Screening of chemical composition and non-enzymatic antioxidant properties of the rhizomes of three commonly cultivated gingers (*Zingiber officinale* Roscoe) of Assam, locally known as jati ada, moran ada and nadia ada'.

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ABSTRACT

Ginger (*Zingiber officinale* Roscoe) is a perennial herbaceous plant of the family Zingiberaceae, whose rhizome is generally used as spice, medicine and flavouring agent. The North-Eastern region of India accounts for about 70 % of the total ginger production of the country. The present study was conducted to detect the bioactive chemical compounds present in the rhizomes of three commonly cultivated gingers (*Zingiber officinale Roscoe*) of Assam locally known as Moran ada, Nadia ada and Jati ada by GC-MS analysis and to estimate and compare the amounts of carbohydrates, starch, protein, flavonoids, total phenolics and ascorbic acid present of the rhizomes of these ginger cultivars. The rhizome of moran ada was found to contain the highest amount of flavonoids and the rhizome of nadia ada was found to contain the highest amount of total phenolic compounds. All the samples of ginger rhizomes were extracted with ethanol and the ethanolic extract was analysed through GC-MS for the identification of different chemical compounds. A total of 30, 27 and 34 chemical compounds were identified in the extracts of rhizomes of jati ada, moran ada and nadia ada respectively. Cis-alpha-bergamotene , 2-methyl-5-((R)-6-methylhept-5-en-2-yl) bicyclo- hex-2-ene, 1,3-cyclohexadiene-5-(1,5-dimethyl-4-hexenyl)-2-methyl, Bicyclo-hept-2-ene-2,6-dimethyl-6(4-methyl-3-pentenyl) and Di-epi-alpha-cedrene were the most abundant compounds identified in the extract of Jati ada; Cis-alpha-bergamotene, 2-methyl-5-((R)-6-methylhept-5-en-2- yl) bicyclo-hex-2-ene, 1,3-cyclohexadiene-5-(1,5-dimethyl-4-hexenyl)-2-methyl, Di-epi-alpha-cedrene, Trans-alpha-bergamotene, 1H-3A,7-methanoazulene,2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, and 2-methyl-5-(R)-6methylhept-5-en-2-yl-bicyclo-hexane were the most abundant compounds identified in the extract of moran ada and 2-methyl-5-(R)-6methylhept-5-en-2-yl) bicyclo-hexane, 2-methyl-5-((R)-6-methylhept-5-en-2- yl)bicyclo hex-2-ene, 1,3-cyclohexadiene-5-(1,5-dimethyl-4-hexenyl)-2-methyl, Bicyclo-hept-2-ene-2,6-dimethyl-6(4-methyl-3-pentenyl) and Di-epi-alpha-cedrene were the most abundant compounds identified in the extract of nadia ada.

Keywords: Ginger, flavonoids, total phenolics, bioactive compounds, GC-MS, Di-epi-alpha-cedrene

INTRODUCTION

Plants have been used as source of medicine and food since the pre-historic period (Hassan, 2015). Different plants and their parts such as leaves, stem, rhizome, fruits, seeds, flowers, roots, bark and bulbs are rich in different phytochemicals which have biological properties such as antioxidant activity, antimicrobial effect, stimulation of immune system and anticancer property (Saxena *et al.*, 2013).

Antioxidants or inhibitors of oxidation are the compounds that have the capacity to protect cells from the damages caused by free radicals (Ayoub *et al.*, 2017; Nigam *et al.*, 2014). Antioxidants are generally categorized into two groups: enzymatic antioxidants which include the enzymes such as superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase etc. and non-enzymatic antioxidants which include compounds such as phenolics, flavonoids, carotenoids, vitamins, and minerals (Ayoub *et al.*, 2017; Mehta *et al.*, 2015; Bunaciu *et al.*, 2016).

Free radicals are constantly generated in our body resulting in extensive damage to tissues and biomolecules leading to various disease conditions. So, the medicinal plants with antioxidant properties are employed as an alternative source of medicine to mitigate the disease associated with oxidative stress (Nigam *et al.*, 2014).

Ginger (*Zingiber officinale* Roscoe) is a perennial herbaceous plant of the family Zingiberaceae, whose rhizome is generally used as spice, medicine and flavouring agent (Hazarika *et al.*, 2013). It is propagated through rhizomes and it is considered as an important spice throughout the world (Hazarika *et al.*, 2013).

The rhizomes of ginger are irregular in shape, branched and palmate. Their colour varies from pale yellow to dark brown and are pungent in taste. The rhizome of ginger is actually an underground modification of stem and therefore bears nodes**,** internodes and scaly leaves. The adventitious roots arise from the lower surface of the nodes.

The rhizome of ginger contains many useful phytochemicals, nutrients, minerals and also exhibit antioxidant properties. It is mainly rich in phenolic compounds, terpenes, polysaccharides, organic acids, lipids and raw fibres. Phenolics are one of the largest and most diverse groups of plant active substances that take part in regulation of seed germination, cooperate in plant growth and also take part in defence responses during infection, abiotic stresses and injuries (Myint *et al.*, 2020). The health benefits of ginger are mainly attributed to its phenolic compounds such as gingerols and shogaols (Nishidono *et al.*,2018). The rhizome of ginger generally possesses multiple biological activities including antioxidant, anti-inflammatory, antimicrobial, anticancer, neuroprotective, cardiovascular protective, respiratory protective, anti-obesity, antidiabetic, antinausea and antiemetic activities. Ginger is also commonly used for the treatment of stomach problems (including motion sickness, diarrhoea, irritable bowel syndrome, nausea, loss of appetite, etc.), menstrual pain, osteoarthritis, cough, respiratory problems, headache, bronchitis, etc (Gupta *et al.*, 2021).

