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GC-MS analysis and antioxidant potential of wild underutilized medicinally important legume, velvet bean (*Mucuna pruriens* L. DC.)

Kamlakar C. MORE[1]*, Deepak B. SHELKE[2]*, Sunil TAYADE[1], Prashant GAWANDE[1], Hiralal B. SONAWANE[3]

[1]*Sant Gadge Baba Amravati University, Department of Botany, Amravati (M.S.), India; kamlakarmore@sgbau.ac.in (*corresponding author); suniltayade002@gmail.com; prashantgawande@sgbau.ac.in*
[2]*Amruteshwar Art's, Commerce and Science College, Department of Botany, Vinzar, Velha, Pune-412213, MS, India; dpk.shelke1@gmail.com*
[3]*PG Research Centre in Botany, Prof. Ramkrishna More Arts, Commerce and Science College, Akurdi, Pune- 411044, India; amolsbr@gmail.com*

Abstract

Mucuna pruriens (L). DC is one of the most promising wild underutilized medicinal legume belonging to family Fabaceae. It is used in ayurvedic as well as various traditional systems of medicine. This plant was widely utilized in treatment of various disorders. Also, it is a rich source of nutrients as well as used as a flavouring agent in bakery industry. The present study was aimed to investigate leaves and seeds antioxidant potential by DPPH assay and phytochemicals by preliminary phytochemical screening and Gas Chromatography-Mass Spectroscopy (GC-MS) analysis in five different solvents. Highest antioxidant activity was found to be 76.96% in seeds extracted with ethanol and 72.50% in leaves extracted with petroleum ether. While preliminary phytochemical screening revealed presence of alkaloids, flavonoids, phenols, tannins, saponins, glycosides, steroids and terpenoids. GC-MS analysis revealed twenty-four and thirty bioactive compounds from the leaves and seeds respectively and it was solvent specific. Antioxidant, antifungal, antimicrobial, anti-malarial, anti-diabetic, anti-cancerous, and hypocholesterolemic properties have been reported to compounds which were found in present study. However, reported bioactive compounds highlight its nutritional importance and validate the use of the plant to cure various disorders by traditional practitioners. While the antioxidant potential and phytochemical investigations will direct their potential for utilization and applicability as a nutraceutical.

Keywords: antioxidants; GC-MS; *Mucuna pruriens*; phytochemicals

Introduction

Herbal remedies were used to treat various diseases from ancient times. The phytoconstituents derived from natural sources create an attention for researchers due to their potential use in therapeutic treatment (Bhusare *et al.*, 2021a). In recent days huge amounts of synthetic medicines are explored but traditional remedies are gaining popularity day by day. Also, structural complexity makes chemical synthesis of important metabolite an unviable option, and makes plants the only source (Bhusare *et al.*, 2021b). However, the

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alternative eco-friendly herbal medicines are gaining huge attention due to ease of availability and their potential benefits (Philomena, 2011; Sahoo and Manchikant, 2013). The plant derived phytoconstituents classified into two categories like primary and secondary metabolites depending upon their role and properties. The primary metabolite includes amino acids, proteins, lipids and carbohydrates etc. which play an important role in growth and development of plants (Jan *et al.*, 2021). Whereas, the secondary metabolites *viz* alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds, cardiac glycosides playing crucial role in plant defence mechanism against different stresses like biotic and abiotic stress (Edeoga *et al.*, 2005). Moreover, these primary metabolites act as an important food source for growth and development of human being and secondary compounds have been used as medicines, flavourings, or relaxing drugs in human life (Savithramma *et al.*, 2011; Umdale *et al.*, 2020; Umdale *et al.*, 2021; Sonawane *et al.*, 2021). The biotic and abiotic factors significantly influencing crop plants leads to radical decreases in crop production (Shelke *et al.*, 2017). On the other hand, ever increasing global population increases food demand day by day. To accomplish global food demands, wild plants gain popularity as an important food source (Duguma, 2020). Wild plants have higher abiotic and biotic stress resistance than cultivated crop plants and also a rich source of nutrients (Nikalje *et al.*, 2019). Moreover, these plants have a traditional history of their utilization as a food. Noteworthy there utilization along with cultivated crop plants helps to realize the global nutritional food demands.

A) B)

Figure 1. A) Flowering twig and B) seeds of *Mucuna pruriens* (L). DC

The *Mucuna pruriens* (L). DC is one of the most promising medicinal plants used in the ayurvedic system, and in various traditional medicinal systems of different countries (Simmons, 2018; Lampariello *et al.*, 2012). It is commonly known as velvet bean and belongs to the family Fabaceae (Figure 1) (Mishra and Wagner, 2006). This plant possesses vital applications such as sedative, to relieve urinary tract infections, treatment of chest complaints, to treat snake bite intoxication and used in flavouring agent in cakes, sweet breads and candy (Devhade *et al.*, 2015). Also, it is a rich source of nutrients (Shanmugavel and Krishnamoorthy, 2018). It was utilized as a herbal drug to treat male infertility and nervous disorders (Lampariello *et al.*, 2012). This plant is also utilized for treatment of Parkinson's disease (PD) (Longhi *et al.* 2011). There are many reports on neurodegenerative diseases including PD causes due to lack of antioxidants. Noteworthy, free radicals generated in cells cause more than forty-five different diseases (Tripathi and Upadhyay, 2002). However, various plants are a rich source of antioxidants. The various metabolites from plants are mainly associated with antioxidant activity and have medicinal significance. Therefore, the present investigation aims to reveal

metabolites from wild underutilized medicinally important legume *Mucuna pruriens* (L.) DC. leaves and seeds and their bioactive potential for medicinal significance.

