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Original Research Article

Isolation and Characterization of Bioactive compound and Its Application from Traditional Dye Plant *Basella rubra Linn*

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Abstract: *Basella rubra Linn* belongs to the family Basellaceae. The people of Manipur have been using plants as indigenous colorants before introducing synthetic colorants in Manipur. In addition traditional natural dyes plants were extinct their exploitation due to unavailability of indigenous knowledge on extracting and dying techniques. Natural dyes are environmental friendly although synthetic dyes rapidly replaced the traditional natural dyes because of availability, cost less, a vast range of new colors and imparted better properties to the dyed materials. Present circumstances, the use of natural dyes has fall-off due to lack of documentation. The traditionally used dye plants *Achyranthus aspera* and *Basella rubra linn* of Manipur were investigated for edible herbal pigment and vegetable dyes. *Achyranthus aspera* is used as natural mordant were as chemical analysis was not done. The chemical analyses of the bioactive compounds present in selected plant Basella rubra were characterized by NMR: (^{1}H NMR, ^{13}C NMR, and DEPT-135 NMR), TLC, UV-Vis, IR, Column Chromatography, LC-MS, profiles were recorded for evaluating the products and identification of the compound. Compound 1,2,3,4 and 5 were isolated by column chromatography from *Basella rubra linn.* Only compound 4 (3, 5-Bis-aminomethyl-cyclohexanone) and compound **5** (3-(2-Amino-ethyl)-2-aminomethyl-4-ethyl-cyclopentylamine) were identified and not reported before. Purple, red, green and brown pigments which are food grade are separated and characterized.

Key words: *Achyranthus aspera, Basella rubra*, Meitei Community, Manipur, Traditional dye.

Introduction

Traditional dyes are used in Manipur from time immemorial. Manipur is located in biodiversity hotspot the plant bio-diversity is very rich. Usually the plant species found in Manipur have medicinal of economic value. It is surrounded by the states of Nagaland to the north, Assam to the west and Mizoram to the southwest and the Myanmar to the south-east. Its important economy is agriculture and forestry. Its area is 8,621 square miles (22,327sq.km). According to 2011 census, Manipur has a population of 2,835,794. The state lies at a latitude of 23^{0}83′ N- 25^{0}68′ N and a longitude of 93^{0}03′ E- 94^{0}78′ E. It is mainly covered by the mountains. Manipur is one of the richest plant bio-diversity among the northeastern state of India. Traditional medicine, cultural heritage, dance, garments of unique design for Manipur which are described detailed in the book archaic Manipuri manuscripts or *Meitei* texts "*puya khunungi leechat shajat"* in Meitei language which is Tibeto-Burman language and the predominant language and lingua franca of the state of Manipur in northeastern

