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Research Article

GC-MS ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS IN THE PETROLEUM ETHER LEAF EXTRACTS OF *MILLETTIA PEGUENSIS*

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ABSTRACT

Millettia peguensis is commonly known as Moulmein rosewood, is a legume tree species in the genus *Millettia* belongs to the family Fabaceae. The powdered leaf was successively extracted with Petroleum ether through Soxhlet apparatus. The leaves of Petroleum ether extract of *Millettia peguensis* by GC-MS analysis clearly showed the presence of 10 compounds. In GC-MS analysis, *Millettia peguensis* showed 3 major and 7 minor compounds. The results revealed that Pentadecane (32.73%), Tetradecane (29.79%) and Octadecane(22.77%) were reported as 3 major components in the Petroleum ether leaves extract of the *Millettia peguensis.* The seven minor compounds such as Eicosane (7.23%), Undecane, 5-methyl- (2.13%), 9-methylheptadecane (1.40%), Sulfurous acid, dodecyl hexyl ester (1.13%), Heptadecane, 2,6,10,15-tetramethyl- (0.99%), 2-Bromo dodecane (0.96%) and Heneicosane (0.87%) were also reported from leaves. The results indicated that the leaf extracts might be utilized as natural drug for the treatment of several infectious diseases.

Keywords: *Millettia peguensis,* GC-MS analysis, Soxhlet apparatus, Petroleum ether

INTRODUCTION

Traditional medicinal plants have been the origin and basis of pharmacology and therapeutics and played an important role in drug discovery. Plants have also contributed valuable drugs for medicines on the basis of traditional practices on medicinal plants. Plants are the goldmines to treat the diseases of men and animals and serve as cure in the natural way[1]. The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is found in 'Rigveda' which is said to have been written between 4500-1600B.C and is supposed to be the oldest repository of human knowledge.

Plants are used for the traditional medicine contains a wide range of substance used to treat chronic as well as infectious diseases. A vast knowledge of the plants used against different illnesses may expected to have accumulated in areas where the use of plants is still of great importances[2, 3]. Plant synthesizes a more amount of chemical compounds, which can be sorted by their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites. Primary metabolites make up the physical integrity of the plant cell and are involved with the primary metabolite process of building and maintaining of living cells.

In many parts of the world medicinal plants are used for antimicrobial and antiviral activity a plant derived drugs serve as a prototype to developed more affective and loss toxic medicinal. Tribal medicines have not been studied extensively. Infections disease is the number one among all causes of death, accounting approximately one-lady all deaths throughout the world. About 50-75% of hospital deaths are reported due to infectious diseases. These numbers are still increasing due to the development of resistance in microorganisms to the existing first line drugs[4]. Scientists from divergent fields are investigating plants with a new age for their antimicrobial usefulness and as an alternative source to existing drugs. Plants with their wide variety of chemical constituents offer a promising source of new antimicrobial activity.

Many studies have been undertaking with the aim of determining the antimicrobial and phyto-constituents of medicinal plants and using them for the treatment of microbial infections as possible alternatives to chemical synthetic drugs to which many infectious microorganisms have become resistants[5, 6]. The antimicrobial activity of plant extracts is due to different chemical agents in the extracts. These compounds are usually the secondary metabolites, which function to attract beneficial and harmful organisms, serve as phyto-protectants and respond to environmental changes in plants. In humans, however the compounds have beneficial effects[7, 8].

Millettia peguensis

Millettia is a genus of legume in the Fabaceae family. It consists of about 150 species, which are distributed in the tropical and subtropical regions of the world. The genus *Millettia* is known for the medicinal importance due to a rich source of variety of compounds. The major class of compounds of this genus is flavonoids. Moulmein Rosewood is a small deciduous tree, planted mostly for ornamental purposes. It is really beautiful when in full bloom. It blooms with racemes of mauve pea-like flowers. Leaves are pinnate and leaflets oval in shape. The tree may be confused with the Pongam Tree as the flowers appear the same. However, Pongam flowers are more

whitish compared to Moulminein Rosewood flowers. Looking just at the buds, one might confuse it with Mexican Lilac, however, Moulminein Rosewood has more drooping clusters like Amaltas.