Materials and Methods

Collection of plant material

The plant material of *Mucuna pruriens* (L.) DC. was collected from Sant Gadge Baba Amravati University Campus, Amravati (M.S) India. According to the phonological calendar the frequent visits were made to field habitat for collection of samples.

Identification of plant material

Identification of plant material was done with the help of standard flora; The Flora of British India, Flora of Amravati District (Hooker, 1875; Dhore, 1986). The herbarium specimens were prepared for individual plants and submitted to the Department of Botany, Sant Gadge Baba Amravati University, Amravati.

Sample preparation

The dry pods and fresh leaves were collected from *Mucuna pruriens* (L.) DC. Further seeds were removed from dry pods. The immature and infected or having diseased condition seeds were sorted out. The fresh and clean seeds were cut into small pieces by using a knife and small pieces of seeds were grind by electric mixture grinder. Further grind seeds and fresh leaves were crushed in liquid nitrogen. Prepared leaves and seeds powder were stored in an airtight plastic container and preserved in the refrigerator for further experimentation.

Extraction

The 10-gram powder was filled in the thimble (made up of filter paper) and extracted successively with petroleum ether, chloroform, acetone, ethanol, and methanol solvent in 180 ml for 24 hours using Soxhlet extraction assembly. The temperature of the apparatus was maintained at the boiling point for each solvent. The extractions were carried out using above different solvents with specific characteristics and in order of increasing values of their polarity. The obtained extracts were filtered through Whatman filter paper no.42 for free and clear extract. These extracts were evaporated and concentrated up to 10 ml. The resultant 10 ml extract was again filtered and stored in small sterile airtight bottles at 4 °C temperatures in the refrigerator.

Evaluation of Antioxidant activity by DPPH

A stable free radical DPPH (1, 1-diphenyl-2-picrilhydrazyl) was used to calculate the antioxidant activity, the effect of test samples on DPPH radical was estimated according to the procedure described by Von Gadow *et al.* (1997). A 50 µl sample (10 mg ml^{-1}) was reacted with 2 ml of 0.06 Mm DPPH (HiMedia, India) prepared in methanol solution. The decrease in absorbance at 515 nm was continuously recorded in a spectrophotometer for 16 minutes at room temperature. The methanolic solution of standard ascorbic acid was tested at 10 mg/ml concentration. The percentage of DPPH radical scavenging ability of the sample was calculated from the absorbance value at the end of 16 min duration. All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh (1994).

Inhibition percentage = $[(AC (0) - AA (t)/ AC(0))] \times 100$

Where: AC (0) is the absorbance of the control at t = 0 min;

AA (t) is the absorbance of the antioxidants at t = 16 min.

Preliminary phytochemical screening

The preliminary phytochemical screening was performed for all the extracts as per standard method (Peach and Tracy, 1955; Harborne, 1998; Tiwari *et al.*, 2011) for testing the different chemical groups such as alkaloids, flavonoids, phenol, tannins, glycosides, saponins, terpenoids and steroids present in petroleum ether, chloroform, acetone, ethanol, methanol and water extracts. For every chemical group two tests were selected for confirming their presence in the above solvent extract. Alkaloids detected by Mayer's reagents and Wagner reagents test, phenols detected by ferric chloride and lead acetate test, terpenoids and steroids were detected by using Salkowski test, tannins were detected by gelatine and lead acetate test, flavonoids detected by alkaline and lead acetate test, glycoside were detected by using killer-killani and sodium hydroxide test, saponin detection were performed by froth and foam test. All these tests were performed with six different solvent extracts obtained from each powder sample.

*Gas Chromatography-Mass Spectroscopy (*GC-MS*) analysis*

The extracts derived from five different solvents viz. petroleum ether, chloroform, acetone, ethanol, and methanol were worked out at Department of Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology (IIT) Bombay, Powai, Mumbai, Maharashtra, India. The aliquots (2 µl) were analysed using the GC–MS system (Agilent technologies 7880) equipped with the HP5 column (30 mm length, 0.25 mm I.D., 0.32 µ film thickness). The helium was used as a carrier gas with 1 ml min^{-1} flow rate. Injector temperature was set at 100 °C. The oven temperature was programmed from 50 °C to 280 °C at 10 °C/ minute to 200 °C then 10 °C /3 minute to 250 °C ending with a 5-minute isothermal at 280 °C. The sample was injected in split mode as 50:1. The electron ionization and detector were used to obtain mass spectra (MS) of compounds at 70eV.

