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# CHROMATOGRAPHIC ANALYSIS OF *Blumea lanceolaria* (Roxb.) Druce LEAF EXTRACT: A TRADITIONAL HERBAL DRUG OF NORTH-EAST INDIA

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## ABSTRACT

**The North-Eastern region of India is hotspot of many medicinal herbs. *Blumea lanceolaria* from family Asteraceae is a traditional medicinal herb of North East India. The present study was aimed to screen the phyto-chemical constituents and identify bioactive compounds in crude extracts of *B. lanceolaria* using gas chromatography-mass spectroscopy. The extracts were obtained by Soxhlet extraction in three solvents (methanol, n-hexane, and chloroform). The identification and characterization of bioactive compounds in the extracts was performed using GC-MS. Qualitative phytochemical screening was done for the presence of flavonoids, phenols, phytosterols, tannins and alkaloids. The study showed that the phytochemicals were higher in methanolic extract than chloroform- and n-hexane-extracts. In GC-MS analysis a total of five compounds were detected in each sample. Chloroacetic acid, tetradecyl ester was found in methanol (18.15%) as well as in n-hexane extracts (18.83%) in diverse quantities. Likewise, compounds including 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol and Z,z-6,28-heptatriactontadien-2-one were found in methanol and chloroform extracts. 1-Heptacosanol was identified in both n-hexane and chloroform extracts. The study reveals that crude extracts of *B. lanceolaria* is a potential source of naturally occurring bioactive compounds, so may be used in traditional medicines.**

**Keywords:** Bioactive, ***B****lumea lanceolaria* extracts, medicinal herb, phytochemicals, solvents

## INTRODUCTION

Medicinal plants since the time immemorial have been used for curing human diseases owing to the presence of phytochemicals and bioactive compounds (Nostro *et al.*, 2000). Medicinal plants synthesize secondary metabolites with unique and complex structures. Around 85-90% of world's population consume traditional herbal medicines (WHO, 2002). *Blumea lanceolaria* (Roxb.) Druce locally known as "Jwglaori" in Assam is a tropical perennial herb that belongs to the family Asteraceae and is found in different parts of North-East India. It has serrate longated leaves, racemose cyme inflorescence and head, yellow coloured flowers and is mostly available during April to September. The plant is utilized in the preparation of traditional medicines. The edible leaves possess aromatic smell but are spicy to taste. In rural Assam, the juice of *B. lanceolaria* leaves is used for the treatment of cough (Saikia *et al.,* 2017). The use of *B. lanceolaria* in the treatment of ulcer, cancer and hemorrhoids has also been reported (Sawmliana, 2003; Chawng-kunga, 2005). In Mizoram of North-East India, a decoction of its leaves is taken orally for the treatment of stomach ulcers, dysentery and wounds (Rai and Lalramnghinglova, 2010) owing to the

presence of analgesic, antipyretic and anti-inflammatory properties in *B. lanceolaria*. Despite the known medicinal utility of *B. lanceolaria* in traditional medicines, the information on its qualitative account of phytochemicals and identity of its bioactive compounds is inadequate. In this paper, main focus is on phytochemical screening and bioactive compound identification in *B. lanceolaria*.

