

Phytochemical and GC-MS profiling of ethanolic extract of *Terminalia myriocarpa*- An endangered plant species of N. E. India.

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ABSTRACT

Terminalia myriocarpa, the East Indian almond, is a tree species in the genus *Terminalia* found in Southeast Asia. Hollock is a timber and landscape tree native to India and Southeast Asia, its natural range extending from the Himalayan foothills east to Myanmar (Burma), Thailand, Malaysia, Indonesia and southern China. Exceptional specimens in natural forests may attain up to 40 m (130 ft) but are typically 15 to 25 m (50 to 82 ft) tall on open sites. The trunk is usually straight and on older trees buttressed, supporting a wide-branching crown made up of long, slender, gently dropping branches. The bark is grey and smooth, with age becoming flaking. The flowers are small with light yellow petals, held tightly packed in long, slender clusters at the ends of the branches. They bloom from summer to autumn, coinciding with the rainy season in its native range, and are followed by clusters of small, green, winged seedpods, becoming bright pink when mature. Qualitative estimation of the biochemical metabolites in the said species was done and the following compounds were present viz. alkaloids, tannins, flavonoids, lignin etc. Quantitative estimation of metabolites such as phenolics, flavonoids, flavonols and GC-MS analysis were done and the plant was found to have high amount of phenol but very low amount of flavonoid, flavonol and phytochemical content.

Keywords: Phytochemical, bioactive compound, *Hollock*, Alkaloids.

1. INTRODUCTION

Plants of the genus *Terminalia* (family Combretaceae) are widely used in traditional medicine all over the world. There are about 250 *Terminalia* species, of which at least 50 are used as food. Many species have biological activities including antitumor, anti-inflammatory, wound healing, antifungal, antibacterial, and antiviral activities [1,2,3,4,5]. In particular, *Terminalia chebula*, an Indian species, is well noted as the king of plants in Ayurveda for its extensive medicinal use [6]. These plants mainly include tannins, polyphenols, triterpenoids, flavonoids, aliphatic compounds, and other active ingredients, among which tannins and polyphenols are the main constituents. *Terminalia myriocarpa* (East Indian Almond) is a tall evergreen tree growing up to 40 m, native to eastern Asia, southern China, northeast India, Nepal, Bhutan, Myanmar, Thailand, Malaysia, Indonesia, Laos, and Vietnam. *Terminalia myriocarpa*, the East Indian almond, is a tree species in the genus *Terminalia* found in Southeast Asia. Hollock is a timber and landscape tree native to India and Southeast Asia, its natural range extending from the Himalayan foothills east to Myanmar (Burma), Thailand, Malaysia, Indonesia and southern China. Exceptional specimens in natural forests may attain up to 40 m (130 ft) but are typically 15 to 25 m (50 to 82 ft) tall on open sites. The trunk is usually straight and on older trees buttressed, supporting a wide-branching crown made up of long, slender, gently dropping branches. The bark is grey and smooth, with age becoming flaking. The flowers are small with light yellow petals, held tightly packed in long, slender clusters at the ends of the branches. They bloom from summer to autumn, coinciding with the rainy season in its native range, and are followed by clusters of small, green, winged seedpods, becoming bright pink when mature. Several compounds were previously reported from the leaves of *T. myriocarpa*, including cinnamic acid, *trans*-ferulic acid, syringic acid, gallic acid, methyl gallate, ethyl gallate, 2,3-(S)-HHDP-D-glucose, ellagic acid, flavogallonic acid, methyl-(S)-flavogallonate, (α/β)-punicalagin, epigallocatechin gallate, vitexin, isovitexin, orientin, iso-orientin, kaempferol-3-O- β -D-rutinoside, rutin, neosaponarin, quercetin, and myricetin [7]. Moreover, β -sitosterol, β -amyrin, oleanolic acid, betulinic acid, maslinic acid, and arjunolic acid were separated from the bark of *T. myriocarpa*. From the standpoint of bioactivity, ellagic acid and methyl-(S)-flavogallonate, isolated from *T. myriocarpa* leaves, were found to possess antioxidant and anti-inflammatory

activities [8]. Several previous studies reported that according to these compounds, *Terminalia* species are recognized to have several curative effects in folk medicine such as antifungal, antibacterial, anticancer, hepatoprotective, wound healing, antidiabetic, antiallergic, antiasthma, and anti-inflammatory against the acute and chronic inflammation. Therefore, it is considered vital to study the chemical composition of *T. myriocarpa* to prove their use in alternative remedies as nutrients and antioxidants.

