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LC-MS-Based Targeted Metabolite Profiling and Atomic Absorption Spectrophotmeter Based Mineral Analysis of Methanol Extract of *Terminalia bellerica* (Gaertn.) Leaves

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Natural products in medicinal plants are one of the important sources for modern drug discovery. Plant-based formulations play an important role in management of numerous diseases. To discover the medical potential of natural products, it is important to understand the bioactive

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compounds present in medicinal plants. Hence the aim of the study is to identify the volatile compounds by LC –MS analysis and minerals by AAS assay present in the leaves of *Terminalia bellerica.*

Study Design: This study investigates metabolites present in leaves of *Terminalia bellerica* have been studied by AAS and LC-MS techniques.

Results: The AAS assay showed that the leaves of *T. bellerica* are particularly rich in iron (260 mg/kg) compared to their zinc, manganese, and copper content. Meanwhile, the LC-MS analysis identified chebulinic acid, chebulagic acid, punicafolin, myricetin, rutin, and galloyl punicalagin as being present in the highest concentrations.

Conclusion: Results highlighted presence of considerable amount of iron and other minerals in methanolic extract of the leaves of *T. bellerica,* it may be used in the development of health supplements. The results also revealed that bioactive compounds in the methanolic extracts of *T. bellerica* leave used for drug design and making it safe to human health.

Keywords: Medicinal herbs; Terminalia bellerica leaves; phytoceutics; atomic absorption spectrophotmeter assay; LC-MS assay.

1. INTRODUCTION

Plants have served as an essential source of drugs and remedies against diseases and health disorders since ancient times. Health benefits may be credited to the presence of the various phytochemicals like polyphenols, terpenes, anthocyanins, flavonoids, alkaloids and glycosides. Plants of antimicrobial activity can be related to their essential oils [1] or other isolated compounds such as alkaloids [2], flavonoids, tannins, phenolic acids [3], among other chemical classes including naturally occurring peptides [4,5]. These compounds possess their antimicrobial activity through various mechanisms such as down regulating the nuclear or ribosomal enzyme(s) synthesis, altering the membrane structure and the electron flow, or affecting the metabolic activity of the microbial cell, as well as inhibiting the secretion of their toxins [6,7,8].

Natural products from medicinal plants are one of the important sources for modern drug discovery. Triphala is an ayurvedic drug prepared by mixing dried fruit powder of *Terminalia chebula*, *Terminalia bellerica* and *Phyllanthus emblica* in the ratio of 1:1:1 and also its individual constituent's fruit powder exhibit immuno-modulatory activity" [2,7]. "The important constituents of the Triphala are chebulagic acid (CA), chebulinic acid (CI),gallic acid. These compounds inhibit TNFα induced pro-angiogenic and pro-inflammatory activities in retinal capillary endothelial cells by inhibiting p38, ERK and NFkB phosphorylation. Polyphenol containing plants, of *Terminalia* species*,* have reportedly shown various health benefits and applications in pharma industry. *Terminalia* have a wide spectrum of pharmacological activities including antioxidant, anti-inflammatory, anti-cancer, hepatoprotective, and antimicrobial activities [8]. Polyphenols have been also proven to lower the risk of cardiovascular diseases, enhance liver regeneration, and increase life expectancy.

Traditional medicinal plants practices are used for the treatment of infectious diseases and they have very low level risk compared to synthetic drugs. Natural compounds in medicinal plants are essential sources for drug discovery [9] and are reported that natural products make up to 35% of the global medicine market [10]. To discover the medical values of natural products, it is important to understand the ethno pharmacological uses of various medicinal plants [11]. Although "the modern medicines is quickly growing, large population still preferring herbal medicines due to the effectiveness, medical effectives, enhancing cost of modern medicines and cultural preferences" [12,13,14]. Based on WHO reports, 80% of the global population depends on traditional medicine, and 60% of the Indian rural population use herbal medicines [14]. These natural medicines are cost-effective and efficient in disease treatment [14]. Apart from the medical values, plants are also widely used as food, health care products [15], veterinary medicine [16], have extensive impacts on daily life. Herbal medicine is still the mainstay of health care in several developing countries. The World Health Organization has estimated that more than 80 % of the world's population in developing countries depends primarily on herbal medicine for basic healthcare needs.

