

ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT OF *Alpinia galangal* (L.) Willd. RHIZOME EXTRACT

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ABSTRACT

The present study was carried out in the year 2022 at Department of Botany, Pravabati College, MayangImphal, to analyse an antioxidant activity and total phenolic content of *Alpinia galanga* rhizome extract. Experiment was carried out to determine the phyto-constituents using solvents such as methanol and ethanol. The total phenolic content in methanol and ethanol rhizome extracts in terms of gallic acid equivalent was 19.14 and 7.14 of extract powder respectively. Total antioxidant activity varied among the solvent used in extract with methanol extract having higher activity than ethanol extract. The total antioxidant activity of methanolic and ethanolic extracts of rhizome were found highest ($33.6 \pm 0.70 \mu\text{g AAE mg}^{-1}$ and $27.6 \pm 0.70 \mu\text{g AAEmg}^{-1}$ respectively) in highest concentration of $90 \mu\text{g ml}^{-1}$ of extract. Methanol was found to be a better solvent for extracting phytochemicals. Total phenolic content, total antioxidant activity and reducing power were highest in methanolic rhizome extracts. The present study indicates rhizome of *Alpinia galanga* possessed antioxidant activity. Methanol was found to be a better solvent for extracting phytochemicals from *Alpinia galanga*. Total phenolic content was highest in methanol extract than ethanol extract. Total antioxidant activity and reducing power was highest in methanolic rhizome extract.

(Key words: Antioxidant, phenolic, methanol, ethanol, *Alpinia galanga*)

INTRODUCTION

Alpinia galanga, also known as greater galangal, is a member of the Zingiberaceae family and is a significant medicinal used to treat a variety of illnesses in various traditional medical systems, including chest pain, fever, liver burning, microbial infections, rheumatic pain, inflammations, kidney disease, tumours, diabetes, and even HIV (Ramesh *et al.*, 2011; Verma *et al.*, 2011). Additionally, it actively contributes to the treatment of cholera, bronchitis, coryza, pityriasis, versicolor, otitis internal, and gastritis. The seed is used to clean the mouth and treat emaciation. It functions as a purgative and increases the appetite and digestive system. Rhizomes are typically used as spices. It is a reliable source of essential oils as well. Additionally, the blooms and young branches are utilised as a spice or as a vegetable (Arambewela and Wijesinghe, 2006). Galangal is also effective for treating fever, abnormal menstruation, and increasing male fertility (Abubakar *et al.*, 2018). Galangal rhizome began to be used in several formulations to prevent cancer and tumours and is also used for the treatment of other diseases such as rheumatism, inflammation, diabetes, neurological disorders along with to treat several chronic diseases (Arambewela and Wijesinghe, 2006; Srivastava and Shanker, 2012). The major active compounds such as 1,8 - cineol, α - fenchyl acetate, β - farnesene, β - bisabolene, α -

bergamotene, β -pinene, and 1'- acetoxychavicol acetate. 1,8 - cineole are known as a marker compound for *Alpinia spp* and was reported as the most abundant compound in most of the studies on *A. galanga* (Abdullah *et al.*, 2015). Additionally, the rhizome of *A. galanga* has the phytochemicals such as galangin, beta-sitosterol, quercetin, and emodin which act as a source of iron, vitamins A and C, sodium (Baldo *et al.*, 2016). The rhizomes also have a wide range of uses as an abortifacient, carminative, anti-tuberculosis, and stimulant. They are additionally used to treat skin conditions like eczema, ringworm, and other skin infections. The Chinese have long utilised galanga to treat a variety of ailments, including motion sickness, nausea, vomiting, nausea, and dyspepsia (Indrayan *et al.*, 2009).

MATERIALS AND METHODS

Collection of plant

Alpinia galanga rhizomes were collected from the Thoubal Leisangthem, Thoubal district, Manipur. Authentication of the plant sample was done in Manipur University, Department of Life Sciences (Botany). The rhizomes were washed thoroughly with tap water followed by distilled water. Then the rhizomes were dried under shaded at room temperature and ground into powder.