India, China, Japan, Indonesia, Australia, Nigeria and West Indies Islands are the major ginger producing regions of the world. The North-Eastern region of India accounts for about 70 % of the total ginger production of the country (Hazarika *et al.*, 2013).

In Assam, ginger is mostly grown in Tinsukia, Dibrugarh, Darrang, Jorhat, Karbi- Anglong, Cachar, Dhemaji, Nagaon, Golaghat, Sibsagar, Barpeta, Kamrup and Sonitpur district (Hazarika *et al.*, 2013; Gohain *et al.*, 2020). The most commonly grown gingers of Assam include Moran ada, Jorhat local, Nadia, Jati ada, Jorhat hard, Bhola ada, Zeng ada and Aizol ada ((Hazarika *et al.*, 2013; Saikia *et al.*, 1992).

MATERIALS AND METHODS

The rhizomes of three commonly cultivated gingers (*Zingiber officinale Roscoe*) of Assam, locally known as Moran, Nadia, and Jati were used were collected from the local growers of Dhemaji district (Assam). The rhizomes of each cultivar of ginger were washed with clean tap water to remove the dirt, dust and other external materials that were attached to the surface of the rhizomes. The outer skin of the rhizomes was removed and washed with distilled water. The rhizomes were cut into small pieces and the pieces were

separately shade dried for 5 days. The dried pieces of ginger were grinded in a mixture-grinder into fine powder. The powder was passed through fine sieve and then stored in separate clean and labelled air tight containers until extraction and phytochemical analysis.

Preliminary qualitative phytochemical screening:

To detect the presence of important phytochemicals viz. alkaloids, flavonoids, phenolics, tannins, saponins, cardiac glycosides, terpenoids, proteins and anthraquinones in the rhizomes of the three gingers selected for the study, the methods described by Mujeeb *et al., (*2014) and Rosenthaler (1930) were followed.

Preparation of sample extract for the estimation of total flavonoids and total phenolics content

10 grams dried rhizome powder of each ginger sample was subjected to Soxhlet extraction by taking ethanol as the solvent. The extract was collected and evaporated by using a rotary evaporator. The dried extract was collected and used for analysis.

Estimation of total flavonoids

The total flavonoids content of ginger samples was determined by the aluminium chloride method. 1 ml of each sample (1mg/ml) was taken and 0.3 ml of 5 % $NaNO_2$ was added to each of the sample and left for 5 minutes. 0.3 ml of 10 % $AlCl_3$ was added to each of the mixture and then left for 5 minutes. 2 ml of 1 M NaOH was then added to all the reaction mixtures and the final volume was adjust to 10 ml with distilled water. The absorbance was measured at 510 nm. Quercetin was taken as reference standard and the results were expressed as milligram flavonoids per gram of dry weight (mg/g DW).

Estimation of total phenolics

The total phenolics content of ginger samples was determined by the folin-ciocalteau method. 0.5 ml of each sample (1mg/ml) was taken and the volume was made upto 1 ml with distilled water. 1.5 ml of 10 % folin-ciocalteau reagent was added and left for 5 minutes. 1.5 ml of 7 % Na_2CO_3 was added to each of the reaction mixture and then allowed to rest at room temperature for 90 minutes. The final volume was made up to 10 ml with distilled water.The absorbance was measured at 750 nm. Gallic acid was taken as reference standard and the results were expressed as milligram total phenolics per gram of dry weight (mg/g DW).

Gas chromatography mass spectrometry (GCMS) analysis

GC-MS analysis of the extracts of the samples were carried out with Perkin Elmer (USA) make GCMS instrument (model: Clarus 680 GC &amp) comprising a liquid auto-sampler. The capillary column used was 'Elite- 5MS' having dimensions- length- 60 m, ID- 0.25 mm and film thickness- 0.25 µm and the stationary phase was 5% diphenyl 95% dimethyl polysiloxane. Helium gas (99.99%) was used as carrier gas (i.e mobile phase) at flow rate of 1 ml/min. An injection volume of 1 µl was employed in splitless mode. Injector temperature was set at 280°C and ion-source temperature at 180°C. The oven temperature was programmed at 60°C (for 1 min), with an increase at the rate 7°C/min to 200°C (hold for 3 min) then again increased at rate of 10.C/min to 300.C (hold for 5 min). The total run time was ~ 39min. Solvent delay was kept for 8 minutes. Mass Spectra was taken in Electron Impact positive (EI+) mode at 70 eV. A solvent delay of 8 min was there for MS scan. Mass range i.e m/z range was 50-600 amu. Interpretation of the peaks appeared in the GC Chromatogram was done by library search of the mass spectrum of corresponding peaks using the database software of National Institute Standard and Technology- 2014 (NIST-2014). The mass spectrums of the unknown components were compared with the spectrum known components of NIST library and the compounds were identified with name, molecular weight and empirical formula.

STATISTICAL ANALYSIS:

ANOVA was performed to obtain the significant differences between the amounts flavonoids and total phenolics of the three ginger samples (jati ada, moran ada and nadia ada) using Least Significance Difference (LSD) at the level of 5 %. Graphs were

created using Microsoft Excel. The average of replicas for each sample was calculated and the results were presented as mean ± standard error.