Terminalia bellirica Roxb. is a deciduous tree that is widely distributed in the tropical regions. The

fruit extract of *T.bellirica* has shown hepatoprotective" [17] and the main phytoconstituents reported are tannins such as chebulic acid, chebulinic acid, chebulagic acid, gallic acid, corilagin, ellagic acid and flavonoids, sterols, amino acids, fructose, resin, fixed oils etc[18]. *T.bellirica* contains different chemical constituents in different parts such as stem bark contains arjungenin and its glycosides, belleric acid, belleriosides. Ripe fruits are used as astringent in combination with chebulic myrobalan (*Terminalia chebula*) and *Phyllanthus emblica* as the famous Triphala drug of Ayurveda are also used for eye diseases such as cataract, glaucoma, progressive myopia, and conjunctivitis.

Secondary plants metabolites possess various compounds differ widely in terms of structure, biological properties and wide mechanisms of actions" [10]. Polyphenols have been also proven to lower the risk of cardiovascular diseases, enhance liver regeneration, and increase life expectancy.These secondary metabolites include flavonoids, phenols, phenolic glycosides, saponins, and glycosides" [19]. Terminalia species exhibited nutraceutical value with numerous health benefits in the treatment of some diseases [20]. For example, fruits of T.*bellirica* (Gaertn.) Roxb usually form Triphala, the well-known polyherbal formulation in Ayurvedic has pharmacological applications as a laxative, detoxifying, and rejuvenating effects [21]. Thus, our present study aims to analyze the phytochemical composition of*T.bellirica* leaves and their antimicrobial activity. In this study, methanol extract of the leaves of *T.bellerica* was analyzed by LC-MS and AAS techniques for the phytochemical profile was also carried.

2. METHODS AND MATERIALS

2.1 Preparation of Extract

Fresh mature plant leaves of *T.bellirica* were collected from trees growing in Orathanadu taulk, Thanjavur District, Tamilnadu. The leaves of *T.bellirica* (250 g) were air-dried, ground, and extracted with 95% methanol at room temperature for 8 hours (6 × 500 ml). The methanol extracts were filtered, and reduced under vacuum at 40 °C.

2.2 LC-MS

Leave methanol extract of *T.bellirica* was analyzed by shimadzu LCMS2010/LCMS-QP8000α and the solvents used were 0.5% (v/v) acetic acid (A) and 100% methanol (B). The isocratic elution was as follows: (i) 55% of solvent A, from 0 to 10 min, (ii) 65%, from 11 to 20 min (iii) 35%, at 21-30 min of total run time. The PDA detector (UPLC LG 500 nm) was monitored at 340 nm and the column temperature was maintained at 30 °C. The mass spectrometer (MS) was operated in the positive ionization mode with the mass range of 150 m/z to 1000 m/z, the capillary voltage of 3.50 kV, cone voltage of 30 V, extractor voltage of 3V, the gas flow of 30 L/Hr and collision gas flow of 0.18 mL/Min. The mass spectrometry (MS) was determined for phytochemicals using a shimadzu LCMS-2010 / LCMS- QP8000α plus instrument by direct injection. The experiment was carried out in NIFTEM, Thanjavur, Tamilnadu. Detection of the ions was performed in electrospray ionization (ESI) and quadrupole ion trap mass analyzer [22].

2.3 Atomic Absorption Spectrophotmeter Analysis

Leaves extract was taken in a precleaned and heated in a muffle furnace at 400C till there was no evolution of smoke. The crucible was cooled at room temperature in a desiccator and carbon–free ash was moistened with concentrated sulphuric acid heated on a heating mantle till fumes of sulphuric acid ceased to evolve. The crucible was sulphated ash was then heated in a muffle furnace at 600C till the weight of the content was constant (~2–3 h). One gram of sulphated ash obtained above was dissolved in 100 mL of 5% HCl for Atomic Absorption Spectroscopy (AAS).Standard solution of each element was prepared and calibration curves were drawn for each element using AAS [23].