India (Sanjenbam *et al*., 2020; Lisam *e*t al*.,* 2011; Sujata W *et al*., 2018; Gourachandra *et al.,* 2005; Madhav *et al.,* 2006). In the early period of time, Manipur has affinity of handlooms and handicrafts. Manipuris had practice dyeing by using different kinds of plants leaves, flowers, roots and barks. They used natural dyes obtained from natural products. But, nowadays most of the people are using synthetic dyes. Kum plants produced indigo. Here in, the extraction of the indigo dyes from fermentation of *S. flaccidifolius Nees. Tghsi* is also a traditional dye of Manipur. It is used by traditional fermentation method in a pot (Latonjamwarjeet Singh *et al*., 2011; Manoranjan *et al*., 2005; Akimpou *et al.,* 2005). *Basella rubra linn* belongs to the family *Basellaceae*. In Manipur *Basella rubra linn* is commonly known as "*Urok-sumban*". Extraction was carried out with the one method in the room temperature in the process of crushing. The part used for making dye is fruits. *Basella rudra* is also known as cyclone spinach (Lomnitski *et al*., 2003) spinach leaves contained several active components including flavonoids (Adhikari *et al*., 2012). *Basella rudra* is a succulent, branched, smooth, twining herbaceous vine. The stem becomes purplish in color when it is matured. Fruits are fleshy and greenish pink in color. When it is matured the color turns purple. The fruits contained anthocyanin was reported (Werner *et al.,* 1993). *Basella rubra* is used as dye in the early periods of time in Manipur. Its leaves and stems are also used as medicinal plants (Deshpande *et al.,* 2003; Samala *et al.,* 2001). Natural products play a dominant role in the development of novel drug which leads to the treatment and prevention of diseases (Ambati *et al.,* 2010; Goyal *et al.,* 2011; Hudaib *et al.,* 2008; Dalirsani *et al.,* 2011; Sanda *et al.,* 2011). Khamenchappa is one of the most beautiful and precious pheijom (dhoti not much similarly) made by kabrang silk. It has lots of printed designs and the colors are scheme of deep purple, deep red and chocolate brown. The design is said to have been adopted from the sight of Lord Pakhangba, the serpent God worship by ethics of Meiteis. There are reports that the seven patterns of the Khamenchappa represent the seven clans of the Meiteis. After the 2ndworld war, Khamenchappa was being used as innaphi (women's shawl), khwangchet (additional clothes used by women around their waist) and turban as well. During the period of Maharaj Churachand and Bodhachandra was a king of Manipur, India from 1941 to 1955 A.D, the Khamenchappa pheijom were presented to the tribal chiefs of hills area (Sana *et al*., 2010; Lunalisa *et al.,* 2008). *Solanum incidum linn* is the major ingredients for making khamenchappa. Ripen fruits are the main parts in making khamenchappa. Khamu plants are also used in the traditional medicinal aspects. It is used to cure the high blood pressure. It is commonly known as Khamu in Manipur. It is a kind of herb. From khamu plant we need to use fruits. A purple dye was prepared from this plant. *Achyranthus aspera* is the secondary ingredients for making khamenchappa. It acts as alkaline mordant. The traditional way of curing anemia is by using the plant leaves. To make the dye fast in color, a mordant usually an alkaline mordant derived from the plant, *Achyranthus aspera* (local name, khuchumpere) could be added. The khamu-dye thus obtained is usually used for dyeing turban. Tannins were used by Perkin as mordant to increase the uptake of cationic dyes (KimHeeJe *et al.,* 2013; Kikim *et al.,* 2013). Without mordant, most natural dyes have poor to moderate light fastness while synthetic dyes represent the full range of light fastness properties from poor to excellent (Siva *et al.,* 2007). The fastness properties of a mordant dye depends on the mordant and mordanting method because different metal or tannin dye complexes are formed, which may differ in their stability to washing, rubbing or light and also because the metal may have positive or negative catalytic effect on the photochemical degradation of the dye (Lee *et l.,* 2013). Natural dyes produce very uncommon, soothing and soft shades as compared to synthetic dyes. Synthetic dyes are widely available at low price and a wide variety of colors; these dyes however produce skin allergy, toxic wastes and other harmfulness to human body (FDA 2011). Production of synthetic dyes is dependent of petrochemical source, and some of the synthetic dyes contained toxic/carcinogenic amines which are not eco-friendly. (Pierce *et al.,* 1993; PI, 2008; Agarwal *et al.,* 2009) Herein we aim to formulate environment friendly dyes and also our valuables

traditional clothes *Khamenchappa*'s fabric is going to lose because of synthetic dyes so we have to conserve our ancient traditional dye plants and dying techniques. *Basella rubra linn* is used for dying of cloths as a natural dye. We already knew that synthetic food colorants and synthetic dyes are harmful to our health as well as environment. Compounds were isolated and separated from *Basella rubra linn* by using the technique of NMR, Mass spectrometry, IR, TLC, UV, LC-MS, Column chromatography for evaluate the structure of the compounds contained in this plant.

Materials and methods

Materials: Ripen fruits of *Basella rubra* was collected during the month of November and December. *Basella rubra* fruits were collected from Longjam Leikai, Keishamthong, Imphal, Manipur, 795001. Latitude: 24°79′ 25′′ N Longitude: 93°92′ 97′′ E on date: 9/11/18 and *Solanum incidum linn* were collected from Langol, Imphal, Manipur, 795001. Latitude: 93°91′ 27′′ N, Longitude: 24°83′ 20′′ E on date 12/11/18. *Achyranthus aspera* leaves were collected from Houbam Marak Chingtham Leikai, Imphal, Manipur, 795001. Longitude: 24°77′ 58′′ N and Latitude: 93°92′ 42′′ E on date: 15/11/19. All the chemicals and solvents were of analytical grade.

Fig. 1. *Basella Rubra*

Extraction: The *Basella rubra* fresh fruits (10.10g and 10.30g) were crushed with the help of mortar and pestle for making paste. The pastes were extracted with 100% methanol for 24 hours Dye solution was filtered by using filter paper. Then the sample liquid was dried using rotary. *Achyranthus aspera* leaves were collected and dried in room temperature. The dried leaves were crushed with grinder and it can be used directly as natural mordent.

Fig. 2. *Achyranthus Aspera*

Separation and Isolation

Open Column Chromatography

Open column is used for chromatography. A solid packing material is filled as the stationary phase. The solid material can vary depending on the mode of separation such as absorption, partition etc. In this open column chromatography, silica gel (SiO2) were used as a packing materials and the mobile phase is a solvent (petroleum ether), also the solvent should be relatively less polar than the compounds to be separated. The method of packing is dry packing.

Sample loading method

Slurry was prepared and loaded on column y was Detection can be done by TLC technique with the fraction of Time. Silica gel (60-120 mesh) was used for open column chromatography. The column elution was started with 100% petroleum ether and the polarity was increased gradually. First yellow colored band was seen and collected for TLC reading to check the presence of compound5. Elution was done with increase of polarity for further characterization then compound4 (greenish purple), compound3 (brown),

compound2 (red) and compound1 (purple) were collected (Asila *et al.*, 2008; Vivek *et al.*, 2016).

