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Potential prospects of *Miliusa tomentosa* an underexploited wild edible fruit

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Abstract
The search for indigenous diets and utilization of wild edible fruits has increased considerably in the recent years. The inadequate dietary diversity of the global food system had led to nutritional inadequacy in terms of malnutrition and hidden hunger to a broad concern. The need of wild edible fruit resources coupled with indigenous knowledge, mode of consumption and their nutritional assessment may serve as an alternate to attain food and nutritional security. The current study reports for the first time the ethnobotanical values, nutritional properties and profiling of bioactive compounds of *Miliusa tomentosa* fruits. The nutritional analysis of *M. tomentosa* fruits revealed the presence of appreciable amount of carbohydrates, proteins, fats, calorific value with other nutrients and vitamins which accentuates and makes them indispensable potential in combating over malnutrition. Structural elucidation of bioactive constituents of the ethanolic fruit extract carried out by different analytical techniques. In GC-MS analysis, about 30 therapeutically significant compounds were detected. From FTIR spectral analysis, it was found that the extract possesses a wide range of functional groups such as alcohols, phenols, aliphatic and aromatic compounds. The findings of the study evinced that *M. tomentosa* fruits encompasses potential nutritional value with bioactive compounds which can be further analyzed for their candidature in terms of therapeutic efficacy and it can play a decisive role in nutritional security.

Keywords: *Miliusa tomentosa*, edible fruit, nutritional, bioactive compounds, GC-MS, FTIR

Introduction
The post pandemic and consecutive wars have led world countries to get side tracked in accomplishing the SDGs (Sustainable Developmental Goals) of FAO, which is intended to achieve zero hunger by 2030. It is also predicted that over the course of this decade, nearly 582 million will persistently lack nutrients which will lead to undernourishment [1]. Moreover, India ranks 105th out of 127 countries in the 2024 Global Hunger Index with the score of 27.3 indicates that the country's degree of hunger is severe [2].

The globe abounds with an inordinate number of plant species. Plants are the natural resources of myriad pool of bioactive phytochemicals benefiting the human society in terms of health and nutrition but still food security and poorer nutrition are one of the key concerns in most of the countries, where modernization and settled agriculture practices focuses in increasing only the production of very few domesticated crops. This has led to the downfall of various indigenous diets obtained from the wild edible plants [3].

The value of foraging for ethnic wild edible fruits is predated since the period of antiquity. Wild edible fruits are the valuable alternate health food, serving as a vital component of the global food basket. The sustainable utilization of these natural sources has prompted immense interest and demand all over the world. Insufficient knowledge about the potential of underutilized fruits and a dearth of study remains the major concern, even though the fruits offer secure sustenance. Hence, intensifying the usage of such underutilized wild fruits would not only support the global dietary supplements but would also uplift the socio-economic subsistence of the inhabitants [4].

The Annonaceae family is one of the oldest angiosperms which had its origin from the period of late Cretaceous [5]. It is one of the primitive families of Angiosperms belonging to Magnoliopsida class, Magnoliidae subclass, Magnoliales order [6].

According to Global Biodiversity Information Facility (GBIF) database lists the Annonaceae family consists of 162 genera and 3049 species, whereas with reference to World Checklist of Vascular Plants (WCVP), it consists of 111 genera with 2444 accepted species [7]. Despite consisting of large number of genera, the predominantly representing genera includes *Annona*, *Goniothalamus*, *Guatteria*, *Polyalthia*, *Uravia*, *Xylopia* constitutes about 42.3% of the entire family.

Miliusa is one of the underexplored genera of the family Annonaceae consisting of about 60 species that are prominently native to India, Bhutan, Australia and New Guinea and predominantly found only in Asian countries. India and Thailand are regarded as Centre of distribution as they harbour most of the *Miliusa* species and many new species have been reported in the former years. *Miliusa tomentosa* is a large deciduous tree, indigenous to India. It has been reported as folk medicine and used by different ethnic tribal communities of Grassia, Gond, Bhil, Koya, Katunaika, Puliyars and Muthuvars all over India.

Generally, *M. tomentosa* fruits are edible and given to cure weakness in children [8-11] and the unripe matured fruits are dried, burnt and the smoke is passed all over the body to cure post-delivery swellings [12].