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Preparation of solvent extracts for qualitative screening

40 g of ground samples were weighed and carried out the process by using 400 ml of methanol and ethanol in soxhlet apparatus respectively. Soxhlet extraction has been used widely (Redfern *et al.*, 2014). The extracts were concentrated by evaporation and stored prior to phytochemical screening.

Phytochemical screening

The extracts were subjected to phytochemical screening to test presence of phytoconstituents such as amino-acids, carbohydrates, proteins, phenol, flavonoids, tannins, steroids and terpenoids, saponins, oils and fats, phlobatanins, etc.

The phytochemical tests were carried out using the standard procedures (Khan *et al.*, 2023; Sharma *et al.*, 2023; Bhaigyabati *et al.*, 2017).

Determination total phenolic content

The Folin-Ciocalteu reagent method was used to determine the amount of phenol content in methanol and ethanol extracts of *Alpinia galanga* rhizome (Bag *et al.*, 2016). 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of Na_2CO_3 (2% w/v) were added to 0.5 ml of the sample (3 replicates) of rhizome extract solution (1mg ml^{-1}). The resulting mixture was incubated at 45°C for 15 min. The absorbance of sample was measured at 760 nm using UV Visible Spectrophotometer (UV-2700). Gallic acid ($50\text{-}300\ \mu\text{g ml}^{-1}$) was used as a standard compound. The gallic acid standard calibration curve was established by plotting concentration ($\mu\text{g ml}^{-1}$) versus absorbance (nm) ($y=0.009675X + 0.004840$; $R^2=0.9584$), where y is absorbance at 760 nm and x is concentration (Figure 1). Total phenolic content in the plant extract was expressed as gallic acid equivalent (mg of gallic acid equivalent g^{-1} of sample) and was calculated by the formula:

$$T = (C \times V) / M$$

Where, T = total content of phenolic compounds, mg g^{-1} plant extract, in GAE; C = concentration of gallic acid established from the calibration curve, $\mu\text{g ml}^{-1}$; V = volume of extract, ml; M = weight of the plant (Bhaigyabati *et al.*, 2017).

Estimation of reducing

Different concentrations of the extracts were prepared and mixed with 2.5 ml phosphate buffer and potassium ferricyanide and the mixture was kept at 50°C in water bath for 20 min. After cooling 2.5 ml of 10% trichloroacetic-acid was added and centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and freshly prepared 1% ferric chloride solution (0.5 ml). The absorbance was measured by using UV spectrophotometer at 700 nm. Control was prepared in similar manner excluding samples. Ascorbic acid ($0.5\ \text{mg ml}^{-1}$) at various concentrations was used as standard (Khan *et al.*, 2023, Sharma *et al.*, 2023).

Determination of total antioxidant activity

The total antioxidant activity of the extracts was evaluated by using the phosphomolybdenum method (Khan

et al., 2023; Sharma *et al.*, 2023). 0.3 ml of the methanolic and ethanolic extract sample (1mg ml^{-1}) as well as ascorbic acid ($0.5\ \text{mg ml}^{-1}$) was mixed with 3.0 ml of the reagent solution (0.6 M sulphuric acid, 28 nM sodium phosphate and 4 nM ammonium molybdate) separately and the mixture was incubated at 95°C for 90 min under water bath. Absorbance of all the mixtures was measured at 695 nm after cooling. Total antioxidant activity is expressed as the number of equivalents of ascorbic acid in $\mu\text{g ml}^{-1}$ of extract. Total antioxidant activity was calculated by using the formula. Total antioxidant = O.D. of test x concentration of standard in $\mu\text{g X}$ made up volume of sample (Khan *et al.*, 2023; Sharma *et al.*, 2023).

Statistical analysis

All sample determinations were conducted in triplicates and the results were calculated as mean \pm standard deviation (SD) (Sharma *et al.*, 2023).

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening of methanol, ethanol and ethyl acetate extract of *Alpinia galanga* rhizome reveals the presence of phytoconstituents as listed in Table 1.