This species is native to Lower Burma and Siam but it is cultivated in Burma, India and Pakistan. The medicinal and pharmacological properties of genus *Millettia* is highlighted below: The species of genus *Millettia* such as *M. conrauai* is well known for it's insecticidal and pisticicidal activities[9]. Some of the species *M. pachycarpa* inhibite the activities of murine retroviral reverse transcriptase and human DNA polymerases. Compounds; rotenone and 3α-hydroxyrotenone isolating from *M. pervilleana* has showed inhibition of TPA-induced ornithine decarboxylase at the level of its m RNA expression and recommended as promising cancer chemo-preventive agents. The prenylated isoflavanone, pervilleanone from *M. pervilleana* showing anticancer activities, while *M. ovalifolia* contains hypotensive agents. The flavonoids chalcones isolated from *M. ovalifolia* showed antimalarial activitiy[10] and Isoflavonoids; griffonianone and maximaisoflavone isolated from *M. griffoniana* showed significant cytotoxicity[9]. *M. dura* contains 6α, 12α-didehydro-6-oxodeguelin has good insecticidal activity and played a key role as a phytotoxin inhibitor of ornithine decarboxylase[11]. *M. griffoniana* contains griffonianone C which showed a potent estrogenic activity[9] and Millepurone has antitumour promoted reported from *M. atropurpurea.* Osajin isolated from *M. auriculata* has antioxidant activity while *M. duchusnei* contains rotenones used as potent insecticides. In some parts of Africa especially in Cameroon the plants of genus *Millettia* plants are used as a potent inhibitor of intestinal parasites in children's as well as colic besides oral treatment for boils by different communities.

MATERIALS AND METHODS

Materials

Plant Name	Family	IUCN status (2016.3)	Distribution
Millettia peguensis Ali	Fabaceae	Data Deficient	**Native:** Myanmar & Thailand **Intro:** India

Collection and Processing of plant materials

The fresh leaves of *Millettia peguensis* were collected from the campus of Gandhigram Rural Institute-Deemed University. The collected leaves were washed thoroughly under running tap water and air dried at room temperature for one month. The dried leaves were crushed into fine powder by using electric blender. The powdered samples were kept in sealed air tight containers until further use (Plate1-A&B).

Preparation of extracts

50g of dried leaf powder of *Millettia peguensis* was successively extracted with 250 ml of Petroleum ether solvents by using Soxhlet apparatus. The extraction procedures were continued for 3-4 hours at 60^0C. After complete extraction, respective solvent extracts were evaporated under reduced pressure and the dried extracts thus obtained were stored in air tight vials at 4°C for further study.

Phytochemical analysis by GC-MS

Gas chromatography-Mass spectrometry (GC-MS) analysis of the methanolic extracts was performed by using a GC-MS (Model; QP 2010 series, Shimadzu, Tokyo, Japan) equipped with a VF-5ms fused silica capillary column of 30 m length, 0.25 mm dia. and 0.25µm film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.99%) was used as a carrier gas at a constant flow rate of 1.51 ml/min. Injector and mass transfer line temperature was set at 200 and 240°C respectively. The oven temperature was programmed from 70 to 220°C at 10°C/min, held isothermal for l min and finally raised to 300°C at 10°C/min. 2 µ1 of respective diluted samples was manually injected in the split less mode, with split ratio of 1:40 and with mass 18 scan of 50-600 amu. Total running time of GC-MS is 35min. The relative percentage of the each extract constituents was expressed as percentage with peak area normalization.

Identification of phytochemical components

The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. NIST08s.LIB (Mc Lafferly, 1989) WILEY8. LIB (Stein, 1990) library sources were used for matching the identified components from the plant material.