To the best of our knowledge, this finding would be the first report on the phytochemical constituents of the fruits of *M. tomentosa*, which would exhibit pharmacological significance, as there is no previous literature available yet on nutritional profile, GCMS and FTIR analysis. Therefore, the present study was focused to appraise the nutritional capacity along with identification of bioactive compounds in the fruits of *M. tomentosa* by GCMS and FTIR analysis.

Materials and Methods

Collection and identification of plant material

The fresh fruit materials of *M. tomentosa* were collected from Veerapanoor village, Coimbatore, Tamil Nadu, India. The taxonomic identity of the plant was confirmed by the Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India (ID. NO. BSI/SRC/5/23/2021/Tech). The fruits were washed under running tap water to remove the surface pollutants and they were chopped into smaller pieces and air dried under shade. The dried samples were powdered and used for further studies.

Botanical description and distribution

M. tomentosa is a large deciduous tree, which grows up to 20 m long with dark greyish brown bark with longitudinally grooved. The leaves are simple, alternate, petiolate, oblong-ovate, acute at apex, with entire margin, tomentose when young. The flowers are bisexual, brownish-green, solitary or few together, pedicellate 4-7 cm long, sepals 3, petals 6 (3+3), stamens many, carpels many, stalked, capitate stigma with 4-6 ovules. The fruits are aggregate berries, sub globose green woody when young and glossy maroon when ripe, fruitlets 2.5 cm across with 3-4 seeds. *M. tomentosa* is indigenous to India. It is globally distributed in India, Nepal and Sri Lanka. In India, it is distributed in states of Tamil Nadu, Karnataka, Maharashtra, Andrapradesh, Chattisgarh, Orissa, Bihar, Rajasthan, Uttarpradesh, Gujarat, etc., Western and Eastern Ghats [13].

A. Flower of *M. tomentosa*, B. Young fruits of *M. tomentosa*, C. Ripen fruits of *M. tomentosa*, D. Cut open of *M. tomentosa* fruit)

Fig 1: *Miliusa tomentosa* flower to ripen fruit

Nutritional analysis

The nutritional composition analysis of *M. tomentosa* was conducted according to the standard methods of A.OA.C. (2000) [14] and the mineral profile was analysed by ICP-OES method.

GC-MS analysis

One µL of the ethanol fruit extract of *M. tomentosa* was employed for GC/MS analysis. The AGILENT, was engaged for analysis. Helium was used as the carrier gas at 1 ml/min. The MS was set to scan from 50 to 650 Da. The MS was also having inbuilt pre-filter which reduced the neutral particles. The data system has two inbuilt libraries for searching and matching the spectrum. NIST4 and WILEY9 each contain more than five million references. Only those compounds with spectral fit values equal to or greater than 700 were considered positive identification.

FT-IR analysis

The spectrum analysis was carried out on a SHIMADZU FTIR Spectrophotometer Model IR Spirit Single Reflectance Attenuated Total Reflection (ATR) Accessory. The internal reflection element QATR-S Single bounce ATR - Diamond Prism Disc. FTIR-spectra was recorded with 4 cm^{-1} resolution and 45 scans were averaged in order to obtain a good signal-to-noise ratio. The ATR measurement area was a circular spot of approximately 1.5 mm diameter. The spectrum was recorded in the wave number range of 400-4000 cm^{-1}.

Results and Discussion

Nutritional analysis

Fruits have become increasingly important in human nutrition because of their nutrient composition and potential beneficial health effects. Vital nutrients play a crucial role in molecular and cellular level by maintaining the homeostasis of enzyme regulation and functioning [15]. The nutritional value and health related properties of fruits depend not only on the concentration of nutrients and phytochemicals but also on the daily intake and bioavailability. The Nutritional composition of *Miliusa tomentosa* is enlisted in Table 1:

Table 1: Nutritional composition of *Miliusa tomentosa* fruits

S. No.	Parameter	*M. tomentosa*
1.	Carbohydrate (%)	77.79
2.	Protein (%)	12.58
3.	Crude fibre (%)	35.25
4.	Moisture (%)	3.13
5.	Ash (%)	4.52
6.	Starch (%)	21.18
7.	Total sugars (%)	5.09
8.	Fat (%)	2.16
9.	Calorific value (Kcal/100 g)	380.92
10.	Vitamin A (IU)	8.08
11.	Vitamin C (mg/100 ml)	15.17
12.	Magnesium (ppm)	890.87
13.	Nickel (ppm)	0.65
14.	Aluminium (ppm)	64.99
15.	Barium (ppm)	1.91
16.	Manganese (ppm)	12.99
17.	Potassium (ppm)	74.80
18.	Copper (ppm)	5.58
19.	Molybdenum (ppm)	0.48
20.	Iron (ppm)	37.35
21.	Calcium (ppm)	50.54