Phytochemicals *sviz.*, amino acids, alkaloids, carbohydrates, proteins, flavonoids, steroids and terpenoids, saponins, cardiac glycosides, phenolic compounds and tannins were present in methanol except oils and phlobatanins and ethanol *viz.*, amino acids, alkaloids, carbohydrates, proteins, flavonoids, steroids and terpenoids, saponins, cardiac glycosides, phenolic compounds and tannins were present except oils and phlobatanins extracts of *Alpinia galanga* rhizome. Important phytochemical considered as active medicinal phytochemical were present in the sample that shows a high level of its possible medicinal value (Khan *et al.*, 2023, Sharma *et al.*, 2023).

Total phenolic content

The phenolic compounds act as free radical terminators and the mechanism of action are through scavenging or chelating process (Platzer *et al.*, 2021, Khan *et al.*, 2023). Phenolic compounds are having wide bioactivity including antioxidant properties/activity. The antioxidant activity of phenolic compound is due to hydroxyl functional group, however other factors eg., presence of electron withdrawing or releasing group in the aromatic ring having hydroxyl moiety will increase or decrease the activity. The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties (Bag *et al.*, 2016). The present study reveals that the total phenolic content in methanol and ethanol rhizome extracts in terms of gallic acid equivalent was 19.14 and 7.14 of extract powder respectively. Result indicates that methanol extract of rhizome of *Alpinia galanga* showed higher total phenolic

content than ethanol (Table 2). The total phenolic content varies according to the solvent use in extract.

Determination of reducing power

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Figure 2 shows the dose dependent reducing power activity of *Alpinia galanga* methanol and ethanol rhizome extract at different concentration using the potassium ferricyanide reduction method. The concentration of extract ranged from 10-90 μg level. As the concentration of extract is increased the reducing power also increased in both the solvent and attained maximum at 100 μg concentration. Increasing absorbance indicates an increase in reductive ability. Methanolic extracts showed higher reducing power than ethanolic extracts. The reducing power activity is due to the presence of reductones (phenolics). As reducing power assay measures the electron donating capacity of an antioxidant, it is associated with the presence of reductones. Reductones exhibit antioxidant action by breaking the chain reactions by donating a hydrogen atom and also reported to react with certain precursor of peroxide thereby preventing peroxide formation (Chanda and Dave, 2009, Olayinka and Aiyegore, 2010, Khan *et al.*, 2023).

Total antioxidant activity

Table 3 shows that total antioxidant activity varied among the solvent used in extract with methanol extract having higher activity than ethanol extract and also indicate the total antioxidant activity increased with the increase in concentration of extract. The total antioxidant activity of methanolic and ethanolic extracts of rhizome were found highest ($33.6 \pm 0.70 \mu\text{g AAE mg}^{-1}$ and $27.6 \pm 0.70 \mu\text{g AAE mg}^{-1}$ respectively) in highest concentration of $90 \mu\text{g ml}^{-1}$ of extract and lowest ($26.4 \pm 0.55 \mu\text{g AAE mg}^{-1}$ and $21.3 \pm 0.54 \mu\text{g AAE mg}^{-1}$ respectively) in lowest concentration of $10 \mu\text{g ml}^{-1}$ of extract. However, the total antioxidant activity depends on the solvent type and its concentration used in extraction. The antioxidant quality or activity of plant-derived products has greatly influenced by the extraction factors like the method of extraction, temperature and solvent used. Several studies evidently show that the effectiveness of antioxidant activity of most plant products was significantly increased by using the aqueous mixtures of organic solvents like ethanol, methanol, acetone, isopropanol, or acetonitrile with water rather than using water alone for extraction (Thamizhinyan *et al.*, 2019).

Table 1. Phytochemical constituents of *Alpinia galanga* rhizome

Phytochemicals	Test performed	Solvent	
		Methanol	Ethanol
Amino acids	Ninhydrin	+	+
Alkaloids	Mayre's test	+	+
Carbohydrate	Benedict's test	+	+
	Fehling's test	+	+
Proteins	Xanthoproteic test	+	+
	Biuret test	-	-
Flavonoids	Alkaline reagent test	-	-
	Lead acetate test	+	+
Steroids and terpenoids	Salkowski's test	+	+
Saponins	Froth test	+	+
Cardiac glycosides	Keller Killiani test	+	+
Phenolic compounds	Lead acetate test	+	+
	Ferric chloride test	+	+
	Lead acetate test	+	+
Tannins	Ferric chloride test	+	+
	Lead acetate test	+	+
Oils	Translucent test	-	-
Phlobatanins	HCl test	-	-