Experimental methods

The IR spectra were taken on a Shimadzu FT-IR Spectrophotometer. The mass spectra were recorded in a MALDI-TOF mass spectrometer. The ^{1}H, ^{13}C and DEPT-135 NMR spectra were recorded on an FT-NMR Bruker Avance (200MHz) in deuterated methanol-d4 at ambient temperature. Silica gel (60-120 mesh) was used for open–column chromatography and silica gel-G for thin-layer chromatography. UV spectra were measured on a UV spectrophotometer. Separation were carried out with open column chromatography with the mass analysis were measured by LC-MS (Agilent Technologies 120 Infinity). Fractions were monitored with TLC (Li *et al.*, 2014; Ettre *et al.*, 2001; Gardner *et al.*, 1968; Lipid *et al.*, 2006; Elhbieta *et al.,* 2016)

Thin-Layer Chromatography (TLC):

Technique : One dimension, ascending
Adsorbent : Silica gel
Layer thickness : 0.2 mm
Distance : 2 by 4 cm
Temperature : Laboratory temperature (28-35°C)
Detection : UV-Visible light

A yellow point was seen under visible light with 20%TLC and it was collected as compound 5. The column chromatography is further carried out with 95% petroleum ether and 5% ethyl acetate and taking 20% TLC a blue dot was obtained and it is collected as compound 4 (greenish purple). The method is further carried out with 80% ethyl acetate and 20% petroleum ether and taking 20%TLC another blue spot is obtained and it is collected as compound 3 (brown). The method is further carried out with 80% ethyl acetate and 20% petroleum ether and taking 20% TLC another blue spot is obtained and collected as compound 2 (red). The method is further carried out with 80% ethyl acetate and 20% petroleum ether and taking 20% TLC another blue spot is obtained and collected as compound 1 (purple).

Chromatographic analysis

Thin layer chromatography (TLC) is applicable to identify different color components in natural dyes to be applied on textiles. Dyes detected were vegetable dyes.

Spectroscopy

The compound obtained was further characterized using the following methods:-

Ultraviolet (UV) Absorption Spectra: The UV spectra were taken on Perkin Elmer UV-Vis Spectrometer lambda 35.

Infrared (IR) Absorption Spectra: The IR spectra were taken on a Shimadzu FT-IR Spectrophotometer (Weinmann *et al.,* 1998; Romer *et al.,* 1998; Deinum *et al.,* 1999; Pearse *et al.,* 1985; Dacie *et al.*, 1991; Silveira *et al.*, 2002).

Nuclear Magnetic Resonance (NMR) Spectra: The ^{1}H NMR, ^{13}C NMR spectra were recorded on an FT-NMR Bruker Avance (200MHz) in deuterated methanol-d4 at ambient temperature (Jane *et al.,* 2006).

Liquid chromatography- Mass Spectrometry (LC-MS): LC-MS (Agilent Technologies 1260 infinity) were taken of the methanol extract of these followings (Inez *et al.*, 2019; Hu *et al.,* 2007).

A) Fruits of *Basella rubra linn.*

B) Compound1, 2, 3, 4, 5.

UV-visible Spectroscophic Study

UV-visible spectra of any colorants/dye show its own peaks at predominating wave length, indicating main color. For natural dyes, the spectra specially indicate different peaks for mixed colorants available in their extract in both UV and visible region. UV-visible spectroscopic studies of different natural dyes were carried out by using solvents as methanol for extraction. Some of the studied dyes are *Basella rubra linn* (Mohamed *et al.,* 2013).

LC-MS Condition

Infinity Column: Particle size 10M
Guard column: RT LIcrocrat
Column temperature: 40°C
Sample cooler temperature: 4°C
Flow Rate: 0.6mL/min
Injection mode: 5µL
Gradient:
At 0 - 1 min, 90% of A & 10% of B
At 1 – 2 min, 60% of A & 40% of B

At 2 – 5 min, only 100% of B

Wavelength of Chromatography: 250nm

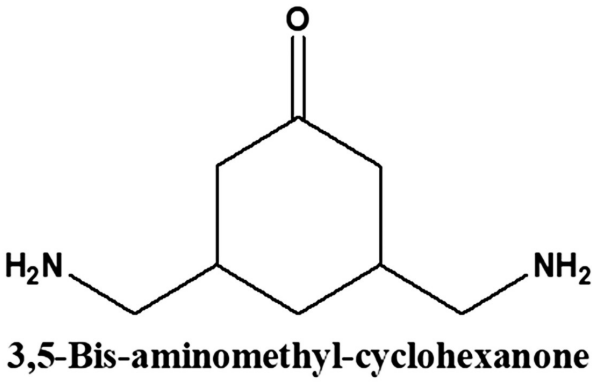
Ethanol extracted food and fabric colorant

The *Basella rubra* fresh fruits were collected in a beaker, and wash with ethanol. Then, the fruits were crush with the help of mortar pestle and are collected in a conical flask while the pastes were mixed with ethanol. The solution was filtered with the help of filter paper and liquid were collected separately in a round bottom flask. The paste was dried using vacuum distillation.