3. RESULTS AND DISCUSSION

The presence of phyto active constituents and early finding son potential biological actives of *T.bellirica* make us to over tone study on the leavesthat has a diverse pharmacological spectrum and has been used in Ayurveda, Siddha, Chinese medicine etc, because of having important phytoconstituents [24,25,26]. LC-MS analysis of T.bellirica leaves recorded 21 compounds and out of these chebulinic acid, chebulagic acid, quercetin and bellericoside were found to be higher in the leaves (Figs.1,2,3). Chebulinic acid is known to possess numerous biological activities including anti tumor activity, anti antherogenic, anti fibrotic, anti inflammatory,

antiulcer, antioxidant, hepatoprotective, antidiabetic and antiviral etc [27]. Chebulagic acid, ellagitannins and proantho cyanidins are the major chemical constituents present in the leaves extract of *T. bellirica.*

Chebulagic acidis benzopyrantannin and anantioxidantthat has many potential uses in medicine. It has been found to beimmuno suppressive [28,29], hepatoprotective [30] and a potentalpha-glucosidase inhibitor [31,32], a human gut enzyme useful in diabetic studies has been shown to be active against *Staphylococcus aureus* and *Candida albicans* [33]. It is formed fromgeraniinthrough aglutathione-mediated conversion [34]. Gallo-tannic acid, bellericanin, ellagic acid, gallic acid, termilignan, thanni lignan, flavone and anolignan B, tannins, ellagic acid, ethyl gallate, galloyl glucose and chebulaginic acid, phenyllemblin,sitosterol, mannitol, glucose, fructose and rhamnose were also found in and these compounds, it showed many of the pharmacological activities such as antisecretory, analgesic, antihypertensive, antidiarrhoeal activity, antimicrobial activity, antidiabetic, antioxidant [35,36] antiulcer, antipyretic, hepatoprotective, anticancer, angiogenesis, antidepressant-like activity [37]. It is useful in the treatment of gastric ulcer, constipation, general debility, piles etc. still, and it has actually not been explored comprehensively. These compounds are traditional used in relief in a cough, asthma, indigestion, dental problems, sore throat and wounds [38].

Glucosides, tannins, galliacid, ellagic acid, ethyl gallate, gallylglucose, chebulanic acid are mainly responsible for its wide therapeutic actions. It has anti-HIV1, antimalarial and antifungal activity. It is used as antioxidant, antimicrobial, antidiarrheal, anticancer, antidiabetic, antihypertensive and hepatoprotective agent [39,40]. It also possesses analgesic, antipyretic and anti- ulcerogenic effect and antimicrobial activity [39,40]. Many phyto constituents have been isolated from the leaves, chebulagic acid, gallic acid have antimicrobial activity of *T.bellerica* leaves. Proteins, three hydrolysable tannins, gallic acid, ellagic acid and methyl gallate, oneflavones, luteolin, two flavonol aglycones, quercetine and kaempferol and four flavonol glycosides, quercetin 3-0-[6"-a-L-rhamnopyranosyl]-(1g6)-B-D-glucopyranoside (rutin),quercetin-3-O-a-L-rhamnopyranoside,quercetin3-0-B-D-glucopyranoside and kaempferol 3-O-B-D-glucopyranoside saponins, tannins, amino acids, proteins, alkaloids, carbohydrates and flavonoids.4-hydroxy-(2-methylbutanol) benzoic acid were present in *T.bellerica* leaves. Apart from vitamins, non-nutritional components of wild green vegetables, notably phenolic compounds are also known to possess powerful radical scavenging properties against reactive oxygen species [14].

Chemically complex compounds from herbs have great therapeutic potential as they have fewer side effects compared to synthetic drugs and also low chances of developing resistance" [27]. Bacteria may develop resistance to medicinal plants treatment if only one active ingredient with a specific target is involved, a condition similar to an antibiotic. Glucosides, tannins, gallic acid, ellagic acid, ethyl gallate, gallyl glucose, chebulanic acid are having wide therapeutic actions. They are used as antioxidant, antimicrobial, antidiarrheal, anticancer, antidiabetic, antihypertensive and hepatoprotective agent. *T.bellirica* also possess analgesic, antipyretic and anti ulcerogenic effect and antimicrobial activity" [40]. The micro minerals included copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn) are shown in Chart 1 and is expressed as mg/g dry plant material, except for Cu which is expressed as µg/g dry plant material. *T.bellirica* contained the highest amount of iron.The search for alternative nutritious foods may hold the key to nourishing many people by rich sources of vitamins, namely β-carotene, ascorbic acid, riboflavin, folate as well as minerals such as iron, calcium and phosphorous. Dietary diversification is critical in order to mitigate the widespread nutrient deficiencies of vitamin A, iron, and zinc. The dietary requirement for a micronutrient refers to the intake level, which meets specified criteria for adequacy; however, threshold levels remain undefined for most dietary nutrients. Nutritional qualities of *T.bellricca* leaves provide critical insights about its potential in adding diversity and may provide the ultimate weapon against dietary deficiency.Based on the analysis, it has been validated that the *T.bellirica* leaves recorded antibacterial activity due to potent bioactive components [35].