Plants have great ability to produce different phytochemicals called secondary metabolites. The preliminary phytochemical screening tests are useful to detect bioactive compounds. The development of drugs starts with the identification of active principles from natural sources. Phytochemicals such as flavonoids, tannins, saponins, alkaloids, and terpenoids have several biological properties, including antioxidant, anti-inflammatory, anti-diarrhea, anti-ulcer, and anticancer activities (Starlin *et al.*, 2019). Phytochemicals study on *B. lacera* by Ahmed *et al.* (2014) showed the presence of carbohydrates, flavonoids, trace amounts of alkaloids, some glucosides, terpenoids and steroids in polar fraction. Pappachen *et al.* (2019) reported the presence of carbohydrate, glycosides, tannin, phenol and flavonoid from *B. mollis* leaves and stem root. Phytochemical studies on the chemicals constituent of *B. laciniata* resulted in the isolation of phenolics compounds *viz*., 3,4-dihydroxybenzoic acid, caffeic acid, vanillic acid, p-coumaric acid, syringic acid, rosmarinic acid, trans-cinnamic acid, catechin, catechol, epicatechin, rutin, quercitin, myricetin and kaempferol which show a wide range of potential bioactivities including antidiabetic, anti-obesity, neuroprotective, and anti-melanogenic along with excess oxidation-reduction activity (Swaraz *et al.*, 2021). Eighteen polyphenolic compounds (17 flavonoids and 1 phenylethanone) were quantified from the leaves of *B. balsamifera* (Qin *et al.*, 2020) while 3,3′,5,7-tetrahydroxy-4′-methoxyflavanone was the most abundant constituent. Phytochemical studies of *B. balsamifera* branches and leaves has led to the isolation of 10 secondary metabolites (Cuong *et al.*, 2021). Among these, three new compounds, namely balsamiferine K, balsamiferosides A & B, and blumeaene J were identified and these compounds exhibited moderate cytotoxicity against LNCaP and SK-Mel2 cancer cell lines. The present study was aimed to identify and characterize the phytochemicals and bioactive compounds present in three different extracts of *B. lanceolaria*.

## MATERIALS AND METHODS

All chemicals and reagents used were of analytical grade purchased from Merck and Co (Germany).

### *Sample collection*

Leaf samples of *B. lanceolaria* were collected from the natural habitat in Besorgaon, Kokrajhar district of Assam (India) during May, 2020. The identity of plant was authenticated and taxonomically confirmed by the expert in the Department of Botany, Bodoland University, Kokrajhar. The shade dried leaves were pulverized using a clean and sterile electric grinder. Dried powder (150 g) was obtained and stored in airtight containers until further use.

### *Extract preparation*

Ground leaf sample (35 g) was taken in a Soxhlet apparatus and extracted using 350 mL of three different solvents *viz*., n-hexane, chloroform and methanol. The extraction temperature was maintained at 60ºC for 24 h. All the solvents used for extraction were evaporated in a rotary vacuum evaporator until a crude viscous semi-solid extract was obtained. Yield percentage of crude extract was calculated by standard formula of Alebiosu and Yusuf (2015).

$$\text{Yield (\%)} = a/b \times 100$$

where, a = dry weight of extract obtained; and b = initial weight of powdered material.

The extraction yield was calculated for each extract.

### *Phytochemical screening*

To detect phytochemicals in *B. lanceolaria*, nine qualitative phytochemical tests were performed using dry crude extract. The screening was carried out for the existence of alkaloids, phenols,

flavonoids and tannins by using the method of Trease and Evans (1983) and Harborne (1998). For the detection of alkaloids, 1mL 2N HCl was added to 2 mL extract and an aqueous layer formed was decanted. Then 0.5 mL Mayer's reagent was added to the decant. The formation of white colour precipitate indicated the presence of alkaloids. For tannins detection, 1 mL of 10% lead acetate was added to 2 mL extract. The presence of tannins was indicated by the development of white precipitate. For saponins detection, 2 mL extract was added to 1 mL distilled water and shaken vigorously for 30 sec. The formation of foamy layer indicated the presence of saponins. For detection of phenols, 2 drops of 5% $FeCl_3$ solution was added to 2 mL extract. The intense colour developed indicated the presence of phenols. For flavonoids, a few drops of 20% NaOH were added to 2 mL extract and the appearance of yellow colour indicated the presence of flavanoids. For phytosterols determination, to 2 mL *B. lanceolaria* extract 2 mL each of chloroform, acetic anhydride and conc. $H_2SO_4$ were added and the development of translucent green colour indicated the presence of phytosterols. For steroids, 2 mL chloroform was added to 2 mL extract, followed by 2 mL conc. $H_2SO_4$. A layer of red colour produced at the bottom of test tube indicated the presence of steroids. For terpenoids, 2 mL chloroform was added to 2 mL *B. lanceolaria* extract and evaporated to dryness. To this, 2 mL conc. $H_2SO_4$ was added and a layer of yellowish green colour formed at the lower portion of solution indicated the presence of terpenoides. For oils and fats detection, 2 mg extract was pressed between two filter papers; oil stain on the filter paper indicated the presence of fixed oil.