Julian *et al*. (2011), have reported the total carbohydrates content of *Annona diversifolia* Safford fruit pulp, pink and deep pink varieties 18.4 ± 0.1 and 20.3 ± 0.1 g/100 g of edible portion respectively, whereas the pulp from white variety of *A. diversifolia* has lower content 13.6 ± 0.2 g/100 g of edible portion when compared with the results obtained with our study and they have also reported protein values for the fruit pulp of *A. diversifolia* 0.89 - 1.14 g/100 g which was found to be lower than that of *M. tomentosa* fruits [16].

In 2008, Qasi and his co-workers in Rajasthan studied the nutrient dynamics of various parts (Except fruits) of composite plant sample *M. tomentosa* tree along with the nutrient level of strata and the litter samples around the tree. They reported that the highest amount of nutrient was recorded in the leaves and they also correlated the results stating that the concentration of nutrient in the plant is inversely proportional to nutrient concentration in soil [17].

The nutritional profile of the sample *Miliusa tomentosa* was compared with the Compendium of Nutritional data of common Indian foods (2023) published by Homeopathy Research Institute for Disabilities Chennai, under Central Council for Research in Homeopathy and Ministry of Ayush, astoundingly some of the nutritional values was found to be higher and moreover similar with the commercially available fruits (per 100 g of edible portion). Namely, the magnesium content of *M. tomentosa* was found to be higher than apple (8.09 mg), gooseberry (6.5 mg), pomegranate (11.07 mg). Compared to apple (0.26 mg), banana (0.28 mg), guava (0.32 mg), orange (0.81), *M. tomentosa* had a higher iron concentration. And moreover *M. tomentosa* exhibited greater total energy content than apple (62.38 kcal), banana (105.16 kcal), gooseberry (23.66 kcal) [18].

Vitamin C is essential for facilitating the transport of long chain fatty acids to mitochondria and produce energy through beta oxidation. Iron as a component of porphyrin ring in heme enzymes, is crucial for cellular energy production. Magnesium plays a vital role in ATP production and its utilization. Each ATP molecule associates with a magnesium ion and these complexes facilitate the transport of mitochondrial ATP, supplying energy to the cells [19].

The presence of appreciable amount of Magnesium, iron and vitamin C envinced scientifically that they play major role in energy yielding mechanism. The higher calorific value of *M. tomentosa* fruit revealed in this study implies its higher efficacy rate which further validates the folkloric claims for consumption of these fruits in curing weakness in children. Hence, the wild edible fruits of *M. tomentosa* could be recommended and encouraged as a good choice for enriching the daily diet.

GC-MS analysis

Interpretation of mass spectrum of GC - MS was done using the data base of National Institute Standard and Technology (NIST4) and WILEY9. The spectrum of the known component was compared with the spectrum of the known components stored in the inbuilt library. A total of 30 compounds were identified from the GC-MS analysis of ethanolic fruit extract of *M. tomentosa* exhibited the presence of various phytoconstituents. The chromatogram is presented in Fig 2, while the bioactive compounds with their therapeutic uses are presented in Table 2.