The traditional method of dyeing using khamu plants, the ripen fruits of *Basella rubra* are made into pieces or powdered to which water is added (about 1 liter of water into half kg of the sample) in the earthen pot or glass vessel. The solution is stirred with the help of a wooden spoon or stick. The solution is kept for about six hours and then filtered using a coarse cloth. The filtrate could be used as such or it is concentrated.

The alkaline mordant is obtained as ash by burning the plant. The ash solution of the mordant and the solution of khamu are mixed in equal amounts and the mixture is boiled till the desired color is obtained.

Results



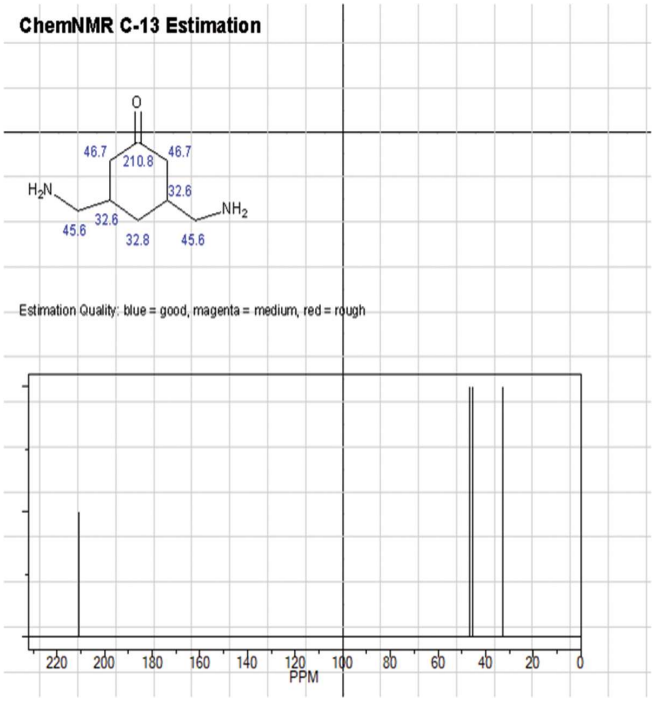


Fig. 3. Compound 4: 3, 5- Bis- amino-methyl- cyclohe xanone

Protocol of the C-13 NMR Prediction:

Node	Shift	Base + Inc.	Comment (ppm rel. to TMS)
CH2	46.7	-2.3	cyclohexane like
		29.3	1 alpha +C(=O)-C from aliphatic
		9.1	1 alpha +C from aliphatic
		18.8	2 beta -C from aliphatic
		-2.5	1 gamma +C from aliphatic
		-5.1	1 gamma +N from aliphatic
		0.3	1 delta +C from aliphatic
		-0.9	general corrections
CH	32.6	-2.3	cyclohexane like
		27.3	3 alpha +C from aliphatic
		0.5	1 beta -C(=O)-C from aliphatic
		9.4	1 beta -C from aliphatic
		11.3	1 beta -N from aliphatic
		-2.5	1 gamma +C from aliphatic
		0.0	1 delta +N from aliphatic
		-11.1	general corrections
CH2	32.8	-2.3	cyclohexane like
		18.2	2 alpha +C from aliphatic
		37.6	4 beta -C from aliphatic
		-2.7	1 gamma +C=O from aliphatic
		-10.2	2 gamma +N from aliphatic
		-7.8	general corrections
CH	32.6	-2.3	cyclohexane like
		27.3	3 alpha +C from aliphatic
		0.5	1 beta -C(=O)-C from aliphatic
		9.4	1 beta -C from aliphatic
		11.3	1 beta -N from aliphatic
		-2.5	1 gamma +C from aliphatic
		0.0	1 delta +N from aliphatic
		-11.1	general corrections
CH2	46.7	-2.3	cyclohexane like
		29.3	1 alpha +C(=O)-C from aliphatic
		9.1	1 alpha +C from aliphatic
		18.8	2 beta -C from aliphatic
		-2.5	1 gamma +C from aliphatic
		-5.1	1 gamma +N from aliphatic
		0.3	1 delta +C from aliphatic
		-0.9	general corrections
C	210.8	193.0	1-carbonyl
		15.2	2 -C-C
		2.6	general corrections
CH2	45.6	-2.3	aliphatic
		9.1	1 alpha +C
		28.3	1 alpha +N
		18.8	2 beta -C
		-2.7	1 gamma +C(=O)-C
		-2.5	1 gamma +C
		0.3	1 delta +C
		-3.4	general corrections
CH2	45.6	-2.3	aliphatic
		9.1	1 alpha +C
		28.3	1 alpha +N
		18.8	2 beta -C
		-2.7	1 gamma +C(=O)-C
		-2.5	1 gamma +C
		0.3	1 delta +C
		-3.4	general corrections