RESULTS AND DISCUSSION

Preliminary qualitative phytochemical screening

The study has revealed the presence of phenolic compounds, flavonoids, terpenoids, saponins, alkaloids, and proteins in the rhizomes of all the three gingers (*Zingiber officinale* Roscoe) selected for the study. But anthraquinones was detected only in the rhizome of Jati ada. Phytochemicals like cardiac glycosides and tannins were not detected in any of the rhizomes of *Zingiber officinale* Roscoe in this study. The result of the preliminary phytochemical analysis of the rhizomes of *Zingiber officinale* Roscoe are displayed in table 4.1.1. The presence of anthraquinones, tannins, steroids, flavonoids, saponins, phenolics, phytate and oxalate in the rhizome of *Zingiber officinale* Roscoe was previously reported by Kela *et al*., (2023). Thakor *et al.*, (2023) reported the presence of flavonoids, steroids, saponins and alkaloids in the rhizome of *Zingiber officinale* Roscoe, but carbohydrates and tannins were not reported.

Table 1: The results of qualitative phytochemical analysis of 3 different gingers (*Zingiber officinale* Roscoe):

Sl. No	Phytochemicals	Tests	Observations		
			Jati Ada	Moran Ada	Nadia Ada
	Flavonoids	Ammonia Test	+	+	+
2	Terpenoids	Salkowski Test	+	+	+
3	Tannins	Braymer's Test	-	-	-
4	Saponins	Foam Test/ Frothing Test	+	+	+
5	Cardiac glycosides	Keller-Kiliani Test	-	-	-
6	Alkaloids	Dragendorff's Test	+	+	+
7	Anthraquinones	Borntrager's Test	+	-	-
8	Phenolics	Lead-acetate Test	+	+	+
9	Proteins	Millon's Test	+	+	+

Note: + indicates positive and - indicates

Flavonoids content

Quantitative estimation of flavonoid content was carried out by aluminium chloride method and absorbance was taken at 510 nm.

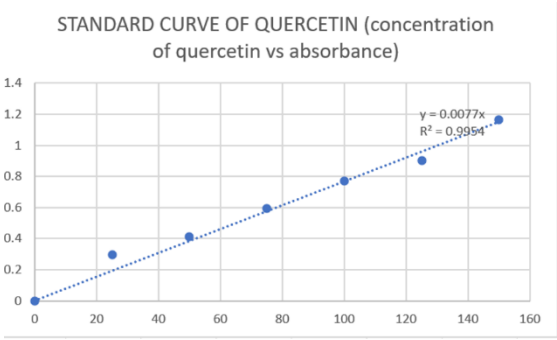

Figure 1: Standard curve for quercetin. Here, x-axis shows the concentration of working stock of quercetin and y-axis shows the absorbance at 510 nm.

Table 2: The amounts of flavonoids present in the dried powder of jati ada, moran ada and nadia ada:

Sl. No	Ginger Sample	Total flavonoids (mg/g)
1	Jati Ada	35.77 ± 0.29
2	Moran Ada	47.8 ± 0.21
3	Nadia ada	17.53 ± 0.15

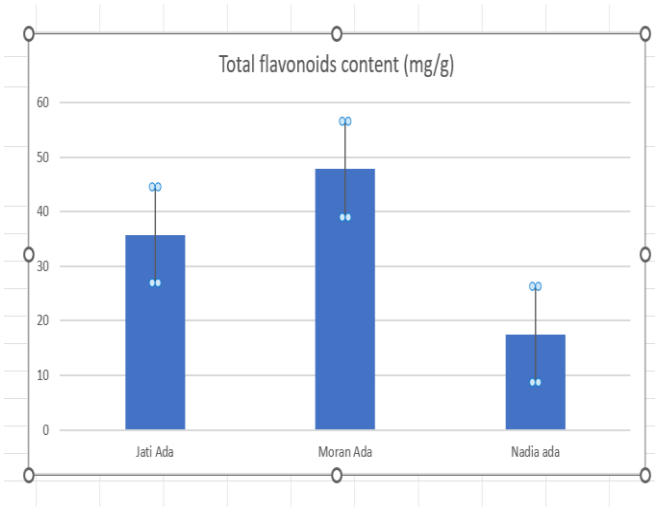

Figure 2: Comparison of flavonoids content present in Jati Ada, Moran Ada and Nadia Ada.

Flavonoids are a group of structurally diverse secondary metabolites in plants that play important role in regulating plant development, pigmentation and biotic and abiotic stress tolerance. The flavonoids content was found to be the highest in Moran ada (47.8 mg/g dry weight) followed by Jati ada (35.7 mg/g dry weight) and Nadia ada (17.5 mg/g dry weight) in this study. A similar study was performed previously by Dhanik *et al.*, (2017) on five accessions of *Zingiber officinale* Roscoe and reported that the flavonoids content was higher in the Bana variety (38.87 mg/g dry weight) than the other varieties viz. Kapkot (30.05 mg/g dry weight), Roorkee (28.3 mg/g dry weight), Chamoli (24.92 mg/g dry weight) and Takula (21.03 mg/g dry weight). A significant difference was observed using ANOVa analysis in the flavonoids content of rhizome of jati ada, moran ada and nadia ada.

Total phenolics content

Quantitative estimation of total phenolics content was carried out by folin ciocalteau method. Absorbance was taken at 750 nm.

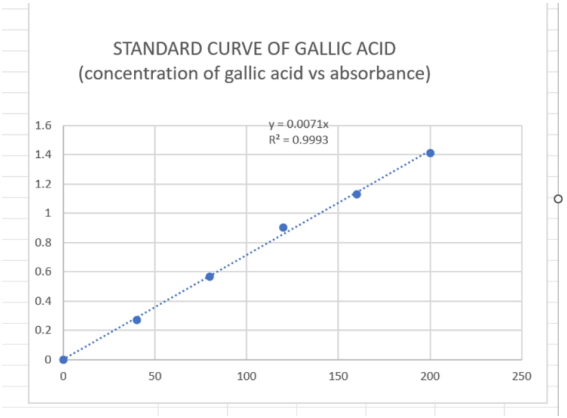

Figure 3: Standard curve for gallic acid. Here, x-axis shows the concentration of working stock of gallic acid and y-axis shows the absorbance at 750 nm.