Identification of compounds

The identification of compounds was based on retention time, fragmentation patterns along with these m/z values. The mass spectra of the unknown compound obtained from sample extract by GC-MS were matched with mass spectra of the known compounds stored in the database of National Institute Standard and Technology (NIST) library. Their structures were defined by the percent (%) similarity values and the name, molecular weight, molecular formula and structure of the compounds were identified. The biological activity was determined by comparing with Dr. Duke's Phytochemical and Ethno-botanical database (Duke's, 2013). For quantitative analysis the relative quantity of each compound was calculated by formula as follows (https://www.nist.gov/).

$$\text{The relative quantity of compounds} = \frac{chromatographic\ peak\ area\ of\ each\ compound}{total\ peak\ area\ of\ all\ chromatographic\ peaks} \times 100$$

Statistical analysis

The antioxidant activities were performed in the triplicate and data were subjected to one-way analysis of variance (ANOVA) test using the SPSS statistical software version SPSS 20 for statistical analysis. The data was expressed as mean ± standard error (S.E.). The mean was compared by Duncan's multiple range tests for significant difference between various solvent at $p < 0.05$ significance level. While student t tests were performed to find significant difference between leaves and seeds antioxidant activity at $p < 0.05$ significance level (Bhusare *et al.*, 2018). Principal component analyses (PCA) were done using the PAST statistical package (Manker *et al.*, 2021). PCA was performed to visualize metabolites difference among used solvents.

Results and Discussion

The awareness about the biological activities of herbal medicines is increasing day by day owing to its cost effectiveness and lesser side effects. Herbal medicines are found to be safer than synthetic medicines because the phytochemicals in the plant extracts targets the biochemical pathways (Philomena, 2011; Sahoo and Manchikant, 2013; Moreira *et al.*, 2014). The medicinal plant species have been used all over the world for the treatment and prevention of various ailments. Medicinal plants are also known to have antioxidants and antimicrobial compounds that can be utilised for sustained resistance of existing antibiotics (Verpoorte, 1998; Kasote *et al.*, 2015; Casagrande *et al.*, 2018). It is now very well understood that each and every part of the plants possesses one or the other natural constituent having an impact of medicinal values (Savithramma *et al.*, 2011; Zia-Ul-Haq *et al.*, 2012; Jahanban-Esfahlan *et al.*, 2019). Similarly, in the present study, leaf and seed extracts of *Mucuna pruriens* exhibited potential free radical scavenging activity. The various compounds act as antioxidants and play an important role to control free radicals generated in cells due to various chemical reactions (Longhi *et al.*, 2011). These generated free radicals can cause 45 different diseases in humans (Tripathi and Upadhyay, 2002; Kehrer and Klotz, 2015; Phaniendra *et al.*, 2015). Plants are an important source of antioxidants which control the free radicals. However, maximum antioxidant activity of *Mucuna pruriens* in the form of percentage of inhibition were exhibited and it was found to be 76.96% in seeds extracted with ethanol, followed by 72.50% in leaves extracted with petroleum ether (Figure 2). Similarly, 54.49% and 43.08% of inhibition were observed in methanolic and chloroform extract of *Mucuna pruriens* leaves respectively, the moderate antioxidants scavenging potential observed in seed extracts were found to be 63.38% in petroleum ether, 32.53% in acetone extracts.

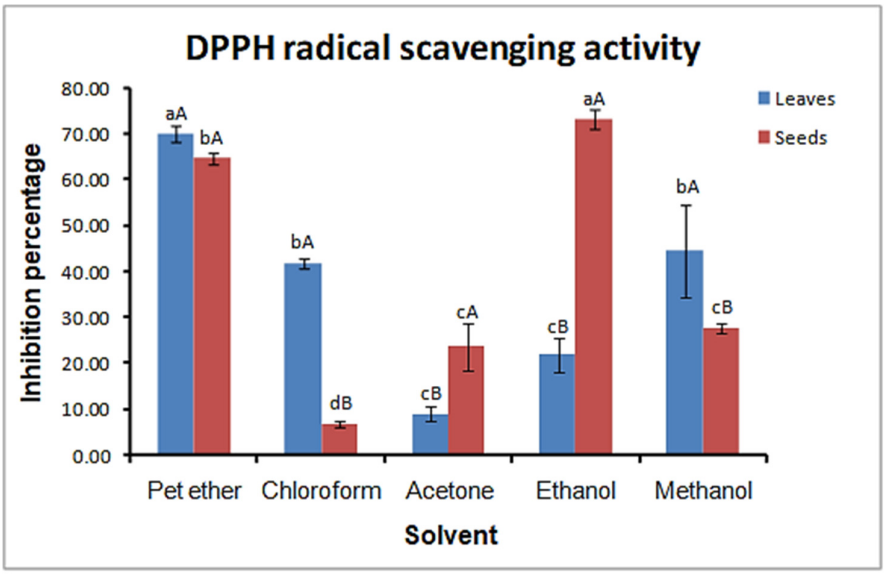

Figure 2. The DPPH radical scavenging activity of leaves and seeds extracts of *Mucuna pruriens* (L.) DC
The small letter on error bars denotes the significant difference between various solvents and capital letter denotes significant difference between leaves and seeds at significance level 0.05.