Key + = presence, - = absence

Table 2. Total phenolic content in *Alpinia galanga* rhizome extract

Solvent	Total Phenolic content (mg GAE g ⁻¹ of extract)
Methanol	19.14 ± 1.94
Ethanol	7.14 ± 1.7

Assays were performed in triplicate. Values are expressed as means \pm SD

Table 3. Total antioxidant of *Alpinia galanga*

Concentration ($\mu\text{g ml}^{-1}$)	Total antioxidant activity in $\mu\text{g AAE mg}^{-1}$ of extract	
	Methanol	Ethanol
10	26.4 ± 0.55	21.3 ± 0.54
30	29.7 ± 0.62	23.4 ± 0.49
50	32.7 ± 0.69	26.7 ± 0.54
70	33.0 ± 0.69	26.7 ± 0.67
90	33.6 ± 0.70	27.6 ± 0.70

Assays were performed in triplicate. Values are expressed as means \pm SD

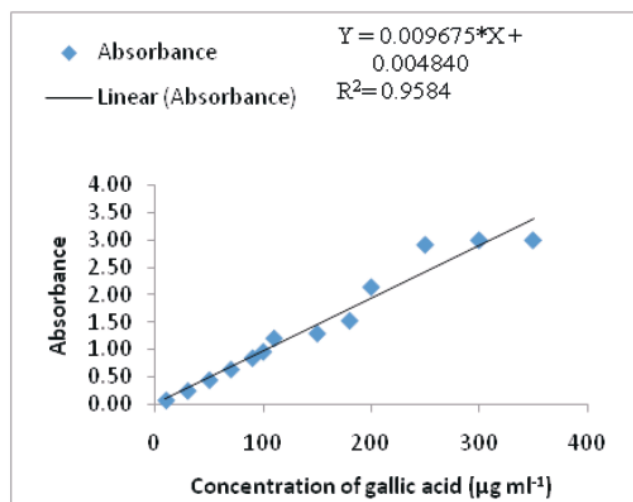


Figure 1. Standard curve of gallic acid ($\mu\text{g ml}^{-1}$)

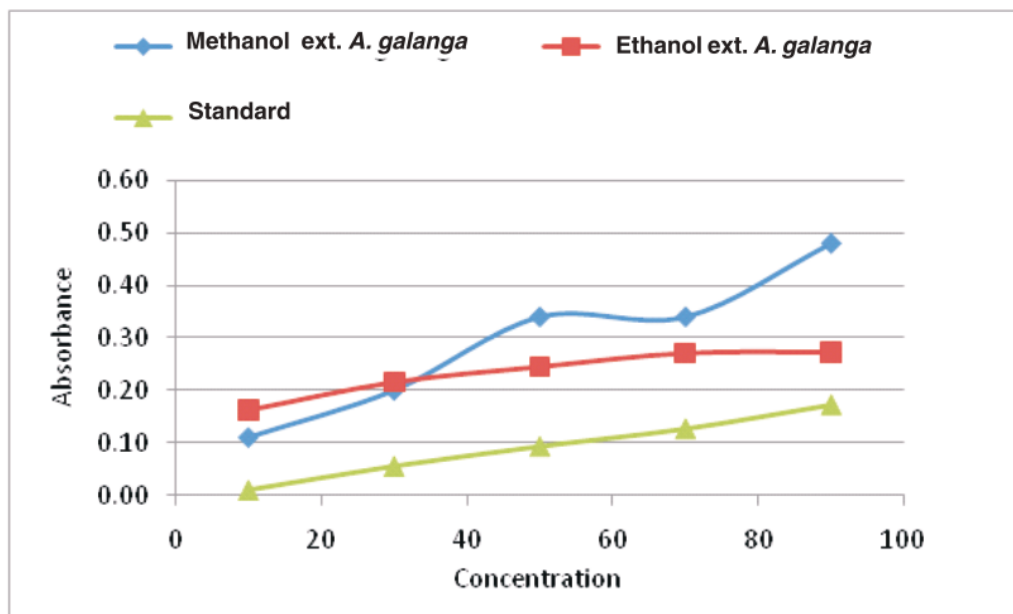


Figure 2. Reducing power of methanolic and ethanolic extracts of *Alpinia galanga*