Table 3: The amounts of total phenolics present in the dried powder of jati ada, moran ada and nadia ada:

Sl. No	Ginger sample	Total phenolics (mg/g)
1	Jati Ada	54.93 ± 0.27
2	Moran Ada	74.63 ± 0.13
3	Nadia ada	102.26 ± 0.69

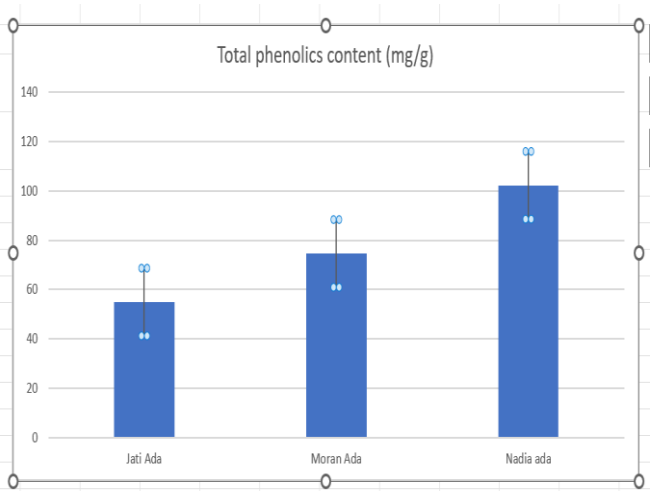

Figure 4: Comparison of total phenolics content present in Jati Ada, Moran Ada and Nadia Ada.

Phenolics are one of the largest and most diverse groups of plant active substances that take part in regulation of seed germination, cooperate in plant growth and also take part in defence responses during infection, abiotic stresses and injuries. The total phenolics content was found to be highest in Nadia ada (102.26 mg/g dry weight) followed by Moran ada (74.63 mg/g) and Jati ada (54.93 mg/g dry weight) in this study. Dhanik *et al*., (2017) also performed a study on five accessions of *Zingiber officinale* Roscoe and reported that the total phenolics content was higher in the Bana variety (46.24 mg/g dry weight) than the other varieties viz. Kapkot (38.55 mg/g dry weight), Roorkee (34.85 mg/g dry weight), Chamoli (33.03 mg/g dry weight) and Takula (29.88 mg/g dry weight). A significant difference was observed using ANOVa analysis in the flavonoids content of rhizome of jati ada, moran ada and nadia ada.

GC-MS analysis

The gas chromatographic spectrums for Jati ada, Moran ada and Nadia ada are presented in figure 5, 6 and 7 respectively. Ten most prominent peaks of each extract were selected for GCMS library search. The retention time, peak area percentage and molecular weight and biological activities of the chemical compounds are shown in table 4, 5 and 6 for Jati ada, Moran ada and Nadia ada respectively. Some compounds were found to be more abundant than the others. The most abundant compounds of present in Jati ada, Moran ada and Nadia ada are presented in table 7. These results showed similarities with those obtained by Chinonye *et al*., (2018) for the rhizome of *Zingiber officinale* Roscoe grown in Eastern part of Nigeria, Momoh *et al*., (2022) for aqueous extract of rhizome of *Zingiber officinale* Roscoe and Madhavan *et al*., (2021) for ethanolic extract of rhizome of *Zingiber officinale* Roscoe.

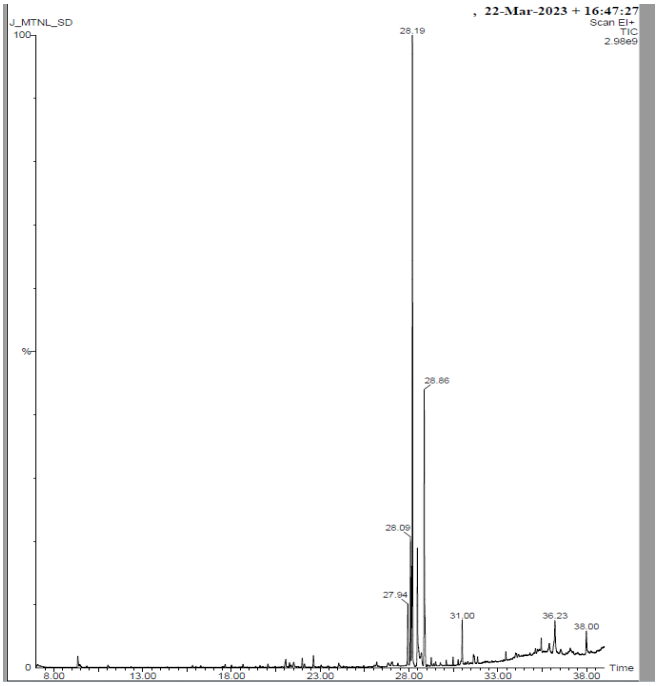

Figure 5. Gas chromatographic spectrum for the extract of Jati ada.