2. MATERIALS AND METHODS

2.1. Study area

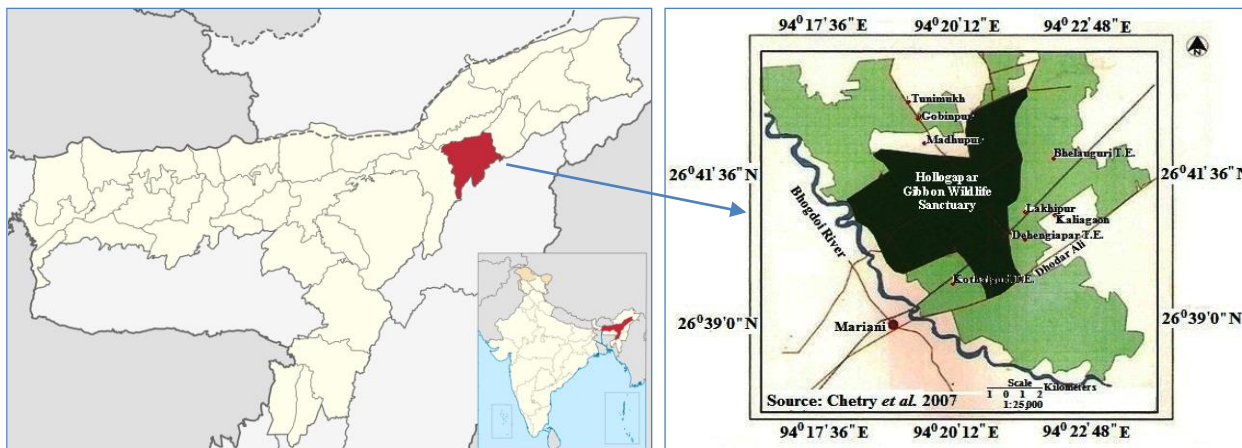


Figure 1: Study area

Hollongapar Gibbon Wildlife Sanctuary (HGWS), Assam, India: HGWS (Fig. 1) is situated at 26° 40' to 26° 45' N and 94° 20' to 94° 25' E, at an altitude of 100 to 120 m, and has an area of 20.98 km² (Figure 1). It is located on the south side of the Brahmaputra River in the district of Jorhat, Assam. The vegetation type in HGWS is Assam plains alluvial semi evergreen forests, sparsely interspersed with wet evergreen forest patches. The vegetation is composed of several canopy layers; most of the components are evergreen in character [9] (Figure 1).

2.2. Preparation of Plant extract

0.5mg of the plant leaves were macerated along with the ethanol in a pestle mortar. The macerated leaves are then kept in a hot air oven for a period of 15 - 20 minutes to evaporate the solution. The solution was then filtered and the extract was obtained for further analysis [10].

2.3. Test for phenol

The total soluble phenols were determined by the Singleton and Rossi (1965) [11] method with slight modification. The ethanolic extract of the plant in desired concentration (0.5mL) was mixed with 0.5mL of Folin Ciocalteu reagent (previously diluted 1: 1 with distilled water). The mixture was then incubated for 5 minutes at room temperature. 1mL of 2% Na₂CO₃ solution was added to it. After incubation at room temperature for 10 minutes, the absorbance was measured at 730nm with a UV - vis - spectrophotometer.

2.4. Test for flavonoid

The flavonoid content was determined according to Zhishen et al (1999) [12] with minor modifications [13] using quercetin as a standard. To 0.25mL of the ethanolic extract of the plant was added to 1.25mL of double distilled water followed by 75μL of 5% NaNO₂. After 5 minutes of incubation at room temperature, 0.15mL of 10% AlCl₃ was added. After a further incubation of 6 minutes, the reaction mixture was treated with 0.5mL of 1mM NaOH solution. Finally, the reaction mixture was diluted with 275 μL of double distilled water. The absorbance was measured at 510nm after incubating the mixture for 20 minutes at room temperature.