REFERENCES

- Abdullah, F., P. Subramanian, H. Ibrahim, S. N. A. Malek, G. S. Lee and S. L. Hong, 2015. Chemical composition, antifeedant, repellent, and toxicity activities of the rhizomes of galangal, against asian subterranean *Alpinia galanga* termites, *Coptotermes gestroi* and *Coptotermes curvignathus* (Isoptera: Rhinotermitidae). *J. Insect Sci.* **15**:1-7.
- Abubakar, I. B., I. Malami, Y. Yahaya and S. Sule, 2018. A Review on the ethnomedicinal uses, phytochemistry and pharmacology of *Alpinia officinarum* Hance. *J. Ethnopharmacol.* **224**:45-62.
- Arambewela, L.S. and A. Wijesinghe, 2006. Sri Lankan medicinal plant monograph and analysis: (10th *Alpinia galanga*). Industrial Technology Institute and National Science Foundation: Colombo.
- Bag, G. C., P. G. Devi, and T. H. Bhaigyabati, 2016. Phytochemical screening and antioxidant activity of *Meynalaxiflora* species found in Imphal West District of Manipur, *Int. J. Pharm. Sci. Rev. Res.* **36**(1):137-143.
- Baldo, D. E. B., J. E. Serrano, C. Diomerl and E. B. Baldo, 2016. Screening for the intestinal anti-inflammatory activity of *Alpinia galanga* against acetic acid-induced colitis in mice (*Mus musculus*). *J. Med. Plants Stud.* **4**:72-7.
- Bhaigyabati, T., P. G. Devi, N. R. Devi and G. C. Bag, 2017. Antioxidant activity, total phenolic and total flavonoid content of *Oenanthe javanica* Blum (D C) collected from Imphal west district, *Int. Res. J. Pharm.* **8**(6):63-68.
- Chanda, S. and R. Dave, 2009. *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview, *Afr. J. Microbiol. Res.* **3**(13):981-996.
- Indrayan, A., P. Agrawal, A. Rathi and A. Shatru, 2009. Nutritive value of some indigenous plant rhizomes resembling ginger. *Nat. Prod. Radiance*, **8**:507-13.
- Khan, M. R., T. Bhaigyabati, Y. D. Mrinamala, L. S. Dinendra and S. S. Jinus, 2023. Phytochemical screening and antioxidant activity of methanolic rhizome extract of *Acorus calamus* collected from Imphal west district of Manipur, *J. Soils and Crops*, **33**(2):328-334.
- Olayinka, A., A. I. Aiyegoro and Okoh, 2010. Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longi folium* DC, *BMC Complementary and Alternative Medicine*, Oxford University Press, **10**:21.
- Platzer M, S. Kiese, T. Herfellner, U. Schweiggert-Weisz, O. Miesbauer and P. Eisner, 2021. Common trends and differences in antioxidant activity analysis of phenolic substances using single electron transfer based assays. *Molecules*. **26**:1244-61.
- Redfern, J., M. Kinninmonth, D. Burdass and J. Verran, 2014. Using Soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties, *J. Microbiol. Biol. Educ.* **15**(1):45-6.
- Ramesh, K. V., S. Garima, S. Pradeep, K. K. Jha and R. L. Khose, 2011. *Alpinia galanga* an important medicinal plant: a review, *Der. Pharmacia Sinica*, **2**(1):142-54.
- Sharma, L. D., M. R. Khan and A. Kikim, 2023. Methanolic and ethanolic phytochemical screening of sweet flag (*Acorus calamus* L.) rhizome, *J. drug deliv.ther.* **13**(9):114-118.
- Srivastava, P. and K. Shanker, 2012. *Pluchea lanceolata* (rasayana): Chemical and biological potential of rasayanat herb used in traditional system of medicine. *Fitoterapia*, **83**(8):1371-85.
- Thamizhiniyan Venkatesan, Young-Woong Choi, and Young-Kyoon Kim, 2019. Impact of Different Extraction Solvents on Phenolic Content and Antioxidant Potential of *Pinus densiflora* Bark Extract. *Bio. Med. Res. Int.* **2019**:14.
- Verma, R. K., G. Mishra G, P. Singh, K. K. Jha and R. L. Khosa, 2011. *Alpinia galanga*-an important medicinal plant: a review. *Der. Pharm. Sin.* **2**:142-54.