Fig. 4. ^{13}C NMR- ∂46.7, ∂32.6, ∂32.8, ∂32.6, ∂46.7, ∂45.6, ∂45.6 (∂210.8)

C-13 NMR prediction of Compound 4:

CH_2 node = ∂45.1, CH node = ∂32.6, CH_2 node= ∂35.6, CH node= ∂32.6, CH_2 node= ∂45.1, CH_2 node= ∂46.5, CH_2 node= ∂46.5, C node= ∂208.2 (Carbonyl)

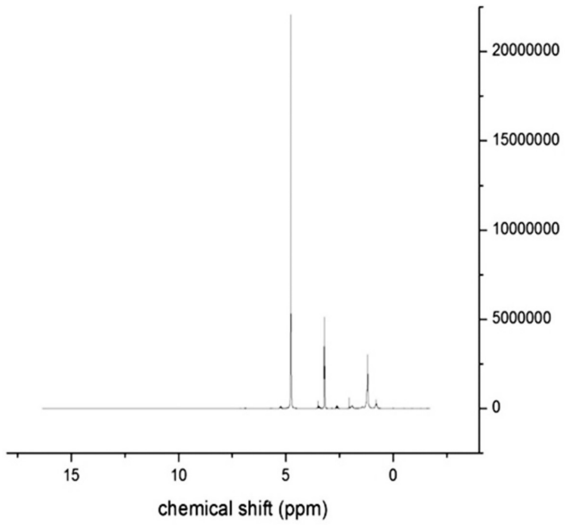


Fig. 5. ^{1}HNMR compound 4: ∂2.1, ∂3.8, ∂4.9

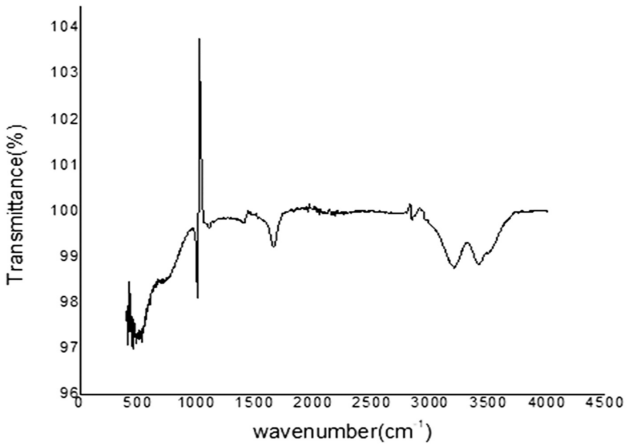


Fig. 6. IR compound 4 (1000 cm^{-1},1720 cm-,^{1}3300 cm^{-1},3550 cm^{-1})

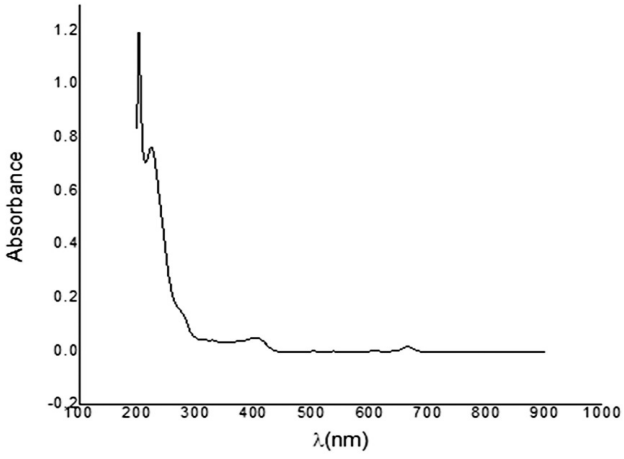


Fig. 7. UV (260nm)

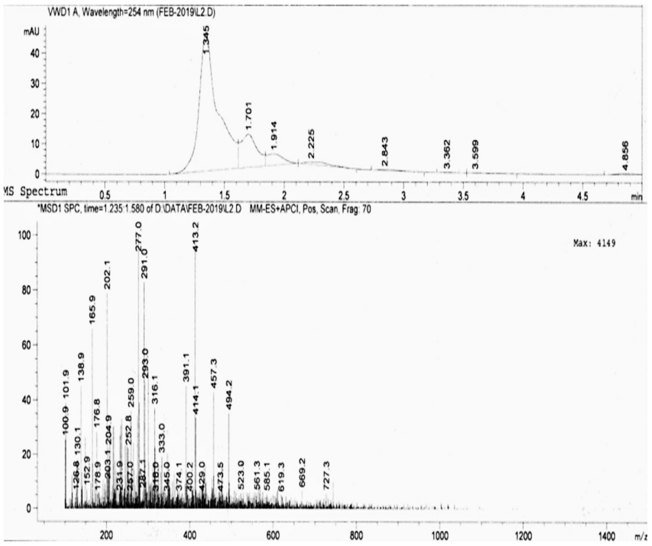


Fig. 8. LC-MS Compound 4 (171, 172 [M+1]+, 195- [M+23]+)