The preliminary phytochemical screening of leaves and seed extracts of *Mucuna pruriens* revealed the presence of different phytoconstituents and it was found to be variable in different solvents (Tables 1 and 2). The leaves and seed extracted in six different solvents namely petroleum ether, chloroform, acetone, ethanol, methanol and water have shown presence of alkaloids, flavonoids, phenols, tannins, saponins, glycosides, steroids and terpenoids. However, the leaves extracted in ethanol and methanol show significant results for flavonoids, phenols, tannins, saponins and glycosides. With the exception of saponins, steroids and terpenoids,

other phytoconstituents like flavonoids, phenols, tannins and glycosides were also observed in ethanolic and methanolic seed extracts. Likewise, Dhawan and Gupta (2017) studied the comparison of different solvents for phytochemical extraction potential from leaves of *Datura metel*. All these phytochemicals derived from leaves were also observed in aqueous extracts except phenols and glycosides. Seeds extracted with water also showed the presence of alkaloids, flavonoids, phenols, tannins and saponins. The leaves extracted with ethanol and acetone has not shown the presence of alkaloids in preliminary screening. However, seed extracted with acetone showed presence of flavonoids, phenols, tannins, glycosides, steroids and terpenoids. The common secondary metabolites which were considered in preliminary phytochemical screening are not observed in petroleum ether and chloroform extracts of leaves but seeds extracted in petroleum ether and chloroform showed presence of tannins, saponins, steroids and terpenoids. The plants synthesized secondary metabolites are the important source for pharmaceutical industries (Revathi *et al.*, 2014; Kabera *et al.*, 2014; Phaniendra *et al.*, 2015).

Table 1. Preliminary phytochemical screening of leaves extracts of *Mucuna pruriens* (L.) DC.

Phyto-chemicals	Phytochemical test	Solvents					
		Pet. Ether	Chloro-form	Acetone	Ethanol	Methanol	Water
Alkaloids	Mayers reagents	-	-	-	-	+	++
	Wagner reagent	-	-	-	-	+	++
Flavonoids	Alkaline reagent	-	-	+	+++	+++	++
	Lead acetate	-	-	++	++	++	++
Phenols	Ferric chloride	-	-	+	+++	+++	-
	Lead acetate	-	-	+	+++	+++	++
Tannins	Gelatine	-	-	-	++	++	+
	Lead acetate	-	-	+	+++	+++	+++
Saponins	Froth	-	-	-	++	++	+++
	Foam	-	-	-	++	++	+++
Glycosides	Sod. hydroxide	-	-	++	+++	++	-
	Killer Killani	-	-	+	++	+++	-
Steroids	Salkowski	-	-	-	+	+	++
Terpenoids	Salkowski	-	-	-	+	+	+++

(+++) = Highly present, (**++**) = Moderately Present, (**+**) = Present, (-) = Absent

Table 2. Preliminary phytochemical screening of seeds extracts of *Mucuna pruriens* (L.). DC.

Phyto-chemicals	Phytochemical tests	Solvents					
		Pet. Ether	Chloro-form	Acetone	Ethanol	Methanol	Water
Alkaloids	Mayers reagents	-	-	-	+	+	++
	Wagner reagent	-	-	-	+	+	++
Flavonoids	Alkaline reagent	-	-	+	++	+++	++
	Lead acetate	-	-	+	++	++	++
Phenols	Ferric chloride	-	-	+	+++	++	++
	Lead acetate	-	-	+	+++	++	++
Tannins	Gelatine	+	-	+	++	++	++
	Lead acetate	+	-	+	++	++	+++
Saponins	Froth	+	+	-	-	-	+++
	Foam	+	+	-	-	-	+++
Glycosides	Sod. hydroxide	-	-	+	+++	++	-
	Killer Killani	-	-	+	++	++	-
Steroids	Salkowski	+	+	+	-	-	-
Terpenoids	Salkowski	+	+	+	-	-	-

(+++) =Highly present, (**++**) = moderately present, **(+)** = present, (-) = absent

The mass spectrum derived from GC-MS was interpreted by using the database of National Institute Standard and Technology (NIST). The mass spectrum of observed unknown compounds was identified with the spectrum of known components of the NIST library. The bioactive principles along with their retention time (RT), relative percentage (%), class, molecular formula, molecular weight (MW) and biological activities are presented in (Tables 3 and 4) and chromatogram in Figures 3 and 4. From the observations it was revealed that the phytoconstituents extracted in five different solvents showed bioactive potential.