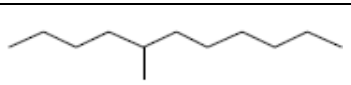
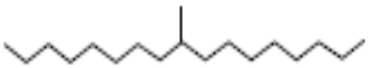
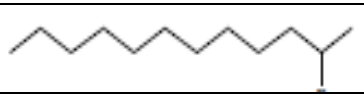
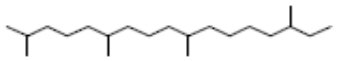
RESULTS

Phytochemical analysis by GC-MS

The GC-MS identification of the chemical constituents was based on comparison of their mass spectra with NIST and WILEY libraries. Structures were defined by percentage similarity values. The identification of the chemical compounds was confirmed based on the peak area, retention time, retention index, molecular formula, molecular weight of the compounds and structure. The active principles with their Retention time (RT), molecular formula, molecular weight of the compounds, structure, retention index (RI) and peak area as in percentage are presented in Table 1 and Plate1-C. The individual fragmentation of the components illustrated in Figure 1- 10.

In this study, the active principles of the leaves of Petroleum ether extract of *Millettia peguensis* by GC-MS analysis clearly showed the presence of 10 compounds. In GC-MS analysis, *Millettia peguensis* showed 3 major and 7 minor compounds. The results revealed that Pentadecane (32.73%), Tetradecane (29.79%) and Octadecane(22.77%) were reported as 3 major components in the Petroleum ether leaves extract of the *Millettia peguensis.* The seven minor compounds such as Eicosane (7.23%), Undecane, 5-methyl- (2.13%), 9-methylheptadecane (1.40%), Sulfurous acid, dodecyl hexyl ester (1.13%), Heptadecane, 2,6,10,15-tetramethyl- (0.99%), 2-Bromo dodecane (0.96%) and Heneicosane (0.87%) were also reported from leaves.

Table 1: List of chemical compounds identified from Petroleum ether leaf extracts of *Millettia peguensis* through GC-MS analysis

S.No	RT	Compound Name	Structure	RI	Molecular Formula	Molecular weight	Area%
1.	5.48	Undecane, 5-methyl-		1150	C12H26	170	2.13
2.	5.78	9-methylheptadecane		1746	C18H38	254	1.40
3.	6.38	Pentadecane		1512	C15H32	212	32.73
4.	9.79	Sulfurous acid, dodecyl hexyl ester		2434	C18H38O3S	334	1.13
5.	10.21	2-Bromo dodecane		1446	C12H25Br	248	0.96
6.	10.93	Tetradecane		1413	C14H30	198	29.79
7.	14.38	Heptadecane, 2,6,10,15-tetramethyl-		1852	C21H44	296	0.99
8.	15.64	Octadecane		1810	C18H38	254	22.77
9.	17.88	Heneicosane		2109	C21H44	296	0.87
10.	20.03	Eicosane		2009	C20H42	282	7.23