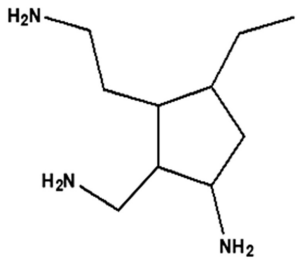


Fig. 9. Structure of Comp. 5 C^{13} NMR (3-(2-Amino-ethyl)-2-aminomethyl-4-ethyl-cyclopentylamine)

Protocol of the C-13 NMR Prediction:

Node	Shift	Base + Inc.	Comment (ppm rel. to TMS)
CH	29.1	-11.4	cyclopentane
		27.3	3 alpha -C from aliphatic
		47.0	5 beta -C from aliphatic
		-2.5	1 gamma -C from aliphatic
		-15.3	3 gamma -N from aliphatic
		-16.0	steric corrections from aliphatic
CH	46.0	-11.4	cyclopentane
		27.3	3 alpha -C from aliphatic
		28.2	3 beta -C from aliphatic
		22.6	2 beta -N from aliphatic
		-5.0	2 gamma -C from aliphatic
		0.3	1 delta -C from aliphatic
		0.0	1 delta -N from aliphatic
		-16.0	steric corrections from aliphatic
CH	44.8	-11.4	cyclopentane
		18.2	2 alpha -C from aliphatic
		28.3	1 alpha -N from aliphatic
		28.2	3 beta -C from aliphatic
		-5.0	2 gamma -C from aliphatic
		-5.1	1 gamma -N from aliphatic
		0.6	2 delta -C from aliphatic
		-9.0	steric corrections from aliphatic
CH2	34.1	-11.4	cyclopentane
		18.2	2 alpha -C from aliphatic
		28.2	3 beta -C from aliphatic
		11.3	1 beta -N from aliphatic
		-7.5	3 gamma -C from aliphatic
		0.3	1 delta -C from aliphatic
		0.0	1 delta -N from aliphatic
		-5.0	steric corrections from aliphatic
CH	32.4	-11.4	cyclopentane
		27.3	3 alpha -C from aliphatic
		37.6	4 beta -C from aliphatic
		-5.0	2 gamma -C from aliphatic
		-5.1	1 gamma -N from aliphatic
		0.0	2 delta -N from aliphatic
		-11.0	steric corrections from aliphatic
CH2	25.3	-2.3	aliphatic
		18.2	2 alpha -C
		18.8	2 beta -C
		-7.5	3 gamma -C
		0.6	2 delta -C
		0.0	1 delta -N
		-2.5	steric corrections
CH3	12.1	-2.3	aliphatic
		9.1	1 alpha -C
		9.4	1 beta -C
		-5.0	2 gamma -C
		0.9	3 delta -C
CH2	39.4	-2.3	aliphatic
		9.1	1 alpha -C
		28.3	1 alpha -N
		18.8	2 beta -C
		-7.5	3 gamma -C
		-5.1	1 gamma -N
		0.6	2 delta -C
		-2.5	steric corrections
CH2	33.8	-2.3	aliphatic
		18.2	2 alpha -C
		18.8	2 beta -C
		11.3	1 beta -N
		-10.0	4 gamma -C
		0.3	1 delta -C
		0.0	2 delta -N
		-2.5	steric corrections
CH2	40.7	-2.3	aliphatic
		9.1	1 alpha -C
		28.3	1 alpha -N
		9.4	1 beta -C
		-5.0	2 gamma -C
		1.2	4 delta -C

Fig. 10. ^{13}CNMR compound 5 (∂38.5, ∂37.4, ∂46.0, ∂44.8, ∂38.5, ∂38.7, ∂32.7, ∂40.5, ∂26.1, ∂12.2 (∂210))

C-13 NMR prediction of Compound 5:

CH node = ∂29.1, CH node = ∂46.0, CH node = ∂44.8, CH_2 node = ∂34.1, CH node = ∂32.4, CH_2 node = ∂25.3, CH_3 node = ∂12.1, CH_2 node = ∂39.4, CH_2 node = ∂33.8, CH_2 node = ∂40.7