2.5. Test for Total Flavonols

The method developed by Kumaran and Karunakaran (2006) [14] was used to estimate the total flavonols using quercetin as a standard. 2mL of 2% AlCl₃ was added to 2mL of the ethanolic plant extract. 1mL of 1% CH₃COONa was added to it and then incubated for 2.5 hours at room temperature. Optical density was taken at 440nm.

2.6. GC-MS analysis

GC-MS analysis of the extracts of the samples were carried out with Perkin

Elmer (USA) make GCMS instrument, Model: Clarus 680 GC & Clarus 600C MS comprising a liquid auto-sampler. The Software used in the system is Turbo Mass Ver. 6.4.2. The peaks were analyzed using data analysis software NIST-2014. The capillary column used is 'Elite- 5MS' having dimensions- length- 60 m, ID- 0.25 mm and film thickness- 0.25 μ m and the stationary phase is 5% diphenyl 95% dimethyl polysiloxane.

GC-Protocol: 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 μ l was employed in splitless mode. Injector temperature is 280°C and ion-source temperature 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 is ~ 39min. Solvent delay was kept for 8 minutes.

MS Protocol: 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 is 50-600 amu

Identification of Peaks: Interpretation of the peaks appeared in the GC Chromatogram were 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, empirical formula etc.

3. RESULTS

The results revealed the presence of medicinally active constituents in *Terminalia myriocarpa*. Qualitative estimation of the phytochemicals in the target plant species depicted the presence of the bioactive compounds such as phenols, flavonoids and flavonols. Quantitative estimation of the phytochemicals revealed the presence of 1150 mg/g of phenols, 2.84 mg/g of flavonoid and 21.6 mg/g of flavonols in the ethanolic extract of the plant species (Figure 2). GC-MS analysis of the plant species revealed the presence of around 136 compounds (Figure 3). Some of the predominant compounds found in the plant species are (E)-.Beta.-Farnesene; 1,2,4-Benzenetriol; 3,7,11-Trimethyl-2,4-Dodecadiene; 3,9-Dodecadiyne; 3-Acetylthymine; 3-Carene; 4,4'-Ethylenedi-M-Toluidine; 4,5-Diamino-6-Hydroxypyrimidine; 4-Tert-Butoxystyrene; 5,6-Decadien-3-Yne, 5,7-Diethyl; 5-Chlorovaleramide, N-Benzyl-N-Phenethyl; 6-Methoxy-3(2h)-Pyridazinone ; Aromandendrene ; Benzene, [(Butylsulfonyl)Ethylyl]; Bicyclo[2.2.1]Heptane, 1,7,7-Trimethyl; Ethyldiethanolamine; Guaia-1(10),11-Diene ; Hexanamide, N-Benzyl-N-Phenethyl; Maltol ; Metoprolol, 2tms Derivative ; N-Benzyl-2-Phenethylamine ; N-Butylbenzylamine ; Phenol, O-(2-Butenylthio, Thymine; Phenethylamine, N-Benzyl-P-Chloro; O-Menth-8-Ene; N-Benzyl-2-Phenethylamine ; N-Hexyloxazolidin-2,4-Dione ; L-Norvaline, N-Ethoxycarbonyl-, Butyl Ester ; Ethyldiethanolamine; Eudesma-4(15),7-Dien-1.Beta. -Ol; Camphene; Beta.-Longipinene; O-Menth-8-Ene; Bamifylline; Maltol; Valdetamide ; Santolina Triene ; Metoprolol, 2tms Derivative (**Table 1**).