RT-Retention Time; RI-Retention Index

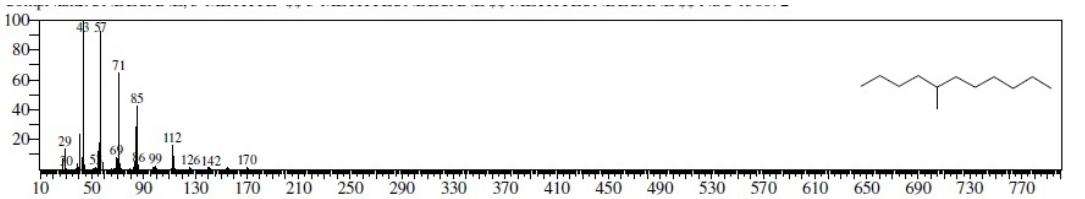


Figure 1: Undecane, 5-methyl-

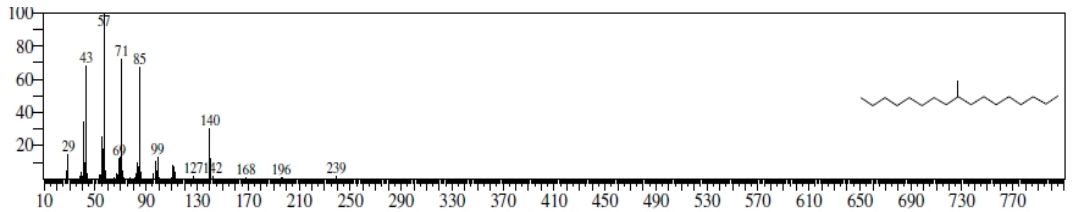


Figure 2: 9-methylheptadecane

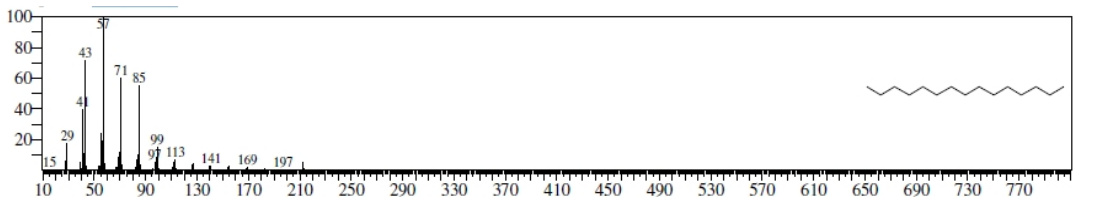


Figure 3: Pentadecane

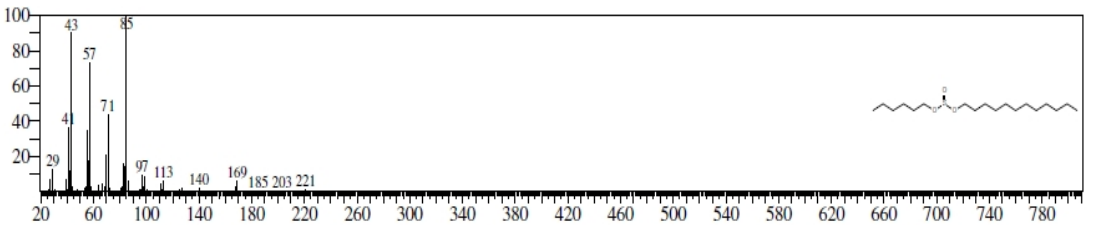


Figure 4: Sulfurous acid, dodecyl hexyl ester

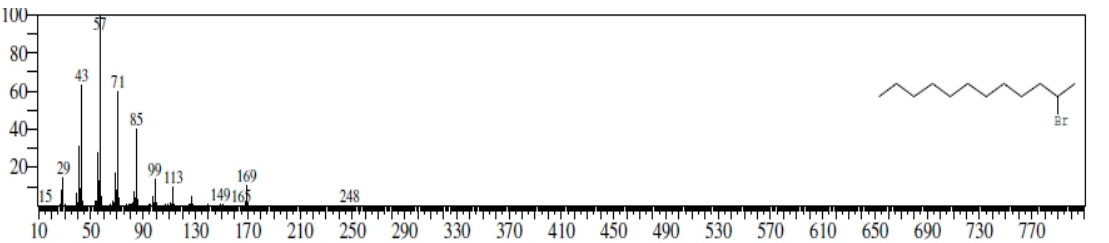