***Gas chromatography-mass spectroscopy (GC-MS) study***

GC-MS analysis of *B. lanceolaria* extracts was performed by using Perkin Elmer (USA) Clarus 680 GC and Clarus 600C MS comprising of a liquid autosampler. The software used in the system was Turbo Mass Ver.6.1.2. For analysis, 2 µL of each plant extract was injected in GC-MS system through auto-sampler in split-less mode. The temperature of injector was fixed at 280°C and helium (99.99% purity) was used as carrier gas (i.e. mobile phase) fixed at flow rate of 1mL $min^{-1}$. The column oven temperature was programmed at 60°C for 1 min, with an increase @ 7ºC $min^{-1}$ to 200ºC (hold for 3 min) then again increased @ 10°C $min^{-1}$ to 300°C (held for 5 min). The total run time of GC-MS system was 39 min. Solvent delay was kept for 8 min. Mass spectra was observed in Electron Impact positive (EI+) mode at 70eV. A solvent delay of 8 min was there for MS scan. Mass range i.e. m/z range was 50-600 amu (Hema *et al.,* 2010).

***Compound identification***

The mass spectra of compounds obtained from GC-MS analysis were recognized by relating their peak, peak area, peak height, retention time, molecular weight and mass spectrum patterns to that of known compounds described by National Institute of Standard and Technology (NIST) (2014) library database (Hema *et al.,* 2010).

## RESULTS AND DISCUSSION

***Extract properties***

The n-hexane extract was yellow orange in colour and semi-solid in appearance. The chloroform extract was sticky, brown in colour and semi-solid in appearance while methanol extract was dark green non-sticky and dry solid. The results showed maximum percentage of yield in methanol extract (2.97%), followed by n-hexane (2.20%) and chloroform (2.17%). However, this was low as compared to reported yield (Saikia *et al.* 2017). The variation in the extraction yield depends on the nature of solvents used and the chemical nature of samples (Terblanche *et al.,* 2017). Results highlighted that methanol is the most efficient solvent for phytochemical extraction from *B. lanceolaria* in comparison to n-hexane and chloroform.

**Table 1: Preliminary phytochemical screening of *Blumea lanceolaria* leaf extracts**

| Phytochemical constituents | Tests performed | Methanol extract | Chloroform extract | n-hexane extract |
|---|---|---|---|---|
| Alkaloids | Mayer's test | + | - | - |
| Tannins | Lead acetate test | + | - | - |
| Saponins | Foam test | + | - | - |
| Phenolics | Ferric chloride test | + | + | + |
| Flavonoids | Alkaline reagent test | + | + | - |
| Phytosterols | Liebermann-Burchard's test | + | + | + |
| Steroids | Salkowski's test | - | + | + |
| Terpenoids | Salkowski's test | - | + | + |
| Oils and fats | Spot test | + | + | - |

(+) = Present; (-) = Absent

***Preliminary phytochemical screening***

Methanolic extracts of *B. lanceolaria* leaf contained the majority of phytoconstituents, except steroids and terpenoids (Table 1). Flavonoids, oil and fats were absent in n-hexane extract; while alkaloids, tannins and saponins were absent in both chloroform and n-hexane extract. It may be due to the poor solubility of these phytochemicals in n-hexane and chloroform extracts. The phytochemicals detected in the sample extracts have medicinal and biological activities as other studied natural substances. Phenolics are used for treatment of skin infection and wounds (Okwu *et al.,* 2001). Lou *et al.* (2007) reported that phenolics and flavonoids have anti-inflammatory activities and the property of preventing oxidative cell damage, carcinogenesis and cardiovascular disease. Tannins are responsible for analgesic and anti-inflammatory activities and provide protection against microorganisms (Chung *et al.,* 1998). The solvent extract contents studied ascertain that methanol is a potent solvent for phenolic extraction from *B. lanceolaria*.