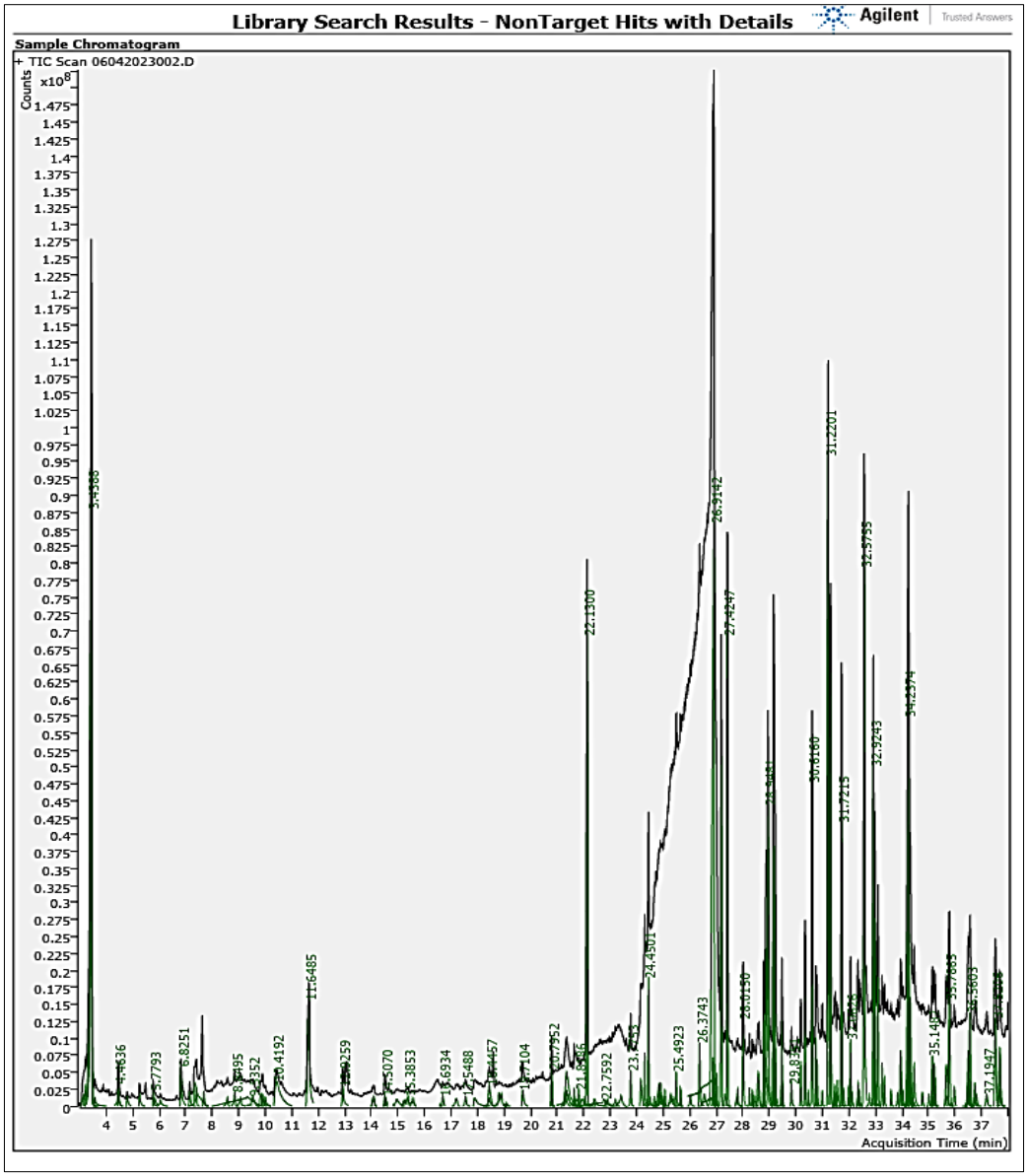

Fig 2: GC-MS chromatogram of ethanolic extract of *M. tomentosa* fruits

Table 2: Bioactive compounds detected in the ethanolic fruit extract of *M. tomentosa* fruit