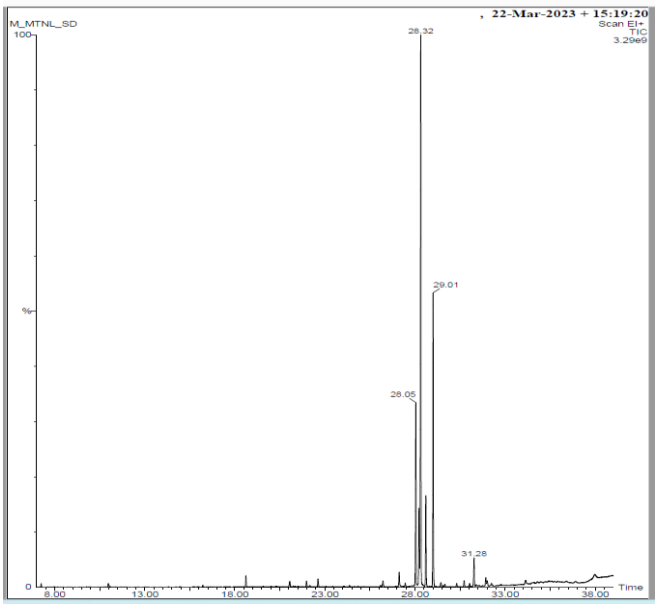

Figure 6. Gas chromatographic spectrum for the extract of Moran ada.

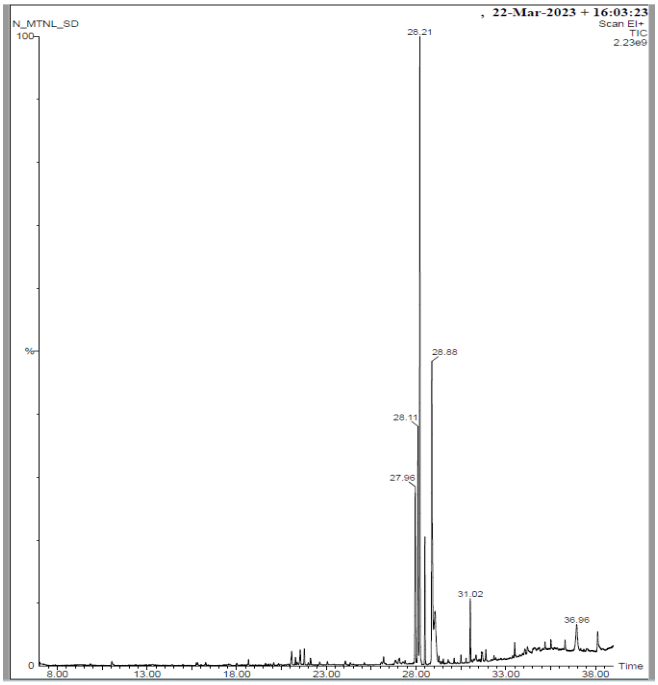

Figure 7. Gas chromatographic spectrum for the extract of Nadia ada.

Table 4: Chemical compounds identified from GC-MS analysis of ethanolic extract of rhizome of Jati Ada

Sl. No	Name of compound	Retention time	Peak area %	Molecular weight	Biological activity	Reference
1	Benzene-1-(1,5-dimethyl-4-hexenyl)-4-methyl	27.937	2.983	202	Anti-microbial, anti-inflammatory	Shareef *et al*., 2016
2	Benzene-1-methyl-4-(1,2,2-trimethylcyclopentenyl)	27.937	2.983	202	Anti-microbial, cardioprotective, anti-oxidant	Sharma *et al*., 2017
3	2-methyl-6-(p-tolyl)-hept-2-en-1-ol	27.937	2.983	218	Anti-inflammatory, anti-helminthic, tonic	Karthick *et al*., 2019; Kelany *et al*., 2020
4	Cycloisolongifol-5-ol	27.937	2.938	220	Anti-bacterial	Naidoo *et al*., 2014
5	Cis-alpha-bergamotene / trans-alpha-bergamotene	28.192	27.402	204	Anti-oxidant, anti-inflammatory	Bayala *et al*., 2014
6	Bicyclo-hept-2-ene-2,6-dimethyl-6(4-methyl-3-pentenyl)	28.192	27.402	204	Anti-bacterial, anti-fungal	Abiodun *et al*., 2021
7	Di-epi-alpha-cedrene	28.192	27.402	204	Anti-microbial anti-inflammatory	Dosoky *et al*., 2018
8	2-Butanone-4-(4-hydroxy-3-methoxyphenyl)	31.003	2.106	194	Anti-oxidant	Kabuto *et al*., 2005
9	4-(1-Hydroxyallyl)-2-methoxyphenol	31.003	2.106	180	Anti-oxidant	Jisha *et al*., 2021
10	Caryophyllene	28.862	12.951	204	Anti-cancer, anti-	Dahham *et al*.,