Polyphenolic compounds detected during preliminary screening indicate the antimicrobial activity of plant extracts (Massih *et al.,* 2019). The presence of alkaloids in methanolic extract of *B. lanceolaria* confirms that it can be used as an analgesic drug. Terpenoids detected in chloroform and n-hexane extracts are the compounds with anti-inflammatory, anti-viral, anti-malaria and anti-bacterial properties (Mahato and Chen, 1997). Similarly, steroids detected in n-hexane and chloroform extract reveal antibacterial properties (Epand *et al.,* 2007). Phytosterols exert many biological activities as anticarcinogenic effects (Awad and Fink, 2000); immunomodulatory and anti-inflammatory properties (Navarro *et al.,* 2001); antioxidant potential, hypocholesterolemic and antidiabetogenic effects (Furlan *et al.,* 2013). The presence of phytosterols in all the three solvent extracts tested indicate that *B. lanceolaria* can act as an anticancer drug. Saponins are the classes of bioorganic compounds (naturally occurring glycosides) having soap-like foaming properties, and consequently form foam when shaken in aqueous solutions. Saponins produce inhibitory effect on inflammation (Just *et al.,* 1998). The presence of saponins in methanolic extract of *B. lanceolaria* indicates that this plant can be exploited for wound healing activity (Razika *et al.,* 2017). Oils and fats found in methanolic and chloroform extracts reportedly have gastroprotective, carminative, antiemetic, antibacterial, antifungal, antiviral, antiprotozoal, insect repellents, antioxidant, anticancer, antidiabetic and antimutagenic properties (Reddy, 2019).

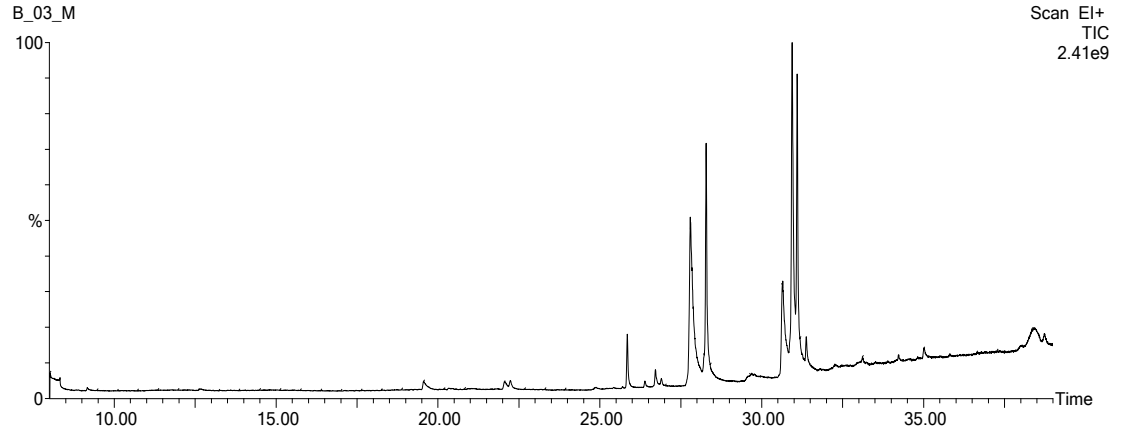
***GC-MS profiling of bioactive compounds***

The bioactive compounds present in methanolic extract and their retention time (RT), molecular formula, molecular weight and peak area are given in Table 2. Based on the area, the 3 major compounds in methanolic extract were identified as chloroacetic acid, tetradecyl ester (18.15%); trans-13-octadecenoic acid, methyl ester (15.45%) and N-tetracosanol-1 (7.88%). The n-hexane crude extract contained chloroacetic acid, tetradecyl ester (29.03%), followed by 1-heptacosanol (8.62%) and phytol (7.34%). The chloroform crude extract had Z,z-6,28-heptatriactontadien-2-one (20.29%), 1-heptacosanol (21.21%) and methyl 6, 9-octadecadienoate (6.8%) as the 3 major

compounds. The GC chromatograms of extracts are given in Fig. 1-3 and show the mass spectrum of the identified bioactive compound.