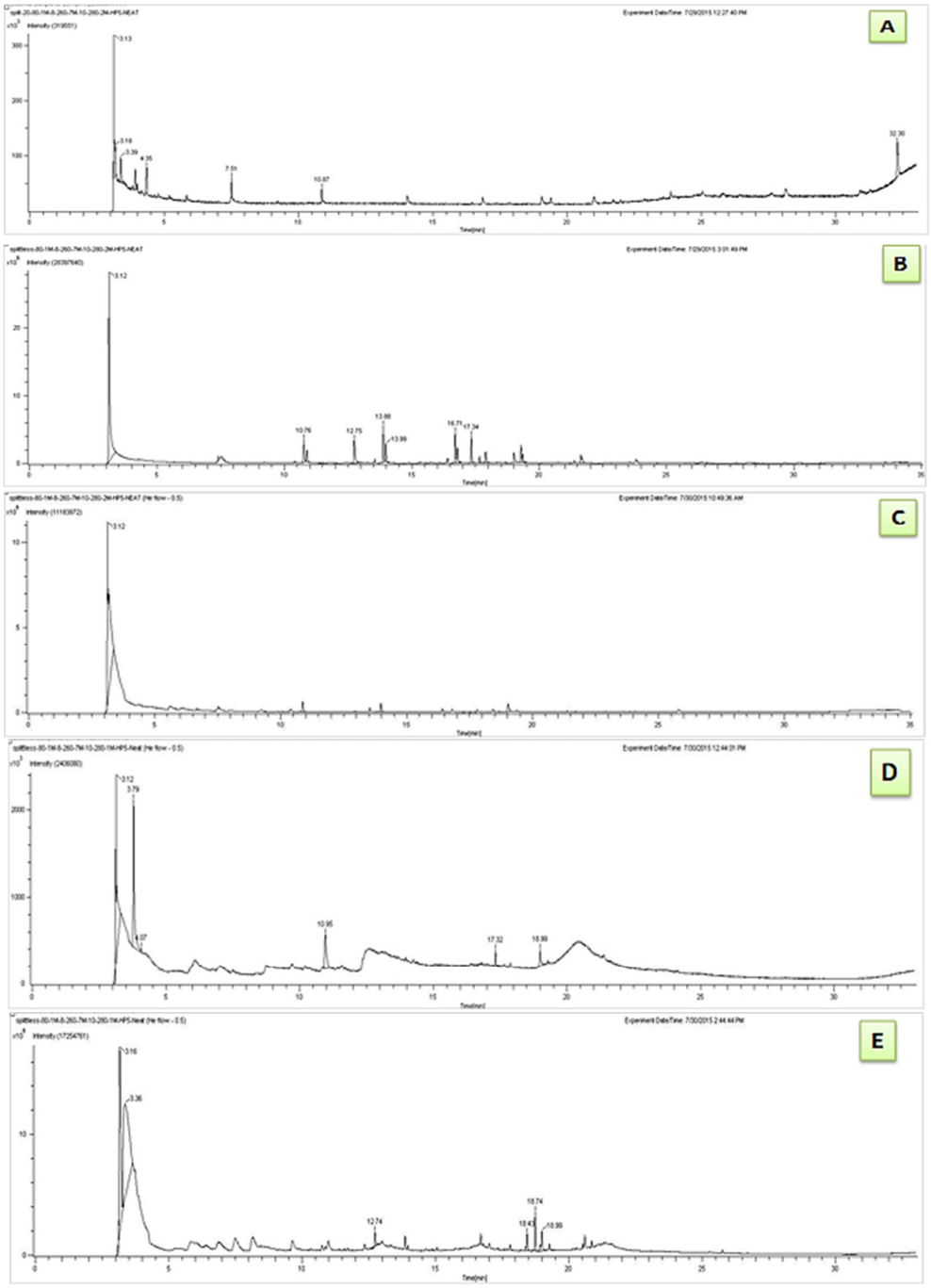

Figure 3. Gas Chromatography-Mass Spectrometry (GC-MS) chromatograms of A) petroleum ether B) chloroform C) acetone D) ethanol E) methanol leaves extract.