					oxidant, anti-microbial	2015
11	Germacrene D	28.862	12.951	204	Anti-fungal, anti-bacterial	Noge *et al*., 2009
12	Chalcone-2,3,4-trihydroxy-4-methoxy	36.225	3.733	286	Anti-inflammatory, anti-oxidant, anti-anti-viral, anti-cancer, anti-tumor	Tielas *et al*., 2016
13	€-Labda-8-(17)-12-diene-15,15-dial	36.225	3.733	302	Glucosidase inhibitory activity	Awin *et al*., 2016
14	Methyl-7,10,13,16-docosatetraenoate	35.905	1.997	346	Cardio-protective	Balamurugan *et al*., 2017
15	Gingerol	35.905	1.997	294	Anti-cancer, anti-inflammatory, anti-oxidant	Wang *et al*., 2014
16	5.8,11,14-eicosatetraenoic acid-ethyl ester-(all-z)	35.905	1.997	332	Anti-fungal, anti-bacterial, anti-tumor	Damhougy *et* al., 2017; Agoramoorthy*et al.*, 2006
17	2,6,10-Dodecatrien-1-ol-3,7,11-trimethyl	37.996	1.667	222	Anti-tumor, analgesic, anti-bacterial	Devi *et al*., 2015
18	Alpha-Farnesene	28.087	5.47	204	Anti-oxidant, Anti-microbial, anti-fungal	Celik *et al*., 2014
19	Beta-bisabolene	28.472	7.798	204	Anti-oxidant	Kazemi *et al*., 2014
20	Methyl stearidonate	28.472	7.798	290	Anti-oxidant, anti-microbial	Hady *et al*., 2018
21	1-(4-hydroxy-3-methoxyphenyl)-dec-4-en-3-one OR Shogaol	28.712	1.688	276	antioxidant, anti-inflammatory, anticancer, antiemetic	Kou *et al*., 2018
22	2-methyl-5-((R)-6-methylhept-5-en-2-yl)bicyclo hex-2-ene	28.192	27.402	204	Not reported	NA
23	Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene	28.862	12.951	204	Not reported	NA
24	4-methylene-1-(R)-6methylhept-5-en-2-yl) bicyclo[3.1.0]hexane	28.862	12.951	204	Not reported	NA
25	2,4,4-trimethyl-3-hydroxymethyl-5A-(3-methyl-but-2-enyl)-cyclohexene	36.225	3.733	222	Not reported	NA

26	1H-Cyclopropazulene, decahydro-1,1,4,7-tetramethyl-[1AR-(1A-alpha)]	36.225	3.733	286	Not reported	NA
27	Trans-alpha-bergamotene	28.087	5.47	204	Not reported	NA
28	2,6,10-Dodecatrien-1-ol-3,7,11-trimethyl-9-(phenylsulfonyl	37.996	1.667	362	Not reported	NA
29	Bicyclo-octane, 8-methylene	28.472	7.798	122	Not reported	NA
30	Bicyclo-non-1-ene	28.472	7.798	122	Not reported	NA

Table 5: Chemical compounds identified from GC-MS analysis of ethanolic extract of rhizome of Moran Ada:

Sl. No	Name of compound	Retention time	Peak area %	Molecular weight	Biological activities	Reference
1	Benzene-1-(1,5-dimethyl-4-hexenyl)-4-methyl	28.047	11.874	202	Anti-microbial, anti-inflammatory	Shareef *et al*., 2016
2	Benzene-1-methyl-4-(1,2,2-trimethylcyclopentenyl)	28.047	11.874	202	Anti-microbial, cardioprotective, anti-oxidant	Sharma *et al*., 2017
3	2-methyl-6-(p-tolyl)-hept-2-en-1-ol	28.047	11.874	218	Anti-inflammatory, anti-helminthic, tonic	Karthick *et al*., 2019; Kelany *et al*., 2020
4	Eudesma-2,4,11-triene	28.047	11.874	202	Antimicrobial, anti-spasmodic	Erdem *et al*., 2017
5	Cycloisolongifol-5-ol	28.047	11.874	220	Anti-bacterial	Naidoo *et al*., 2014
6	1,3-cyclohexadiene-5-(1,5-dimethyl-4-hexenyl)-2-methyl	28.322	35.137	204	Anti-oxidant, anti-inflammatory, anti-nociceptive	Shareef *et al*., 2016
7	Cis-alpha-bergamotene	28.322	35.137	204	Anti-oxidant, anti-inflammatory	Bayala *et al*., 2014
8	Trans-alpha-bergamotene	28.322	35.137	204	Anti-oxidant, anti-inflammatory	Bayala *et al*., 2014
9	Di-epi-alpha-cedrene	28.322	35.137	204	Anti-microbial, anti-inflammatory	Dosoky *et al*., 2018

10	1-Methylene-2B-hydroxymethyl-3,3-dimethyl-4B-(-3-methylbut-2-enyl)-cy	28.607	16.87	222	Anti-microbial, anti-inflammatory, anti-hyperlipidemic	Murugan *et al*., 2016
11	Beta-bisabolene	28.607	16.87	122	Anti-oxidant	Kazemi *et al*., 2014
12	Aromandendrene	28.607	16.87	204	Anti-bacterial	Mulyaningsih *et al*., 2011
13	Alpha-Farnesene	28.227	11.58	204	Anti-oxidant, Anti-microbial, anti-fungal	Celik *et al*., 2014
14	Methyl-stearidonate	28.227	11.58	290	Anti-oxidant, anti-microbial	Hady *et al*., 2018
15	Caryophyllene	29.007	17.263	204	Anti-cancer, anti-oxidant, anti-microbial	Dahham *et al.*, 2015
16	Germacrene D	29.007	17.263	204	Anti-fungal, anti-bacterial	Noge *et al*., 2009
17	Cedrene	29.007	17.263	204	Anti-microbial anti-inflammatory	Dosoky *et al*., 2018
18	2-methyl-5-((R)-6-methylhept-5-en-2-yl)bicyclo-hex-2-ene	28.322	35.137	204	Not reported	NA
19	2-methyl-5-(R)-6methylhept-5-en-2-yl) bicyclo-hexane	28.322	35.137	204	Not reported	NA
20	1H-3A,7-methanoazulene,2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl	28.322	35.137	204	Not reported	NA
21	Bicyclo-octane, 8-methylene	28.607	16.87	122	Not reported	NA
22	Bicyclo-non-1-ene	28.607	16.87	122	Not reported	NA
23	Arsenous acid, tris(trimethylsilyl)ester	37.981	1.699	342	Not reported	NA
24	Cyclotrisiloxane,hexamethyl-	37.981	1.699	222	Not reported	NA