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STATUS OF COFFEE PRODUCTION IN NAGALAND, INDIA

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ABSTRACT

Nagaland is the sixteenth state of India inaugurated on 1st December 1963. Nagaland at present cultivates two types of coffee i.e., *Coffea Arabica* and *Coffea Robusta*. Nagaland coffee is known for its unique taste and aroma. In recent years, it was observed that coffee cultivation has gained a particularly healthy popularity among farmers from different regions of the state. Thus, a research was conducted to understand the status of coffee production over the years in Nagaland, India in the year 2023. The study was conducted in 6 different blocks as per the availability of requisite respondents (Kohima RD block, Mangkolemba RD block and Ongpangkong (South) RD block, Sanis RD block, Wokha RD block and Niuland RD Block) located in 4 selected districts, namely Kohima, Mokokchung, Wokha and Niuland. A structured interview schedule was prepared to collect primary data from a total of 120 coffee cultivators from 8 villages (Touphema, Nerhema, Khar, Khensa, Lakhuti, Wokha, Hovishe, Ghotovi). It is revealed from the data that majority of the respondents were middle aged and male. Majority acquired education till secondary level, married, had semi-pucca houses, semi- medium sized of land holding with a medium level of income from coffee. Most of the respondents had attended training on coffee cultivation and had active in social life. The study further inferred that, with regard to production trend of coffee cultivation, a significant linear trend of production under coffee over the study period was observed. 99 per cent of the variation under coffee was captured by the considered trend model. From the regression coefficient and figure-1 the area under coffee was expected to increase in the coming years. It was clearly revealed that the area under coffee cultivation had been increasing significantly from 2018 (45 kgs) to 2022(66 kgs).

(Key words: Coffee, area, production, socio-economic, Nagaland)

INTRODUCTION

Coffee is an aromatic beverage that is enjoyed and savoured by many across the globe. It is grown and sourced from all around the world, and there is also a vast array of its kinds available to us all. One such variety is the coffee that originates from the African seeds. These seeds are harvested then roasted, which gives it its unique flavour and aroma, and finely ground to be made ready to brew the beverage for us to enjoy it. Coffee in India is more than an agricultural export product. It is also a social, institutional and cultural fabric of southern states of India, in the heart of rural societies in traditional coffee growing areas. The two most important species of coffee grown in India are arabica (*Coffea arabica*) and Robusta (*Coffea canephora*). Minhas (2023) in his article stated that India's total coffee estimated cultivated area was more than 471 thousand hectares in the fiscal year 2022. "India is among the top 10 coffee-producing countries with about 3% of the global output in 2020" (Bhawan, 2022). Karmakar (2022) identified that, "the total coffee planted area in the Northeast is 4,618.26 ha, with

1,394.21 ha of coffee-bearing area yielding an average annual 150 metric tonnes of clean coffee". Vidya and Kadam (2018) in their study found that the total production of coffee in the year 2017-18 stood at 316000 metric tonnes of which 221000 metric tonnes (69.9%) of Robusta and 95000 metric tonnes (30.1%) of Arabica variety in India.

The coffee production in Nagaland has been increasing over the years and with increase in new growers every year the yield of coffee beans is expected to increase more. Kiho (2020) revealed that, "Nagaland has immense potential for coffee plantation owing to the climatic conditions for both Arabica and Robusta coffee. Nagaland is organic by default which will further enhance the productivity however to enhance the soil health application of manures may be encouraged as per the study done by Ezung *et al.* (2020). They found that, "vermin-compost in greengram not only increased the yield but enhanced the productivity of the system and maintained the sustainability of the soil". It has been estimated that a total area of 10.40.100 hectares (Robusta 3.55,300 and Arabica 6,84,800) is suitable for coffee plantation in Nagaland which is About 62.7% of the state's total geographical area of 16.57.900 ha

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