S. No.	List of Bioactive compounds	Nature of compounds	Therapeutic uses
1	2-Propenoic acid	Fatty Acid Methyl Ester	Antimicrobial, Anti-inflammatory, Antineoplastic, Antiurolithic, Antiobesity and Antiprotozoal
2	4-Cyclopentene-1, 3-dione	Alkyne	Antitumor
3	Butanoic acid, 4-hydroxy-	Ester	Flavor, Perfumery, Antioxidant.
4	beta.-Alanine, N-methyl-, ethyl ester	Amino acids	Antioxidant, antimicrobial and used in human protein synthesis
5	3-Carene	Bicyclic monoterpene	Antimicrobial and Antibacterial
6	1, 2, 3-Propanetriol, 1-acetate	Phospholipids	Used in food products, nutritional supplements, pharmaceutical products, personal-care products, and oral-care products
7	Geranyl formate	Vitamin E	Antioxidant
8	DL-Leucine, N-acetyl-, ethyl ester	Amino acid	Nutritional therapy, used in supplementation, muscle-building
9	DL-Proline, 5-oxo-, ethyl ester	Amino acid	Nutritional therapy, used in supplementation, muscle-building
10	2, 4-Di-tert-butylphenol	Phenol	Analgesic, Anesthetic, Antibacterial, Antioxidant, Antiprostatitic, Antipyruvetic, Antiseptic, Antiviral
11	Pentanoic acid, 5-hydroxy-, 2, 4-di-t-butylphenyl Esters	Monocarboxylic acid	Anti-cancer, anti-diabetic, antihypertensive, anti-inflammatory, and immunomodulatory
12	Dodecanoic acid	Fatty acid methy esters	Anticancer and antioxidant
13	Tetradecanedioic acid (Myristic acid)	Saturated fatty acid	Anti-inflammatory, antioxidant, antiproliferative, antimicrobial and Analgesic
14	Borinic acid, diethylthio-, methyl ester	Acids	Anticancer, antibacterial, antiviral
15	Pentadecanoic acid	Fatty acid	Antibacterial and antifungal
16	Hexadecanoic acid, ethyl ester	Fatty acid ester	Antimicrobial and antioxidant, acts as a neurotransmitter in the central nervous system.
17	Phytol	Chlorophyll pigment	Antioxidants, Antibacterial, Antiseptic, Antispirochetal, Anticataract, Antidiarrheal, Antileprosy, Antihyper lipoproteinemic, Antirickettsial, Anticarcinogenic, Antiviral.

18	Linoleic acid ethyl ester	Fatty acid	Anti - inflammatory
19	Dimethylmalonic acid, di(2-formylphenyl) ester	Dicarboxylic acid	Used as a perfumes and artificial flavorings
20	Eicosanoic acid, ethyl ester	Monounsaturated omega-9 fatty acid	Antiinflammatory
21	Morphinan-4-ol-6-one, 2-methoxy-N-methyl-	Alkaloid	Analgesics and antibiotics
22	Heneicosane	Fatty acid	Antimicrobial and Antioxidant
23	Homopterocarpin	Isoflavones	Anti-SARS-CoV-2 (severe acute respiratory syndrome-related coronavirus-2), antioxidant, antiplasmodial, antioxidant, antiplasmodial, antimicrobial and antimicrobial
24	Plastoquinone 3	Terpenoid	Antioxidant
25	9, 12-Octadecadienoic acid (Z, Z)-, 2-hydroxy-1- (hydroxymethyl)ethyl ester	Fatty Acid Methil Ester	Antimicrobial, Anti-inflammatory, Antineoplastic, Antiurolithic, Antiobesity, Antiprotozoal, Antifibrinolytic, antialcoholic, Antipsoriatic, Antioxidant.
26	N-Methylcoclaurine	Alkaloid	Analgesics, antibiotics, antitumor agents
27	(+)-(S)-Isocorydine	Alkaloid	Analgesic and antinociceptive
28	Cholesta-4, 6-dien-3-ol, (3.beta.)-	Steroid	Anti inflammatory, Steroid hormones are involved in the functioning of the control of metabolism, development of sex characters, functions related to the immune system
29	Glutaric acid, 2, 2, 3, 3-tetrafluoropropyl dodec-2-en-1-yl ester	Amino acids	Human metabolite, Prevent liver damage, Anti-allergies, anti -asthma
30	Ethyl stearate, 9, 12-diepoxy	Fatty acid methyl esters	Anti-inflammatory, Anti-diarrheal, cytotoxic, and anti proliferative activity

Similarly, Ezerioha *et al*., (2024) has reported the presence of 33 phytochemicals in the ethanolic fruit extract of *Annona muricata* by GC-MS analysis with glycerine and levoglucosenone as the major compounds [20]. The current GC-MS Analysis of *M. tomentosa* and the reported *Annona muricata* have few similar compounds namely 4H-pyran-4-one, 2, 3-dihydro-3, 5-dihydoxy-6-, n- Hexadecenoic acid, Phytol and Hexadecanoic acid, 2-hydroxy-1-(hydromethyl) ethyl ester. The presence of various other bio-active compounds in *M. tomentosa* are reported to have significant pharmacological properties which could play a vital role in therapeutics.

FT- IR analysis

The FT-IR spectrum was performed to identify the functional groups of the active compounds present in the fruit extract of *M. tomentosa*, based on the peak values presented in the region of IR radiation is depicted in the Fig 3.