25	4-methylene-1-(R)-6methylhept-5-en-2-yl) bicyclohexane	29.007	17.263	204	Not reported	NA
26	Benzene butanal-gamma-4-dimethyl	28.047	11.874	176	Not reported	NA
27	Tris(tert-butyldimethylsilyloxy)arsane	37.981	1.699	468	Not reported	NA

Table 6: Chemical compounds identified from GC-MS analysis of ethanolic extract of rhizome of Nadia Ada

Sl. No	Name of compound	Retention time	Peak area %	Molecular weight	Biological activities	Reference
1	Alpha-Farnesene	28.107	7.586	204	Anti-oxidant, Anti-microbial, anti-fungal	Celik *et al*., 2014
2	Cis-alpha-bergamotene	28.107	7.586	204	Anti-oxidant, anti-inflammatory	Bayala *et al*., 2014
3	Trans-alpha-bergamotene	28.107	7.586	204	Anti-oxidant, anti-inflammatory	Bayala *et al*., 2014
4	Methyl stearidonate	28.107	7.586	290	Cardio-protective	Balamurugan *et al*., 2017
5	Benzene-1-(1,5-dimethyl-4-hexenyl)-4-methyl	27.957	6.1	202	Anti-microbial, anti-inflammatory	Shareef *et al*., 2016
6	Benzene-1-methyl-4-(1,2,2-trimethylcyclopentenyl)	27.957	6.1	202	Anti-microbial, cardioprotective, anti-oxidant	Sharma *et al*., 2017

7	1,3-cyclohexadiene-5-(1,5-dimethyl-4-hexenyl)-2-methyl-	28.212	20.469	204	Anti-oxidant, anti-inflammatory, anti-nociceptive	Shareef *et al*., 2016
8	Di-epi-alpha-cedrene	28.212	20.469	204	Anti-microbial, anti-inflammatory	Dosoky *et al*., 2018
9	Bicyclo-hept-2-ene-2,6-dimethyl-6(4-methyl-3-pentenyl)	28.212	20.469	204	Anti-bacterial, anti-fungal	Abiodun *et al*., 2021
10	Beta-bisabolene	28.492	3.966	122	Anti-oxidant	Kazemi *et al*., 2014
11	Aromandendrene	28.492	3.966	204	Anti-bacterial	Mulyaningsih *et al*., 2011
12	Caryophyllene	28.882	14.951	204	Anti-cancer, anti-oxidant, anti-microbial	Dahham *et al*., 2015
13	Cedrene	28.882	14.951	204	Anti-microbial anti-inflammatory	Dosoky *et al*., 2018
14	1-(4-hydroxy-3-methoxyphenyl)-dec-4-en-3-one OR Shogaol	36.955	3.633	276	antioxidant, anti-inflammatory, anticancer, antiemetic	Kou *et al*., 2018
15	Gingerol	36.955	3.633	294	Anti-cancer, anti-inflammatory, anti-oxidant	Wang *et al*., 2014
16	Chalcone-2,3,4-trihydroxy-4-methoxy	29.062	7.182	286	Anti-inflammatory, anti-oxidant, anti-anti-viral, anti-cancer, anti-tumor	Tielas *et al*., 2016

17	2-Butanone-4-(4-hydroxy-3-methoxyphenyl)	31.023	2.093	194	Anti-oxidant	Kabuto *et al*., 2005
18	Panaxydol	34.209	1.472	260	Anti-microbial, anti-fungal, anti-inflammatory, neuroprotective, antimutagenic, antiproliferative activity	Knispel *et al*., 2013
19	1-Propyl-3-(propen-1-yl) adamantane	34.209	1.472	218	Anti-inflammatory, anti-oxidant	Pitushkin *et al*., 2023
20	18-Norabietane	38.126	1.727	262	Anti-viral, cytotoxic	Lin *et al*., 2010
21	Lupeol	38.126	1.727	426	Anti-cancer, anti-protozoal, and anti-inflammatory	Gallo *et al*., 2009
22	1-Methylene-2B-hydroxymethyl-3,3-dimethyl-4B-(3-methylbut-2-enyl)0-cy	28.107	7.586	222	Not reported	NA
23	2-methyl-5-((R)-6-methylhept-5-en-2- yl)bicyclo-hex-2-ene	28.212	20.469	204	Not reported	NA
24	2-methyl-5-(R)-6methylhept-5-en-2-yl) bicyclo-hexane	28.212	20.469	204	Not reported	NA
25	Bicyclo-octane, 8-methylene	28.492	3.966	122	Not reported	NA
26	Bicyclo-non-1-ene	28.492	3.966	122	Not reported	NA
27	Cyclohexene,3-(1,5-dimethyl-4hexenyl)-6-methylene	28.882	14.951	204	Not reported	NA

28	(E)-beta- famesene	28.882	14.951	204	Not reported	NA
29	Tricyclo-octane-3-carboxamide,N-(4-ethoxyphenyl)	29.062	7.182	271	Not reported	NA
30	9-Oxabicyclo- non-6-en-2-one,3-bromo-, endo	34.209	1.472	216	Not reported	NA
31	3,4-Nonadien-6-yne, 5-ethyl-3-methyl	34.209	1.472	162	Not reported	NA
32	(2R,3R,4AR,5S,8AS)-2-hydroxy-4A, 5-dimethyl-3-(prop-1-en-2-yl) octahydro	38.126	1.727	236	Not reported	NA
33	(1R,2S,8R,8AR)-8-hydroxy-1-(2-hydroxyethyl)-1,2,5,5-tetramethyl-trans-	38.126	1.727	254	Not reported	NA
34	1-Naphthalenepropanol, alpha-ethyldecahydro-5-(hydroxymethyl)-	38.126	1.727	308	Not reported	NA