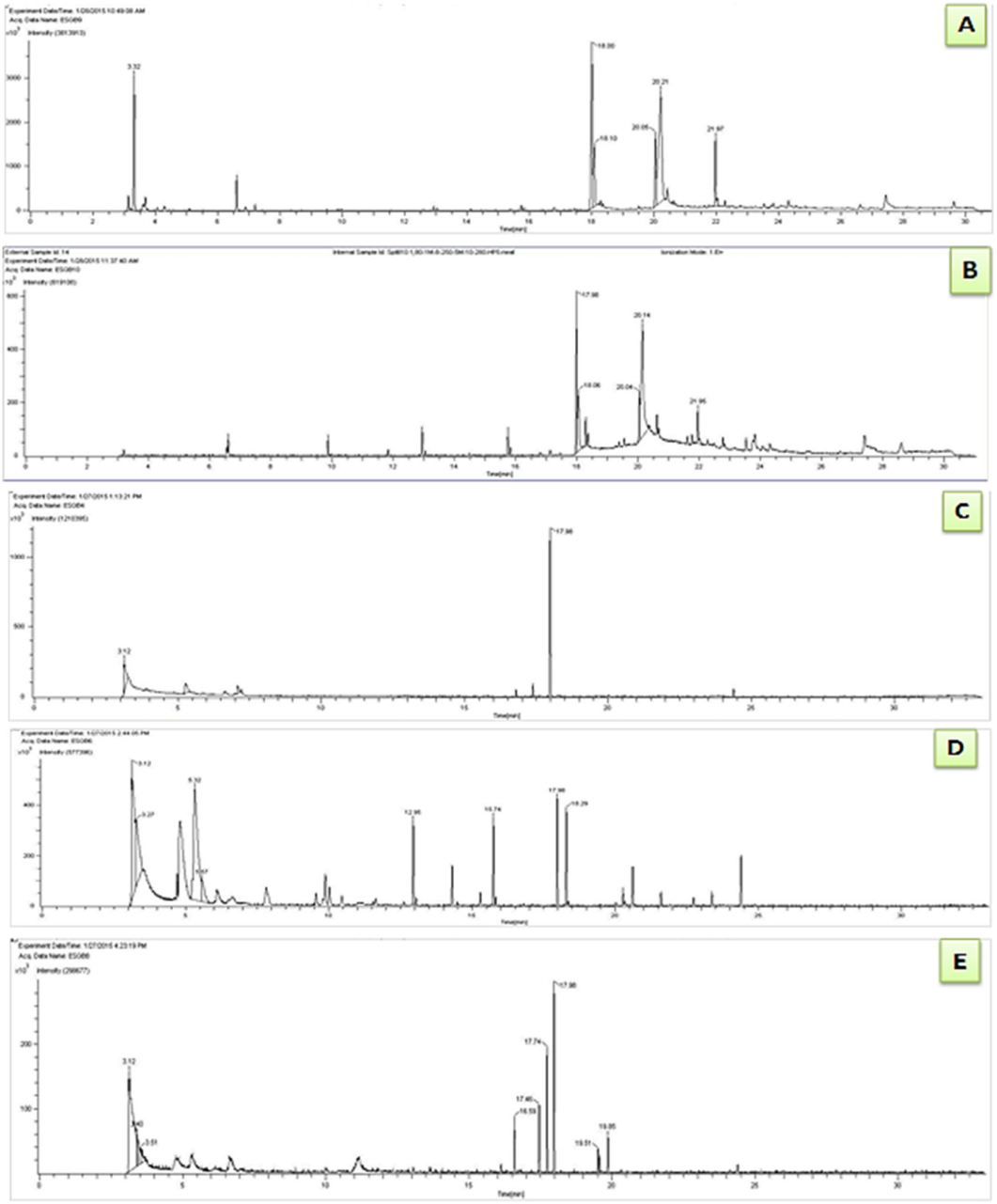

Figure 4. Gas Chromatography-Mass Spectrometry (GC-MS) chromatograms of A) petroleum ether B) chloroform C) acetone D) ethanol E) methanol seeds extract

Leaves methanolic extract were showed 2.25% of Benzenepropanoic acid, 3,5-bis (1, 1-dimethylethyl)-4-hydroxy-methyl ester compound has antioxidant, antibacterial and antifungal potential (Bashir *et al.*, 2012) which were also reported in *Azadiracta indica* leaves extracted in hexane (Akpuaka *et al.*, 2013; Airaodion *et al.*, 2019). 1-Tetradecene found in *Mucuna pruriens* leaves chloroform extract showed 5.34% with anti-tuberculosis and antifungal potential as per earlier investigation in *Croton bonplandianum* leaves extracted in chloroform (Kalaivani *et al.*, 2013). Tetradecane extracted in chloroform from leaves of *Mucuna pruriens* were found to be 2.60% and in acetone extract 3.94 with antioxidant, antimicrobial and preservative properties earlier reported in *Azadiracta indica* leaves extracted in hexane (Akpuaka *et al.*, 2013; Airaodion *et al.*, 2019).

Phenol 2,4-bis[1,1-dimethyl ethyl] present in chloroform leaves extract of *Mucuna pruriens* contained 6.11% and methanol extract 2.05% with antioxidant and antimicrobial properties as reported in fungus *Monochaetia kansensis* by Yogeswari *et al.* (2012). 1-Hexadecene observed in chloroform leaves extracts of *Mucuna pruriens* where 2.97% is an antibacterial agent observed in fungus *Monochaetia kansensis* by Yogeswari *et al.* (2012). Nonadecane obtained from chloroform leaves extracts of *Mucuna pruriens* showed 2.93% with antioxidant properties previously observed in *Epiphyllum oxypetalum* leaves alcoholic extract (Dandekar, 2015). Hexadecane identified in leaves acetone extracts of *Mucuna pruriens* were 2.63% identified as antioxidant and antimicrobial compounds reported in hexane extract of Malaysian red algae by Zakaria *et al.* (2011). 9,12,15-Octadecatrienoic acid methyl ester derived from *Mucuna pruriens* leaves methanolic extract contained 1.55% as a potent anti-inflammatory anti-arthritic, anti-coronary, cancer preventive significance (Ganesh and Mohankumar, 2017). Compound obtained from leaves extracted in petroleum ether are 2-Phenyl-hex-5-en-5-ol (3.20%), Guanidine, methyl (10.80%), Benzamide, N-[2-[4-[[5-[3,3-dimethyloxiranyl]-4-hydroxy-3-methyl-2 pentenyl]oxy]phenyl]ethyl] (15.83%), 2,4,5-Trihydroxypyrimidine (7.30%), Nonane (5.16%) possess remarkable therapeutic potential like antioxidant, antimicrobial, antidiabetic, antiviral, antiinflamatory and antifungal activity. In addition ethanolic extracts revealed the occurrence of 4-Methyl [trimethylene] silyloxyoctane (3.94%), α -D-Glucopyranoside, O-α-D-glucopyranosyl-[1,fwdarw3]-β-D-fructofuranosyl (2.63%) with preservative potential previously reported in ethanolic extract of whole plant shade dried *Mussaenda frondosa* (Gopalkrishanan and Vadivel, 2011). Benzofuran, 2, 3-dihydro (3.14%) found in methanolic extract showed antioxidant and anti-inflammatory properties also mentioned in *Burguiera cylindrica* leaves extracted in ethanol (Revathi *et al.*, 2014).