Table 1. Compounds identified in LC-MS assay

S. No	Tentatively identified compounds	m/z
1.	7- Hydroxy3,4(methylene dioxyflavan)	270
2.	Luteolin	286
3.	Beta sitosterol	414
4.	Termilignan	296
5.	Anolignan	266
6.	Gallic acid	170
7.	Ellagic acid	302
8.	Methyl Gallate	184
9.	Phyllemblin	198
10.	Chebulinic acid	956
11.	Chebulagic acid	955
12.	3-0- alpha glucopyranosylL-aminopyranose	326
13.	Hexahydroxydiphenic acid	338
14.	Arjungenin	505
15.	Bellericoside	666
16.	Quercetine	302
17.	Kempherol	286
18.	Rutin	610
19.	Quercetin – 3-07-0-alpha rhamnopyranoside	756
20.	Quercetin-3-beta-glucoside	464
21.	Dihydroclerodin	436
22.	Quinic acid	191
23.	Mailc acid	133
24.	Galloyl hexoside	933
25.	puigluconin	801
26.	Grahatin	951
27.	corilagin	633
28.	Galloyl punicalagin	1235
29.	Trigaloxylhexoside	635
30.	Elgatannin	967
31.	Punicafolin	937
32.	Quercetin coumaroyl glucoside	609
33.	Vitexin	431
34.	Chebulic acid	355
35.	Cauarictin	935
36.	Myricetin rutine	935
37.	Myricetin glucoside	479

