellou A 2013. In vivo potential anti-inflammatory activity of *Melissa officinalis* L. essential oil. *Advances in pharmacological sciences* Article ID 101759, 7 <http://dx.doi.org/10.1155/2013/101759>.
- Carvalho NCD, Correa-Angeloni MJF, Leffa DD, Moreira J, Nicolau V, Amaral PDA, Rossatto AE and Andrade VMD 2011. Evaluation of the genotoxic and antigenotoxic potential of *Melissa officinalis* in mice. *Genetics and Molecular Biology* **34**(2): 290-297.
- Chaiyana W and Okonogi S 2012. Inhibition of cholinesterase by essential oil from food plant. *Phytochemistry* **15**(19): 836-9.
- Chitravadivu C, Manian S and Kalaichelvi K 2009. Qualitative analysis of selected medicinal plants, Tamilnadu, India. *Middle-East Journal of Scientific Research* **4**(3): 144-146.
- De S, Dey YN and Ghosh AK 2010. Phytochemical Investigation. (Araceae)- *International Journal on Pharmaceutical and Biomedical Research* **1**(5): 150-157.
- Edeoga HO, Okwn OE and Mbaebie B 2005. Phytochemical constituents of some Nigerian medicinal plants. *African journal of biotechnology* **4**(7): 685-688.
- Egwaikhide PA and Gimba CE 2007. Analysis of phytochemical content and anti-microbial activity of *Plectranthus glandulosus* whole plant. *Middle-East. Journal of Scientific Research* **2**(3-4): 135-138.
- Ezeabara CA and Egwuoba GC 2016. Comparative screening of phytochemical and proximate constituents of leaf, stem and roots of *Oldenlandia corymbosa* L. and *Oldenlandia aherbacea* (L.) Roxb. *American Journal of Life Science Research* **4**(3): 113-118.
- Goel PK and Trivedi RK 1992. *Chemical and biological methods for water pollution, Soil and plant analysis*. 1st Ed., Published by Env. Publ., Karad
- Goldo SS 2019. Effects of environmental factors on the accumulation of phytochemicals in plants. *Phytochemistry* **3**.
- Ibrahim MH, Jaafar HZE, Karimi E and Ghasemzadeh A 2013. Impact of organic and inorganic fertilizers application on the phytochemical and antioxidant activity of Kalip Fatimah (*Labisiapumila* Benth.). *Molecules* **18**:10973-10988.
- Jalal Z, Yassine E A, Lyoussi B and Abdellaoui A 2015. Phytochemistry of the essential oil of *Melissa officinalis* L. growing wild in Morocco: Preventive approach against nosocomial infections. *Asian Pacific Journal of Tropical Biomedicine* **5**(6):458-461.
- Lang G and Buchbauer G 2012. A review on recent research results (2008-2010) on essential oils as antimicrobials and antifungals. *A review. Flavour and Fragrance Journal* **27**: 13-39.
- Majaw S and Moirangthem J 2009. Qualitative and Quotative analysis of some *Clerodendron colebrookianum* Walp. leaves and *Zingiber cassumunar* Ronb. Rhizomes. *Ethno botanical leaflets* **13**: 578-589.
- Malik EP and Singh MP 1980. *Plant Enzymology and Hittoenzymology*. Kalyani publishers, New Delhi. 286p.
- Maqbool M, Shafi S and Rehman NZ 2017. Comparison between forest and agriculture land uses in relation to physico-chemical properties and nutrient status of soil in district Ganderbal, J and K. *Research Journal of Agricultural Sciences* **8**(3): 559-566.
- Mervat MMEIF and Hanan AA 2009. Antioxidant activities total anthrocyanine, phenolics and flavonoids content of some sweet potato genotypes under stress of different concentrations of sucrose and sorbitol. *Australian Journal of Basic Applied Science* **3**: 3609-3616.
- Mutalib LY 2015. Physicochemical, phytochemical and biological study of *Melissa officinalis* growing naturally in Kurdistan region / Iraq: Comparative study. *IOSR Journal of Pharmacy and Biological Sciences* **10**(5): 67-72.
- Nair R, Kalariya T and Chanda S 2005. Antibacterial activity of some selected Indian medicinal flora. *Turkish Journal of Biology* **29**: 41-47.
- Pirbalouti AG, Mirbagheri H, Hamed B and Rahimi R 2014. Antibacterial activity of the essential oils of myrtle leaves against *Erysipelothrix husiopathiae*. *Asian Pacific Journal of Tropical Biomedicine* **4**(Suppl 1): S505-509.
- Re R, Pelleorini N, Proteggente A, Pannala, A, Yang M and Rice Evans C 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical Biology and Medicine* **26**: 1231-1237.
- Roux D, Alnaser O, Grayev E, Baghdikian B, Elias R, Chiffolleau P, Ollivier E, Laurent S, Maataoui ME and Sallanon H 2017. Ecophysiological and phytochemical characterisation of wild populations of *Inula Montana* L. (Asteraceae) in Southern France. *Flora, Elsevier* **236-237**: 67-75.
- Shaaban M, Ali M, Tala MF, Hamed A and Hassan AZ 2018. Ecological and Phytochemical studies on *Euphorbia retusa* (Forssk.) from Egyptian Habitat. *Journal of Analytical Methods in Chemistry*, Article ID 9193683
- Sharma YK, Sharma A and Sharma SK 2013. An appraisal of physico-chemical characteristics and soil fertility status of forest and rice land use systems in mokokchung district of Nagaland. *Journal of the Indian Society of Soil Science* **61**(1): 38-43.
- Stanojevic L, Stanojevic M, Nikolic V, Nikolic L, Ristic J, Canadanovic and Brunet V 2009. Antioxidant activity and total phenolic and Flavonoid contents of *Hieracium pilosella* L. extracts. *Sensors* **9**: 5702-5714.
- Stanton-Geddes J, Shaw RG and Tiffin P 2012. Interactions between soil habitat and Geographic Range Location affect plant fitness. *PLOS One* **7**(5): 1-8
- Trease GE and Evans WC 2002. *Pharmacognosy*. 15th edition, London, Saunders Publisher.
- Wang S, Fu BJ, Gao GY, Yao XL and Zhou J 2012. Soil moisture and evapotranspiration of different land cover types in the Loess Plateau China. *Hydrology and Earth System Sciences* **16**: 2883-2892

Yihene GS, Fentanesh A and Solomon A 2015. Effects of land use types, management practices and slope classes on selected soil physico-chemical properties in Zikre Watershed, North-Western Ethiopia. *Environmental Systems Research* 4: 3.

Yitbarek T, Gebrekidan H, Kibret K and Beyene S 2013. Impacts of land use on selected physico-chemical properties of soils of Abobo area, Western Ethiopia. *Agriculture, Forestry and Fisheries* 2(5): 177-183

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