Table 3. Bioactive compounds identified in different solvent extract of *Mucuna pruriens* (L.). DC. leaves

S.N.	RT	Phytochemicals	Rel. %	Class	MF	MW	Biological activity
Petroleum ether							
1	3.18	2-phenyl-hex-5-en-3-ol	3.20	Phenol	$C_{12}H_{16}O$	176	Not reported
2	3.39	Guanidine, methyl	10.80	Purine base	$C_2H_7N_3$	73	Anticancer, Antibiotic, Anti-diabetic (Saczewski and Balewski, 2009)
3	3.93	Acetic acid, sodium salt	12.14	Carboxy-Licacid	$C_2H_3NaO_2$	82	Antibacterial, Antifungal, Antioxidant (Sallam, 2007)
4	4.35	Benzamide,N-[2-[4-[5-[3,3-dimethyloxiranyl]-4-hydroxy -3-methyl-2-pentenyl]oxy] phenyl]ethyl	15.83	Amine	$C_{25}H_{31}NO_4$	409	Not reported
5	7.51	2,4,5-Trihydroxypyrimidine	7.30	Amine	$C_4H_4N_2O_3$	128	Antioxidant, Antimicrobial (Milanović *et al.*, 2020; Olayinka *et al.*, 2015)
6	10.87	Nonane	5.16	Alkane	C_9H_{20}	122	Not reported
Chloroform							
7	7.39	1-Dodecene	0.98	Alkene	$C_{12}H_{24}$	168	Antioxidant, Antimicrobial (Sallam, 2007)
8	10.76	1-Tetradecene	5.34	Alkene	$C_{14}H_{28}$	196	Antifungal, Antituberculosis (Kalaivani *et al.*, 2013)
9	10.88	Tetradecane	2.60	Alkane	$C_{14}H_{30}$	198	Antioxidant, Antimicrobial (Akpuaka *et al.*, 2013; Faridha Begum *et al.*, 2016)

10	12.75	Phenol 2,4-bis[1,1-dimethyl ethyl]	6.11	Alkylate Phenol	$C_{14}H_{22}O$	206	Antioxidant, Antimicrobial (Yogeswari *et al.*, 2012)
11	13.99	1-Hexadecene	2.97	Alkene	$C_{16}H_{32}$	224	Antibacterial (Zakaria *et al.*, 2011)
12	16.80	Nonadecane	2.93	Alkane	$C_{19}H_{14}$	268	Antioxidant (Dandekar, 2015)
13	21.64	1-Docosene	2.76	Alkene	$C_{22}H_{44}$	308	Antioxidant, Antimicrobial (Golubović *et al.*, 2014)
Acetone							
14	3.17	2-Pentanone,4-hydroxy-4-methyl	35.52	Ketone	$C_6H_{12}O_2$	116	Not reported
15	10.87	Tetradecane	3.94	Alkane	$C_{14}H_{30}$	198	Antioxidant, Antimicrobial (Akpuaka *et al.*, 2013; Faridha Begum *et al.*, 2016)
16	13.98	Hexadecane	2.63	Alkane	$C_{16}H_{34}$	226	Antimicrobial, Antioxidant (Faridha Begum *et al.*, 2016)
Ethanol							
17	3.79	2-2-Dimethyl butanedioic acid	35.52	Carboxy-licAcid	$C_6H_{10}O_4$	146	Not reported
18	10.95	4-Methyl [trimethylene] silyloxyoctane	3.94	Silicon Ether	$C_6H_{26}OSi$	214	Not reported
19	17.33	ά-D-Glucopyranoside, O-ά- D glucopyranosyl [1,fwdarw3]-β-D fructofuranosyl	2.63	Sugar	$C_{18}H_{32}O_{16}$	504	Not reported
Methanol							
20	3.36	2-Pentanone.4-methoxy-4-methyl	47.12	Ketone	$C_7H_{14}O_2$	130	Not reported
21	8.15	Benzofuran.2-3-dihydro	3.14	Coum-arine	C_8H_8O	120	Antioxidant, Anti-inflammatory (Andiappan *et al.*, 2017)
22	12.73	Phenol 2,4-bis[1,1-dimethyl ethyl]	2.05	Phenol	$C_{14}H_{22}O$	206	Antioxidant, Antimicrobial (Faridha Begum *et al.*, 2016)
23	18.73	Benzenepropanoic acid. 3.5-bis(1,1-dimethylethyl) -4-hydroxy– methyl ester	2.25	Ester	$C_{18}H_{28}O_3$	292	Antibacterial, Antifungal (Bashir *et al.*, 2012)
24	20.59	9,12,15-octadecatrienoic acid methyl ester	1.55	Fatty acid	$C_{19}H_{32}O_2$	292	Anti-arthritic, Cancer, preventive (Ganesh and Mohankumar, 2017)

RT= Retention Time, **Rel%** =Relative Percent, **MF** =Molecular Formula, **MW** = Molecular Weight

Table 4. Bioactive compounds identified in different solvent extract of *Mucuna pruriens* (L.). DC. Seeds