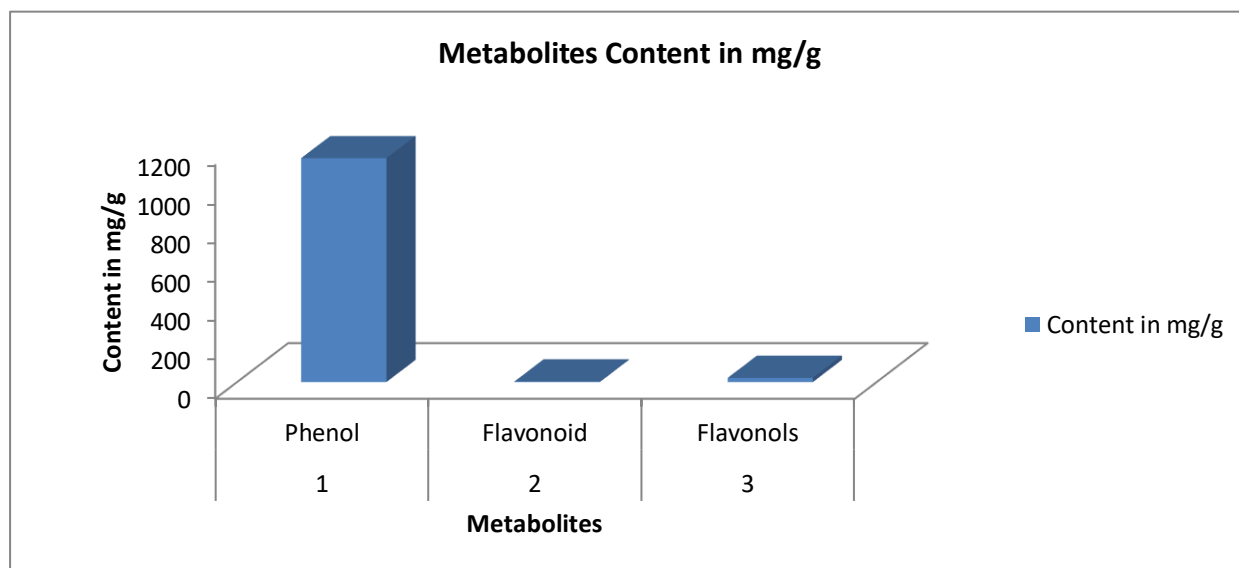


Figure 2: Metabolites (Phenol, flavonoid and flavonol content in mg/g)

Literature survey has revealed that genus *Terminalia* is a rich source of tannins and pseudotannins, including gallic acid and its simple gallate esters, chebulic and non-chebulic ellagitannins, ellagic acid derivatives and ellagic acid glycosides. In a similar study [15], phytochemical research has led to the isolation of different classes of compounds including, tannins, flavonoids, phenolic acids, triterpenes, triterpenoidal glycosides, lignan and lignan derivatives. Crude extracts and isolated components of different *Terminalia* species showed a wide spectrum of biological activities. Habibulla et al., 2016 [16] studied the GC-MS profiling of *M. nilagirica* and found 55 compounds from the leaf extract of *M. nilagirica*. The main compounds in the plant extract were identified as 1Butanol, 3 – Methyl (13.25 %), 2H-Cyclohepta[b]furan-2-one (11.11 %), 5-O-Methyl-d-gluconic acid dimethylam (7.50%), Xanthanin (3.94%), 3.alpha.,6.alpha.-Dihydroxy-5.beta.-preAllopregnane-7.alpha.,11.alpha.-diol-3 (3.47%) and 2,5-Anhydro-1,6-Dideoxyhexo (3.29 %), 1,3-Cyclohexadiene (3.04%) 2,3-Dihydro-5- Hydroxy-6-Met4H-Pyran-4-one, 2,3-dihydro-3,5-dihyd (3.04%) L-Norvaline, N-Ethoxycarbonyl-, Pentyl Ester. In another study [17], GC-MS analytic chromatogram revealed the presence of 24 and 22 compounds in TCE and TCA, respectively, which were identified mainly as phenolic compounds, terpenes/ terpenoids, fatty acids, and other phytochemicals.