The peak obtained at 3338.98 and 3205.31 indicated O-H stretching, where as peaks at 2917.84 was due to asymmetric stretching of - $CH(CH_2)$ and the peak at 2850.28 was symmetric stretching of - $CH(CH_2)$. Peaks at 1778.01, 1710.46, 1650.09, 1602.65 corresponds to the C = O stretching. The IR peaks at 1525.04 and 1433.05 revealed the C = C stretching. The presence of O-H bend, alcoholic group was confirmed by the peak at 1397.11. Similarly, the peaks at 1325.24, 1282.12, 1182.95, 1182.95 showed the presence of C-N stretch, which was followed by the PO_3 stretch at the peaks of 1050.71 and 1013.34. The peaks at 863.85, 814.98, 760.36, 704.31 corresponds to the presence of = CH_2 bending. Based on the functional group assignment, some of the compounds were predicted and the results revealed the presence of phenols, alcohols, aliphatic, aromatic, amine, alkene etc., which could further validate the therapeutic outcomes of *M. tomentosa* fruits.

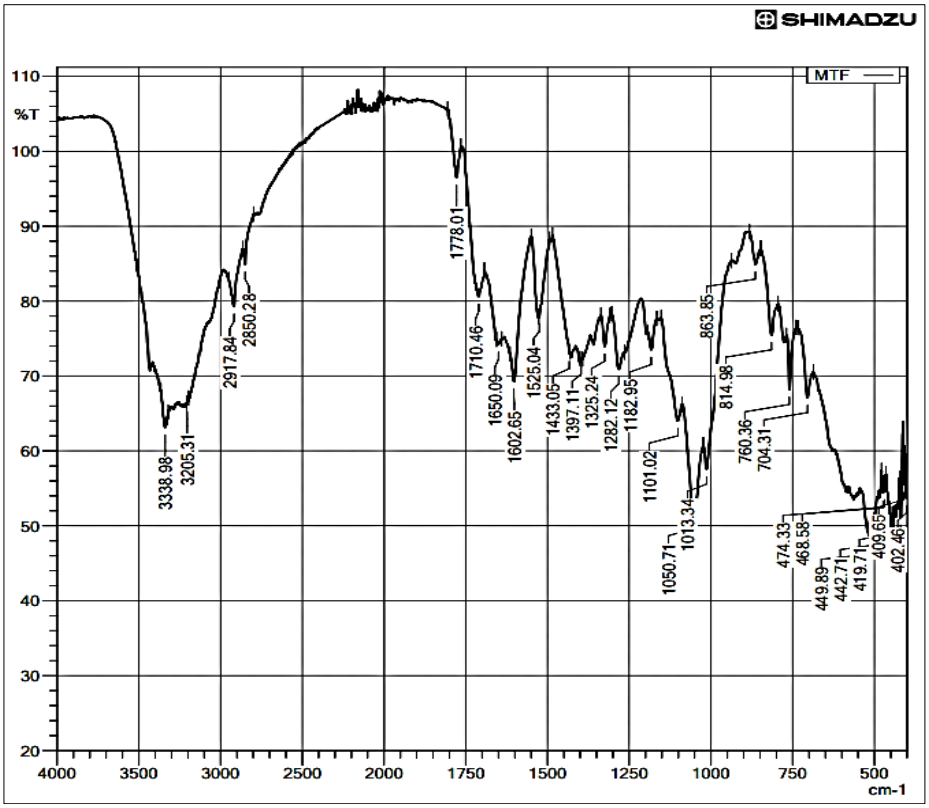

Fig 3: FT-IR Analysis of ethanolic extract of *M. tomentosa* fruits

Conclusion

Current status of drug discovery from natural sources revealed that interest in the screening of nutritional compounds, phytoconstituents for anti-oxidant and related activities has been increased. The finding of this study provided scientific validation to substantiate the use of *Miliusa tomentosa* fruit which has been used traditionally as an edible fruit by various tribes to cure weakness in children and used in treating various health disorders and paves way to open a new avenue of recommending this as a nutritional food source. The primary information of bioactive compounds reported in the fruits of *M. tomentosa* will facilitate in performing further studies on isolating the bioactive compounds and resolve their efficacy by *in vivo* studies and demonstrating their safety and efficacy in clinical trials. Furthermore, these edible wild fruits species could be an alternative source of income generation for local inhabitants of the area and may be used as germplasm by horticulturists and fruit farmers to introduce new varieties and to provide healthy and more delicious fruits for consumers.

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