Figure 5: 2-Bromo dodecane

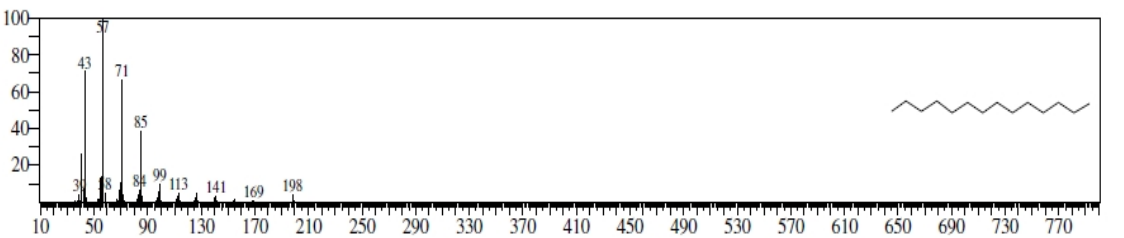


Figure 6: Tetradecane

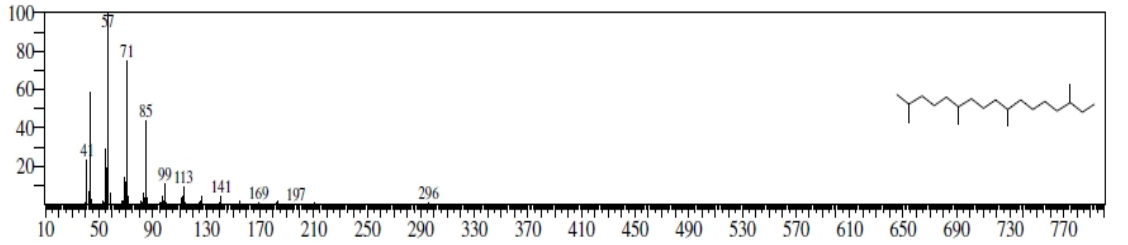


Figure 7: Heptadecane, 2,6,10,15-tetramethyl-

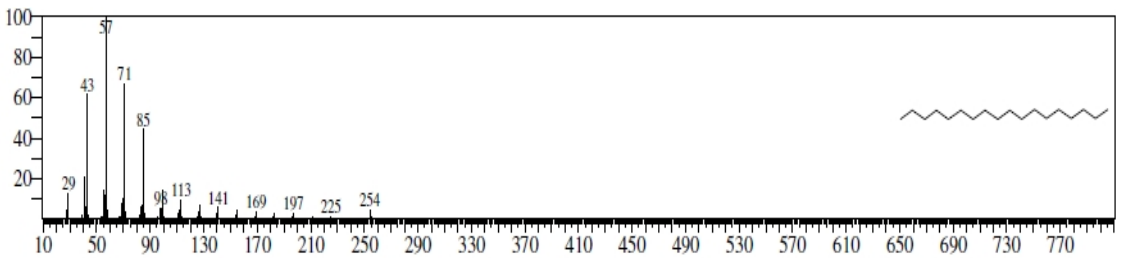


Figure 8: Octadecane

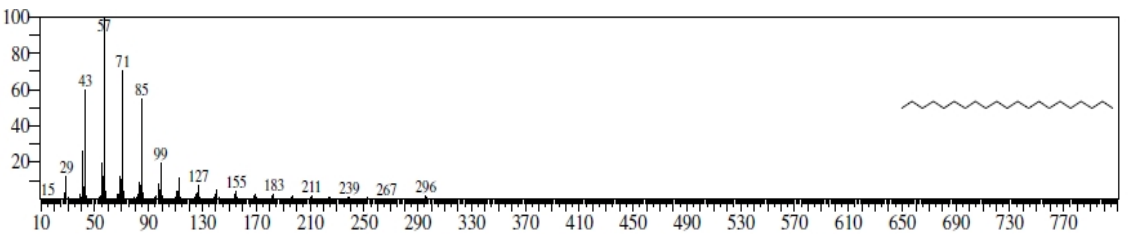


Figure 9: Heneicosane

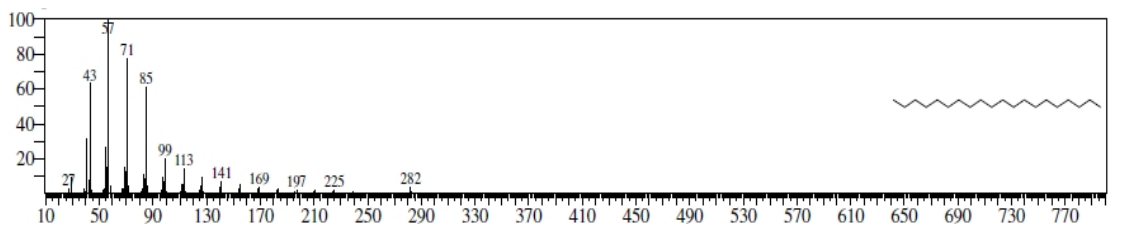
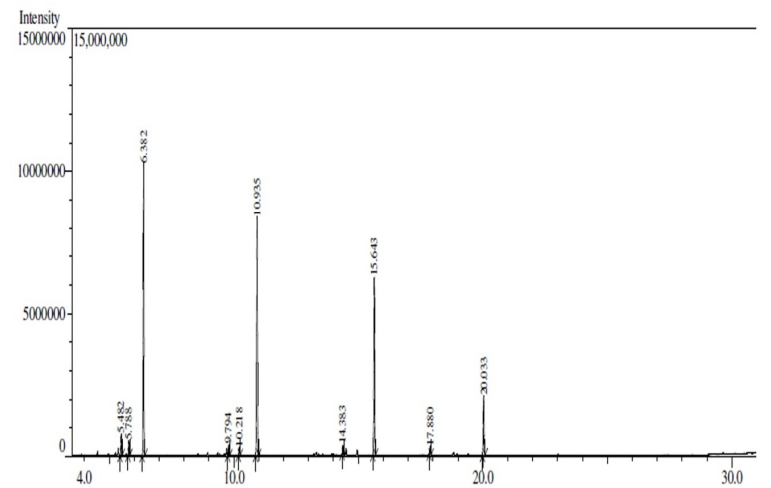