**Table 2: GC-MS profile of methanol extract of *B. lanceolaria***

| Peak | Retention time | Name | Peak area% | Molecular weight | Molecular formula |
|---|---|---|---|---|---|
| Methanol extract | | | | | |
| 1 | 25.845 | 3,7,11,15-tetramethyl-2-hexadecen-1-ol | 2.548 | 296 | $C_{20}H_{40}O$ |
| 2 | 26.716 | Z, z-6,28-heptatriactontadien-2-one | 1.088 | 530 | $C_{37}H_{70}O$ |
| 3 | 27.791 | Chloroacetic acid, tetradecyl ester | 18.154 | 290 | $C_{16}H_{31}O_2Cl$ |
| 4 | 30.647 | N-tetracosanol-1 | 7.883 | 354 | $C_{24}H_{50}O$ |
| 5 | 30.937 | Trans-13-octadecenoic acid, methyl ester | 15.453 | 296 | $C_{19}H_{36}O_2$ |
| N-hexane | | | | | |
| 1 | 27.786 | Chloroacetic acid, tetradecyl ester | 18.832 | 290 | $C_{16}H_{31}O_2Cl$ |
| 2 | 30.617 | 1-heptacosanol | 12.934 | 396 | $C_{27}H_{56}O$ |
| 3 | 30.928 | Phytol | 5.065 | 296 | $C_{20}H_{40}O$ |
| 4 | 36.625 | 1,2-benzenedicarboxylic acid, diisooctyl ester | 5.058 | 390 | $C_{24}H_{38}O_4$ |
| 5 | 38.676 | Squalene | 3.588 | 410 | $C_{30}H_{50}$ |
| Chloroform extract | | | | | |
| 1 | 25.856 | Z, z-6,28-heptatriactontadien-2-one | 9.078 | 530 | $C_{37}H_{70}O$ |
| 2 | 26.726 | 3,7,11,15-tetramethyl-2-hexadecen-1-ol | 3.471 | 296 | $C_{20}H_{40}O$ |
| 3 | 27.816 | 1-heptacosanol | 9.463 | 396 | $C_{27}H_{56}O$ |
| 4 | 28.277 | Hexadecanoic acid, methyl ester | 4.090 | 270 | $C_{17}H_{34}O_2$ |
| 5 | 31.083 | Methyl 6,9-octadecadienoate | 6.266 | 294 | $C_{19}H_{34}O_2$ |