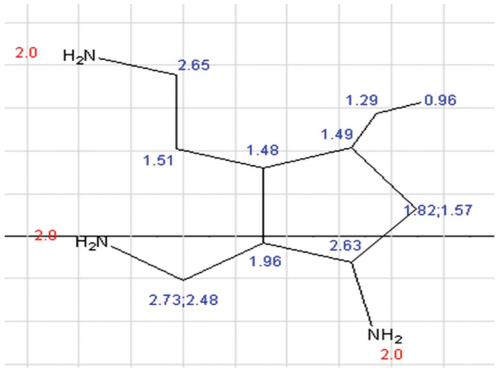
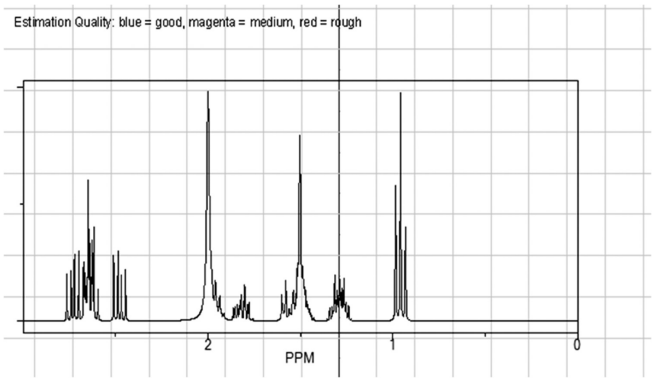


Fig. 11. `HNMR prediction of Compound 5:



Protocol of the H-1 NMR Prediction:			
Node	Shift	Base + Inc.	Comment (ppm rel. to TMS)
NH2	2.0	2.00	amine
CH2	2.73;2.48	1.37	methylene
		1.32	1 alpha -N
		-0.08	2 beta -C
CH	1.48	1.51	cyclopentane
		-0.01	1 beta -C from methine
		-0.01	1 beta -C from methine
		-0.01	1 beta -C from methine
CH	1.96	1.51	cyclopentane
		0.23	1 beta -N from methine
		-0.01	1 beta -C from methine
		0.23	1 beta -N from methine
CH	2.63	1.51	cyclopentane
		1.13	1 alpha -N from methine
		-0.01	1 beta -C from methine
CH	1.49	1.51	cyclopentane
		-0.01	1 beta -C from methine
		-0.01	1 beta -C from methine
CH2	1.82;1.57	1.51	cyclopentane
		0.22	1 beta -N from methylene
		-0.04	1 beta -C from methylene
NH2	2.0	2.00	amine
CH2	1.51	1.37	methylene
		-0.08	2 beta -C
		0.22	1 beta -N
CH2	2.65	1.37	methylene
		1.32	1 alpha -N
		-0.04	1 beta -C
NH2	2.0	2.00	amine
CH2	1.29	1.37	methylene
		0.00	1 alpha -C
		-0.08	2 beta -C
CH3	0.96	0.86	methyl
		0.10	1 beta -C-R

Fig. 12. ^{1}HNMR compound 5 (∂2.0, ∂2.48, ∂1.48, ∂1.96, ∂2.63, ∂1.49, ∂1.57, ∂1.51, ∂2.65, ∂2.0, ∂1.29, ∂0.96)

CH_2 node = ∂2.0, CH_2 node = ∂2.73; ∂2.48, CH node = ∂1.48, CH node = ∂1.96, CH node = ∂2.63, CH node = ∂1.49, CH_2 node = ∂1.57; ∂1.82, NH_2 node = ∂2.0, CH_2 node = ∂1.51, CH_2 node = ∂2.65, NH_2 node = ∂2.0, CH_2 node = ∂1.29, CH_3 node = ∂0.96

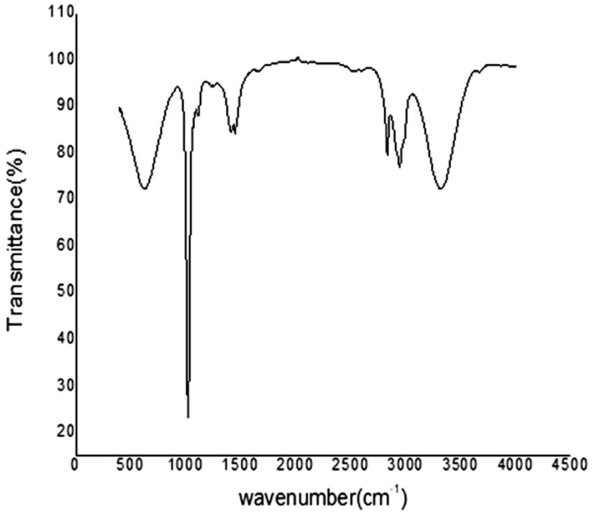


Fig. 13. IR compound 5 (700 cm^{-1}, 1000 cm^{-1}, 1500 cm^{-1}, 3200 cm^{-1}, 3550 cm^{-1})