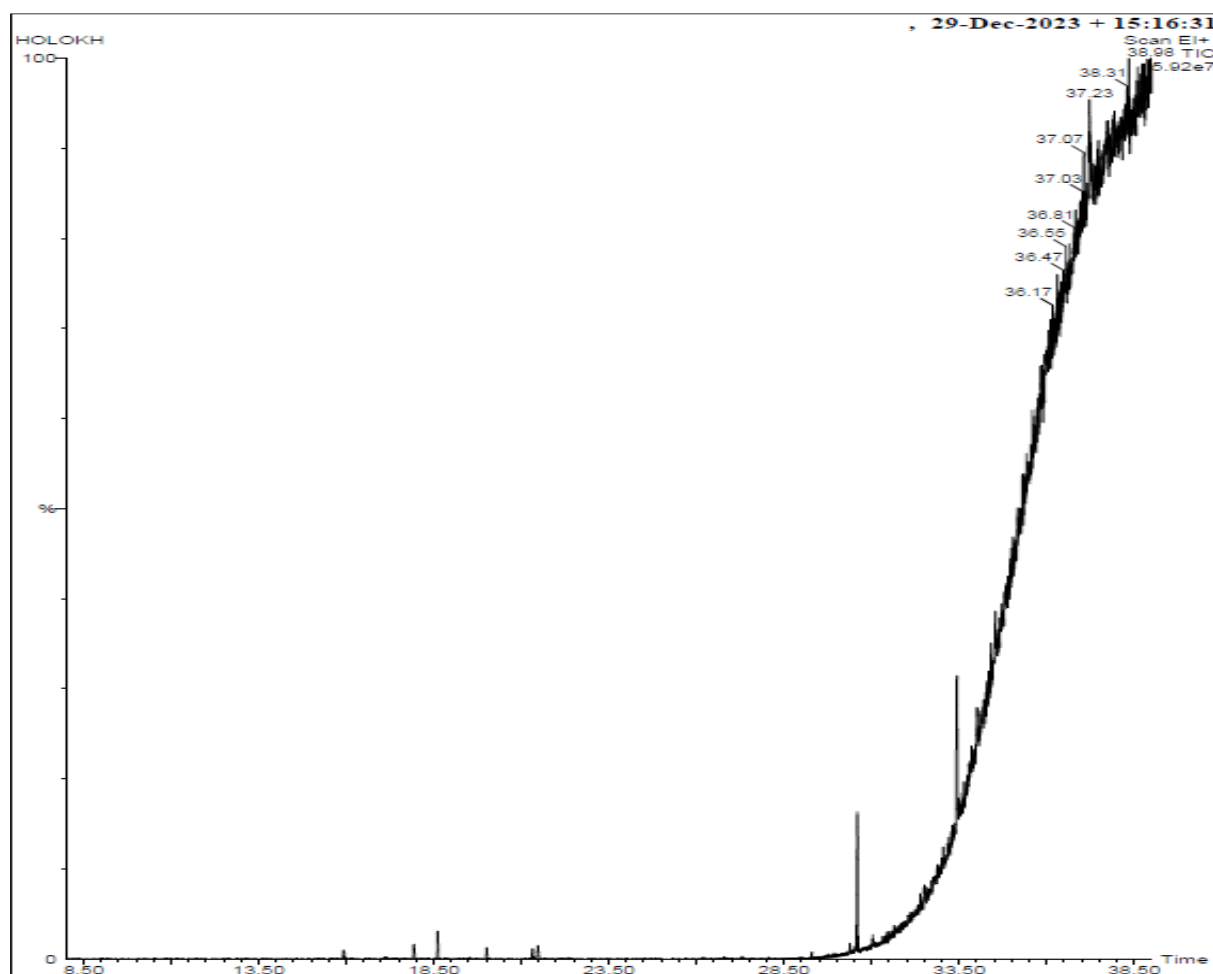


Figure 3: GC- MS spectrum of the plant species

4. CONCLUSION

According to the research, this plant has parts that may have therapeutic uses. Prior research has demonstrated the bioactivity of the phytochemical compounds discovered during this study. It is feasible to ascribe the efficacy of the plants' leaves in treating since prior studies have demonstrated that a number of these compounds have both physiological and therapeutic activities. It is advised that more investigation be carried out to locate, separate, and maybe describe the active ingredients that provide these plants their action.

5. ACKNOWLEDGEMENT

I acknowledge the help received from Guwahati Biotech Park Incubation Centre and Dr. Rajiv Chandra Dev Goswami, Research Associate, Guwahati Biotech Park for providing the technical help as well as funding

agency (DBT project No: BT/BP/19011/1/2008 dt. 24.02.2009) and Sanjib Neog, forest official of gibbon wildlife sanctuary for assisting us in the collection of the specimens.

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