**. Fig. 1: GC-MS Chromatogram of methanol extract of *B. lanceolaria***

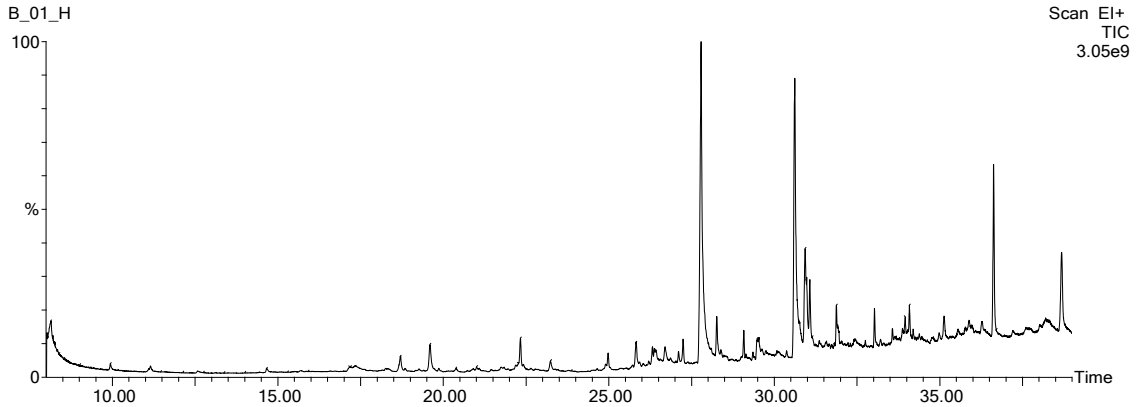



**Fig. 2: GC-MS chromatogram of n-hexane extract of *B. lanceolaria***

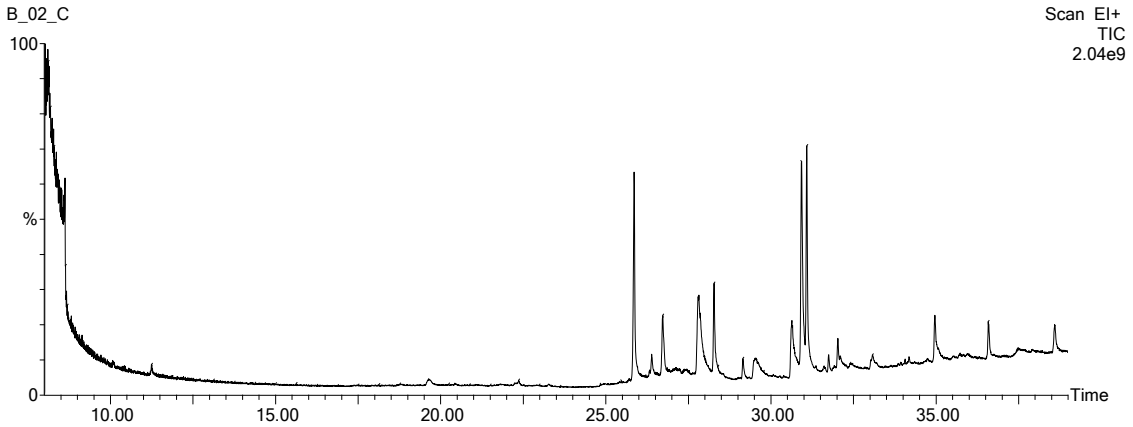



**Fig. 3: GC-MS chromatogram of chloroform extract of *B. lanceolaria***

The reliability of medicinal plant for its usage is evaluated by relating the phytochemical compounds with their biological activities (Belkacem *et al.,* 2013). Chloroacetic acid, tetradecyl ester were present in both methanol (18.154%) and n-hexane extract (18.832%) in different quantities and is well known for its antioxidant properties (Shyam *et al.,* 2013). 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol is a diterpene and has antimicrobial, anti-inflammatory, anticancer and diuretic activities (Rajalakshmi and Mohan, 2016). Z, z-6,28-heptatriactontadien-2-one possesses vasodilatory properties (Mallikadevi *et al.,* 2012). 1-heptacosanol is a long chain alcohol possessing antimicrobial and anti-oxidant properties (Al-Abd *et al.,* 2015) which was found to be present in both n-hexane and chloroform extracts. However, the three crude extracts did not contain a common major compound in them. N-tetracosanol-1, an alcoholic compound present in methanolic extract of *B. lanceolaria* is known for its antioxidant and antibacterial properties (Garaniya and Bapodra, 2014). Trans-13-octadecenoic acid, methyl ester is a bioactive compound having numerous pharmacological activities like anti-inflammatory, antiandrogenic, anticancerous, dermatitigenic, hypocholesterolemic, anemiagenic and insectifuge properties (Krishnamoorthy *et al.,* 2014). Phytol which is present in n-hexane is a precursor of synthetic vitamin E and vitamin K and was also found to be showing cytotoxicity activity against breast cancer cell lines (MCF7) (Satyal *et al.,* 2012). 1,2-Benzenedicarboxylic acid, diisooctyl ester is a compound having antioxidant property (Li *et al.,* 2012). Squalene has antioxidant, antitumor, chemopreventive, and hypocholesterolemic properties (Spanova *et al.,* 2011). Hexadecanoic acid, methyl ester contains several bioactive properties including antimicrobial, antioxidant, hemolytic, 5-alpha reductase inhibitor cancer enzyme inhibitors in pharmaceuticals (Ouyang *et al.,* 2012). Berdeaux *et al.* (1998) reported methyl 6, 9-octadecadienoate for its antioxidant activities.

**Conclusion:** The data obtained from the preliminary phytochemical screening and GC-MS technique has clearly revealed the presence of large spectra of pharmacological and chemo preventive property in *B. lanceolaria* leaves which can be correlated with the claims held by traditional medicine practitioners of North East India. This generated information can be highly valuable for validation of traditional medicine preparations by traditional means. Isolation of active constituent will help in development of novel drugs used in the treatment strategies of certain diseases.

**Conflict of Interest**: The authors declare that they do not hold any conflict of interest.

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