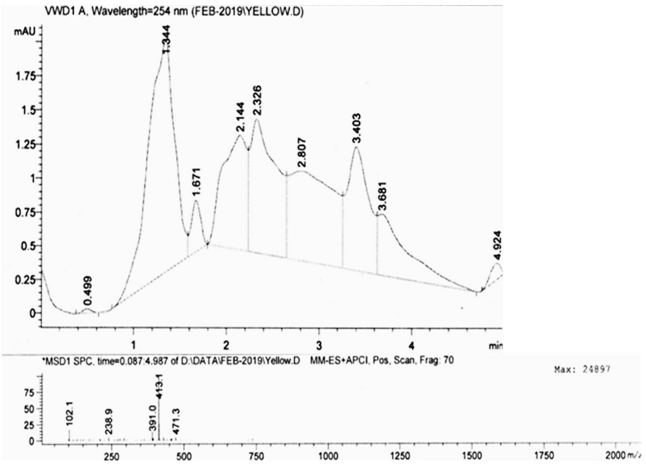


Fig. 14. LC-MS compound 5 (157,178.9- [M+23 -1])

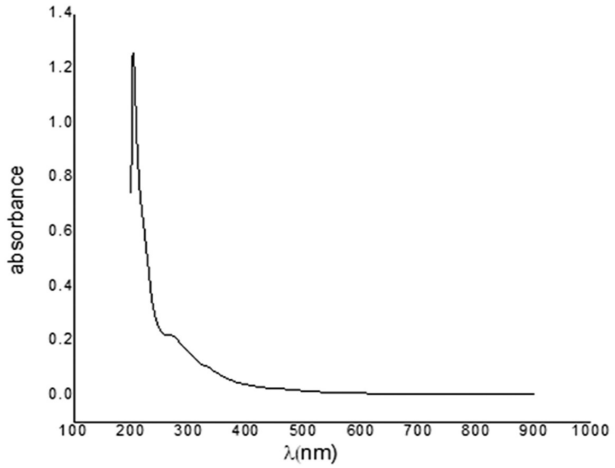


Fig. 15. UV compound 5 (260nm)

From the above data of C-13NMR spectra, ^{1}HNMR spectra, IR spectra, LC-MS and UV spectrum data we identified compound 4 and compound 5 as 3,5-Bis-aminomethyl-cyclohexanone and 3-(2-Amino-ethyl)-2-aminomethyl-4-ethyl-4-ethyl-cyclopentylamine respectively. The compound found in the data has not been reported before.

Extracted dyes were tried in foods and cloths.

Fig. 16. Ice-cream

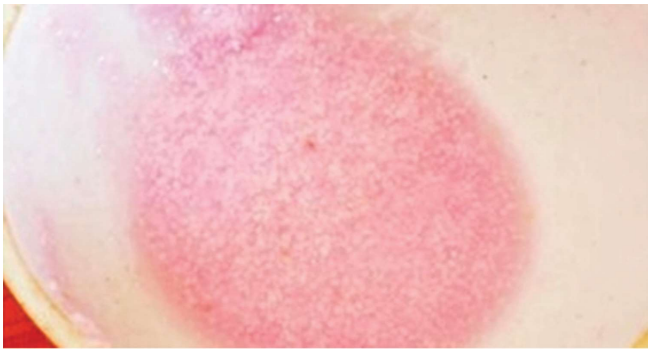


Fig. 17. Halwa

Fig. 18. Cotton

Fig. 19. Silk

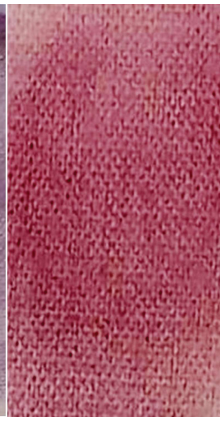


Fig. 19. Nylon

Discussion

Chemicals are synthesized for making synthetic dyes. Some of the synthetic dyes contain metals too (Koren *et al.,* 1995; Irobechni *et al.,* 2009; Rosenberg *et al.,* 2008). Data suggest that the natural dyes are superior to artificial dyes in terms of health related issues. This synthetic dye contained chemical compounds like chlorine, toluene, lead, chromium, copper, arsenic, sodium, benzene and mercury. These chemicals are dangerous to the human body as well as environment (FDA 2010; FDA, FAC, 2011). In spite of the better performance of synthetic dyes, recently the use of natural dye on textile materials and food colorant has been attracting more and more. Artificial food dyes can cause a number of potential health problems, most notably certain types of cancer and attention-deficit disorder and hyperactivity in children. Some individuals can have allergic reactions to particular food colors. Artificial food colors have been found to cause damage to DNA or genotoxicity, bladder tumors and other forms of cancer were linked to certain artificial coloring. Artificial food coloring increased hyperactive in sensitive children and infertility. Herbal dyes produced can be a safe substitute. Moreover, the traditional indigenous natural dyes were extinct of their exploitation due to lack of documentation about our ancient indigenous traditional dying techniques. Nowadays, people demanding more synthetic dyes gained immense attention and replaced natural dyes due to a wide variety of reasons such as low-cost production, availability, easy application to used, a vast range of new color, stability of the

color and cheap. After a few generation will not know about our old culture of traditional dying techniques. Most natural dyes are on the brink of extinction as artificial dyes are being used as substitutes. So, we have to focus on conserving our valuables traditional knowledge of natural dye plants and dye making techniques correlated with the indigenous people.

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