Table 7: Chemical compounds found in abundance in the ethanolic extracts of Jati Ada, Moran Ada and Nadia Ada (as detected by GC-MS analysis):

Chemical compounds in Jati Ada	Peak area %	Chemical compounds in Moran Ada	Peak area %	Chemical compounds in Nadia	Peak area %
Cis-alpha-bergamotene	27.402	Cis-alpha-bergamotene	35.137	2-methyl-5-(R)-6methylhept-5-en-2-yl) bicyclo-hexane	20.469

2-methyl-5-((R)-6-methylhept-5-en-2-yl) bicyclo- hex-2-ene	27.402	2-methyl-5-((R)-6-methylhept-5-en-2- yl) bicyclo-hex-2-ene	35.137	2-methyl-5-((R)-6-methylhept-5-en-2-yl)bicyclo [3.1.0] hex-2-ene	20.469
1,3-cyclohexadiene-5-(1,5-dimethyl-4-hexenyl)-2-methyl	27.402	1,3-cyclohexadiene-5-(1,5-dimethyl-4-hexenyl)-2-methyl	35.137	1,3-cyclohexadiene-5-(1,5-dimethyl-4-hexenyl)-2-methyl	20.469
Bicyclo-hept-2-ene-2,6-dimethyl-6(4-methyl-3-pentenyl)	27.402	Cycloisolongifol-5-ol	11.874	Bicyclo-hept-2-ene-2,6-dimethyl-6(4-methyl-3-pentenyl)	20.469
Di-epi-alpha-cedrene	27.402	Di-epi-alpha-cedrene	35.137	Di-epi-alpha-cedrene	20.469
Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene	12.951	Benzene-1-(1,5-dimethyl-4-hexenyl)-4-methyl	11.874	Cyclohexene,3-(1,5-dimethyl-4hexenyl)-6-methylene	14.951
(1S,5S)-4-methylene-1-(R)-6methylhept-5-en-2-yl) bicyclohexane	12.951	(1S,5S)-4-methylene-1-(R)-6methylhept-5-en-2-yl) bicyclohexane	17.263	Cedrene	14.951
Caryophyllene	12.951	Caryophyllene	17.263	Caryophyllene	14.951

Germacrene D	12.951	Germacrene D	17.263	(E) -beta- famesene	14.951
		Trans-alpha-bergamotene	35.17		
		Benzene butanal-gamma-4-dimethyl	11.874		
		Eudesma-2,4,11-triene	11.874		
		2-methyl-6-(p-tolyl)-hept-2-en-1-ol	11.874		
		Cedrene	17.26		
		1H-3A,7-methanoazulene,2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl	35.137		
		2-methyl-5-(R)-6methylhept-5-en-2-yl) bicyclo[3.1.0]hexane	35.173		
		Benzene-1-methyl-4-(1,2,2-trimethylcyclopentenyl)	11.874		

CONCLUSION

The present study is a comparative study of the biochemical characteristics of the rhizomes of three commonly cultivated gingers (*Zingiber officinale* Roscoe) of Assam, locally known as jati ada, moran ada and nadia ada. The preliminary phytochemical analysis has revealed the presence of phenolic compounds, flavonoids, terpenoids, saponins, alkaloids and proteins in the rhizomes of all the gingers (*Zingiber officinale* Roscoe) selected for the study.

The flavonoids content has been found to be the highest in Moran ada followed by Jati ada and Nadia ada and the total phenolics content has been found to be highest in Nadia ada followed by Moran ada and Jati ada. In the rhizomes of all the samples of ginger (*Zingiber officinale* Roscoe) selected for the study, appreciable amounts of flavonoids and phenolic compounds have been estimated. This indicates that jati ada, moran ada and nadia ada are good sources of flavonoids and phenolic compounds.

A total of 30, 27 and 34 chemical compounds were identified in the extracts of rhizomes of jati ada, moran ada and nadia ada respectively. Cis-alpha-bergamotene , 2-methyl-5-((R)-6-methylhept-5-en-2-yl) bicyclo- hex-2-ene, 1,3-cyclohexadiene-5-(1,5-dimethyl-4-hexenyl)-2-methyl, Bicyclo-hept-2-ene-2,6-dimethyl-6(4-methyl-3-pentenyl) and Di-epi-alpha-cedrene were the most abundant compounds identified in the extract of Jati ada; Cis-alpha-bergamotene, 2-methyl-5-((R)-6-methylhept-5-en-2- yl) bicyclo-

hex-2-ene, 1,3-cyclohexadiene-5-(1,5-dimethyl-4-hexenyl)-2-methyl, Di-epi-alpha-cedrene, Trans-alpha-bergamotene, 1H-3A,7-methanoazulene,2,3,4,7,8,8a-hexahydro-3,6,8,8-tetramethyl-, and 2-methyl-5-(R)-6methylhept-5-en-2-yl-bicyclo-hexane were the most abundant compounds identified in the extract of moran ada and 2-methyl-5-(R)-6methylhept-5-en-2-yl) bicyclo-hexane, 2-methyl-5-((R)-6-methylhept-5-en-2- yl)bicyclo hex-2-ene, 1,3-cyclohexadiene-5-(1,5-dimethyl-4-hexenyl)-2-methyl, Bicyclo-hept-2-ene-2,6-dimethyl-6(4-methyl-3-pentenyl) and Di-epi-alpha-cedrene were the most abundant compounds identified in the extract of nadia ada.

Further studies in this field may reveal the lead metabolites present in the rhizomes of Jati ada, moran ada and nadia ada and their structural characteristics. This may help in establishing the basis of their biological functions.

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