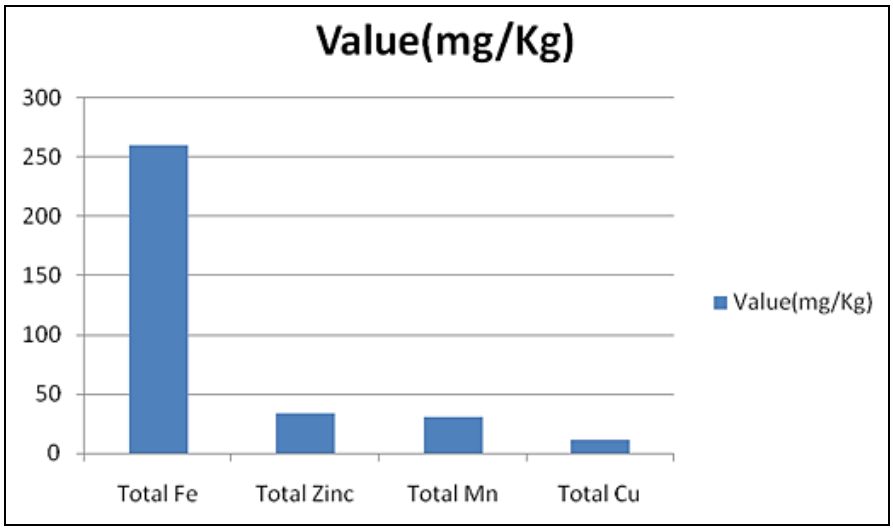

Chart 1. Minerals Assay by AAS in leaves of *T.bellerica*

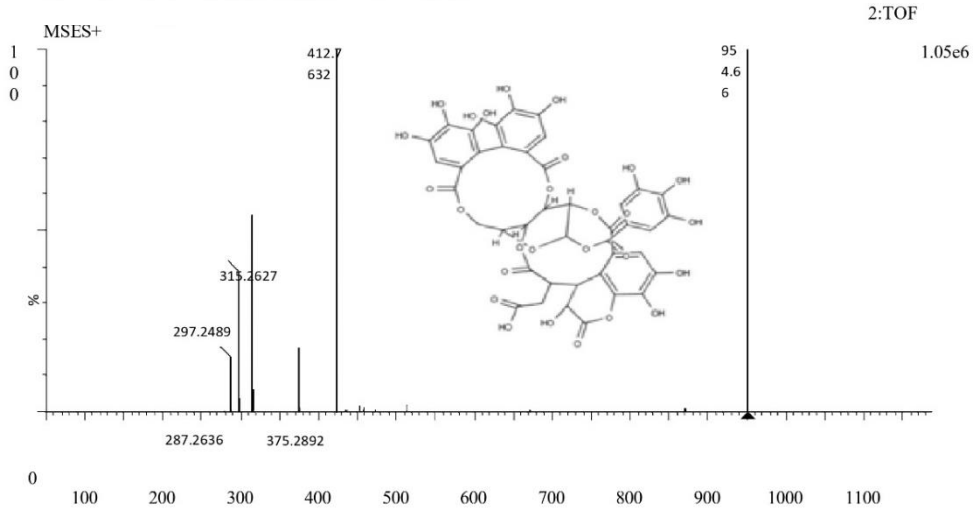

Fig. 1. Chebulagic acid, C41H30O27, Exact Mass:954.66

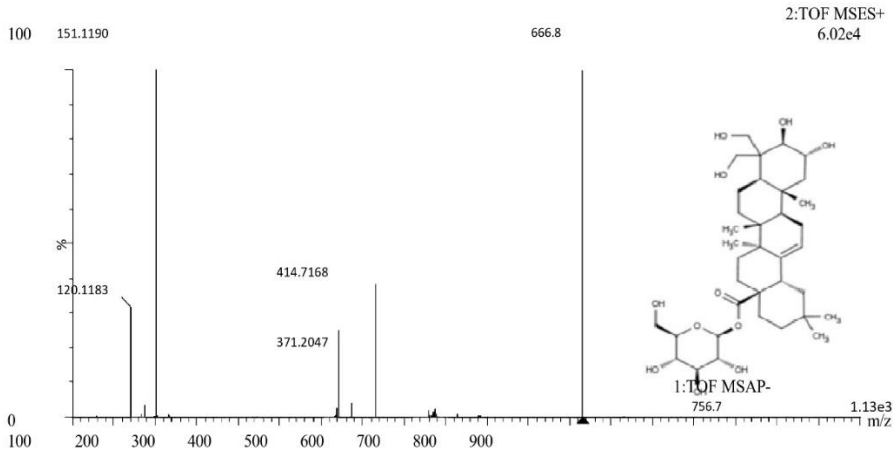

Fig. 2. Bellericoside,C36H58O11,Exact Mass:666.8

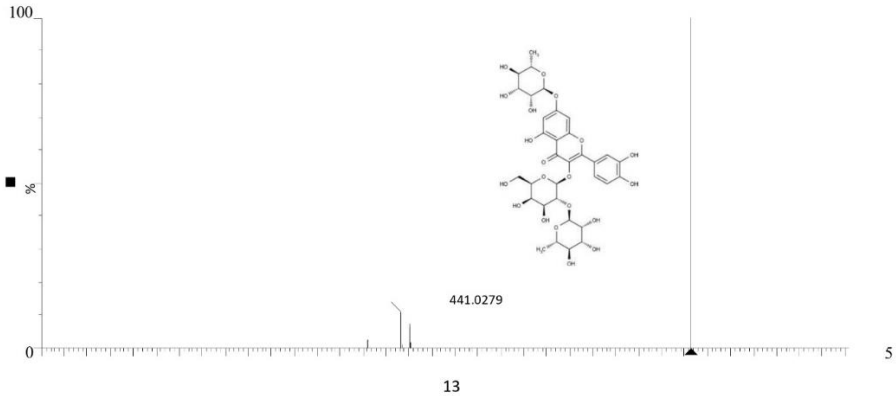

Fig. 3. Quercetin3-O-7-O-alpha-L-rhamnopyranoside,C33H40O20 Exact Mass:756.7

4. CONCLUSION

The LC-MS analysis recorded the leaves of *T.bellerica* consist of chebulinic acid, chebulagic acid, punicafolin, myricetin rutine, galloyl punicalagin in high amount. Based AAS study, leaves of *T.bellerica* could provide reservoir of iron and it could be great therapeutic potential with fewer side effects than to synthetic drugs.Due to the rich phyto nutrients, leaves of *T.bellerica* may be used in development of health supplements. Use of health hazards of chemicals in drug design and making it safe to human health, *T.bellirica* leaves are actively explored on the molecular docking studies for drug development in the pharma industry. Currently effectives of phyto constituents in plants to reduce many diseases are still in experimental stage and could be the source of scientific community for efficient drug development.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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