Figure 10: Eicosane

PLATE 1



C

A - Leaf twig of *Millettia peguensis*; B - Flower twig of *Millettia peguensis*;
C - GC-MS Chromatogram of leaves extract (Petroleum ether) *Millettia peguensis*

The first compound identified with less retention (5.48 min.) was Pentadecane whereas Eicosane was the last compound which took longest retention time (20.03 min.) to identify. At (6.38) retention time Pentadecane compound were found to be very high (32.73%) and the lowest percentage (0.87%) was found to be Heneicosane. The above mentioned isolated compound from the Petroleum ether extract of *Millettia peguensis* leaves have a medicinal and economic important.

DISCUSSION

Natural products perform various functions and many of them have interesting and useful biological activities[12]. Plant species being used in various human cultures around the world for medicinal purpose. Use of herbal medicine represents a long history of human interactions with the environment. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases[13].

The use of plant extracts and phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments. In this, present study the leaves of Petroleum ether extracts of *Millettia peguensis* clearly showed the 10 compounds identified through GC-MS analysis. The results revealed that Pentadecane (32.73%), Tetradecane (29.79%), Octadecane(22.77%), Eicosane (7.23%), Undecane, 5-methyl- (2.13%), 9-methylheptadecane (1.40%), Sulfurous acid, dodecyl hexyl ester (1.13%), Heptadecane, 2,6,10,15-tetramethyl- (0.99%), 2-Bromo dodecane (0.96%) and Heneicosane (0.87%) were rported. At (6.38) retention time Pentadecane compound were found to be very high (32.73%) and the lowest percentage (0.87%) was found to be Heneicosane

was observed in Petroleum ether leaf extract of *Millettia peguensis*. All the compounds have a medicinal and economic important.

Yayli *et al.*[14] reported that the essential oil of *Minuartia meyeri* was analyzed by GC-MS with an HP-5 column and 52 components were identified on the basis of a typical library search, selecting only the components showing matches exceeding 80%, which represented about 68.2% of the total detected constituents. Nonacosane (6.2%), 6, 10, 14- trimethyl-2-pentadecanone (5.1%), nonanal (4.6%), and β-caryophyllene (2.9%) were the major compounds in the essential oil. The essential oil extract of *M. meyeri* showed antibacterial activity against *Y. pseudotuberculosis, E. faecalis* and *S. aureus,* but no considerable antimicrobial activity was observed against the *E. coli, K. pneumoniae, S. marcescens* and *B. subtilis,* and the fungi *C. albicans* and *C. tropicalis.* Results obtained were in accordance with the present research work.

Garicina cowa are an important source of bioactive compounds and the fruit, twig and stem are the best source of metabolites, thirty compounds have been isolated from *G. cowa*, i.e. one depsidone, one *α,*β -unsaturated cyclohexenone, three flavonoids, six phloroglucinols and nineteen xanthones. Some of these compounds showed interesting pharmacological activities against pathogenic microbes. *α*-Mangostin, cowanol and cowanin are commonly found in all parts of *G. cowa*[15].

CONCLUSION

The analysis of *Millettia peguensis*, an important medicinal tree species suggests that the phyto-chemical constituents identified through GC-MS analysis. In, the present study the leaves of Petroleum ether extract of *Millettia peguensis* by GC-MS analysis clearly showed the presence of 10 phyto-compounds. The results indicated that the leaf extracts might be utilized as natural drug for the treatment of several infectious diseases. So, the results thus concluded that *Millettia peguensis* leaves possess various potent bioactive compounds and is recommended as a plant of phytopharmaceutical importance.

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