S.N.	RT	Phytochemicals	Rel%	Class	MF	MW	Activity
Petroleum ether							
1	3.32	Oxalic acid cyclohexyl pentyl ester	13.37	Carbo-xylic	$C_{13}H_{22}O_4$	242	Antioxidant, Antibacterial (Devhade *et al.*, 2015)
2	6.61	Azulene	3.93	Aromatic hydro-carbon	$C_{10}H_8$	128	Antioxidant, Antimicrobial (Shanmugavel and Krishnamoorthy, 2018)
3	18.10	n-Hexadecanoic acid	11.28	Fatty acid	$C_{16}H_{32}O_2$	256	Antibacterial, Antifungal (Devhade *et al.*, 2015)
4	20.05	Decanamide,N-[2-hydroxy ethyl]	7.92	Amine	$C_{12}H_{25}NO_2$	215	Anticonvul-sant (Chandrasekaran *et al.* 2011)
5	20.21	9,12-octadecadienoic acid[zz]	39.08	Fatty acid	$C_{18}H_{32}O_2$	280	Antioxidant, Cancer Preventive (Lampariello *et al.* 2012)
6	21.97	Octanamide,N-[2-hydroxy ethyl]	7.12	Amine	$C_{10}H_{21}NO_2$	187	No reported
Chloroform							
7	6.61	Azulene	3.37	Aromatic hydro-carbon	$C_{10}H_8$	128	Antioxidant, Antibacterial (Shanmugavel and Krishnamoorthy, 2018)
8	12.94	Oxalic acid, allyldodecyl ester	4.32	Carbo-xylic acid	$C_{17}H_{30}O_4$	298	No reported
9	15.74	Oxalic acid, allyltridecyl ester	4.29	Carbo-xylic acid	$C_{18}H_{32}O_4$	312	No reported
10	18.06	n-Hexadecanoic acid	5.71	Fatty Acid	$C_{16}H_{32}O_2$	256	Antioxidant, Antimicrobial (Devhade *et al.*, 2015)
11	20.04	Valeric acid,3-tridecyl ester	9.35	Carbo-xylic acid	$C_{18}H_{36}O_2$	298	No reported
12	20.14	1-octadecyne	19.08	Alkyne	$C_{18}H_{34}$	250	No reported
13	23.80	β-Tocopherol	3.39	Vitamin E	$C_{28}H_{48}O_2$	416	Antioxidant, Antimicrobial (Syeda *et al.*, 2011)
Acetone							
14	5.26	2-Cyclohexen-1-one, 3,5-dimethyl	10.65	Cyclic alkene	$C_8H_{12}O$	124	Not reported
15	7.09	1H-Pyrazole,4,5-dihydro-5,5-dimethyl-4-isopropylidene	4.60	Aromatic amine	$C_8H_{14}N_2$	138	No reported
16	7.20	5-Methyl-1-heptanol	2.02	Alcohol	$C_8H_{18}O$	130	Antimicrobial (Mannaa and Kim, 2018)
17	24.38	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	2.23	Carbo-xylic acid	$C_{16}H_{22}O_4$	278	Antioxidant, Anticancer, Antidiabetic (Pietro *et al.*, 2010; Bagavathi and Ramasamy, 2012; Balachandran *et*

							al., 2012)
Ethanol							
18	4.84	Propane,1,1,3-triethoxy	2.89	Ether	$C_9H_{20}O_3$	176	No reported
19	5.30	2-Cyclohexen-1-one, 3,5-dimethyl	9.90	Cyclic alkene	$C_8H_{12}O$	124	Not reported
20	12.95	Oxalic acid, allyldodecyl ester	6.62	Fatty acid	$C_{17}H_{30}O_4$	298	No reported
21	14.31	2-Buten-1-one,1-(2,2,5a -trimethylperhydro-1 -benzoxiren-1-yl)	3.58	Ketone	$C_{13}H_{20}O_2$	208	No reported
22	15.74	Oxalic acid, allyltridecyl ester	6.88	Fatty acid	$C_{18}H_{32}O_4$	312	No reported
23	18.29	Pentadecanoic,2,6,10,14-tetramethyl-,methyl ester	8.45	Fatty acid	$C_{20}H_{40}O_2$	312	Antibacterial (Nurettin *et al.*, 2006)
24	23.38	Fumaric acid, 2dimethylaminoethyl heptyl ester	1.33	Carbo-xylic	$C_{15}H_{27}NO_4$	285	No reported
25	24.39	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	4.44	Carboxy lic Acid	$C_{16}H_{22}O_4$	278	Antioxidant, Anticancer, Antidiabetic (Pietro *et al.*, 2010; Bagavathi and Ramasamy, 2012; Balachandran *et al.*, 2012)
Methanol							
26	3.12	2-Pentanone.4-methoxy-4-methyl	47.64	Ketone	$C_7H_{14}O_2$	130	No reported
27	16.59	Benzoic acid,3,4dimet oxy- , 4-[ethyl[2-[4-methoxy phenyl]-1,methylethyl]amino]but yl ester	4.67	Aromatic acid	$C_{25}H_{35}NO_5$	429	Antimicrobial and preservative (Revathi *et al.*, 2014)
28	17.46	Tridecanoic acid, methyl ester	5.27	Fatty acid	$C_{14}H_{28}O_2$	228	Antioxidant, Antibacterial (Devhade *et al.*, 2015)
29	17.74	Benzene propanoic acid,3,5,bis [1,1-dimethyethyl]-4 hydroxy, methyl ester	9.11	Aromatic acid	$C_{18}H_{28}O_3$	292	Antibacterial Antifungal (Bashir *et al.*, 2012)
30	19.85	Octadecanoic acid methyl ester	1.77	Acid	$C_{19}H_{38}O_2$	294	Antioxidant, Antimicrobial, (Edeoga *et al.*, 2005)

RT= Retention Time, **Rel%** =Relative Percent, **MF**= Molecular Formula, **MW** = Molecular Weight.

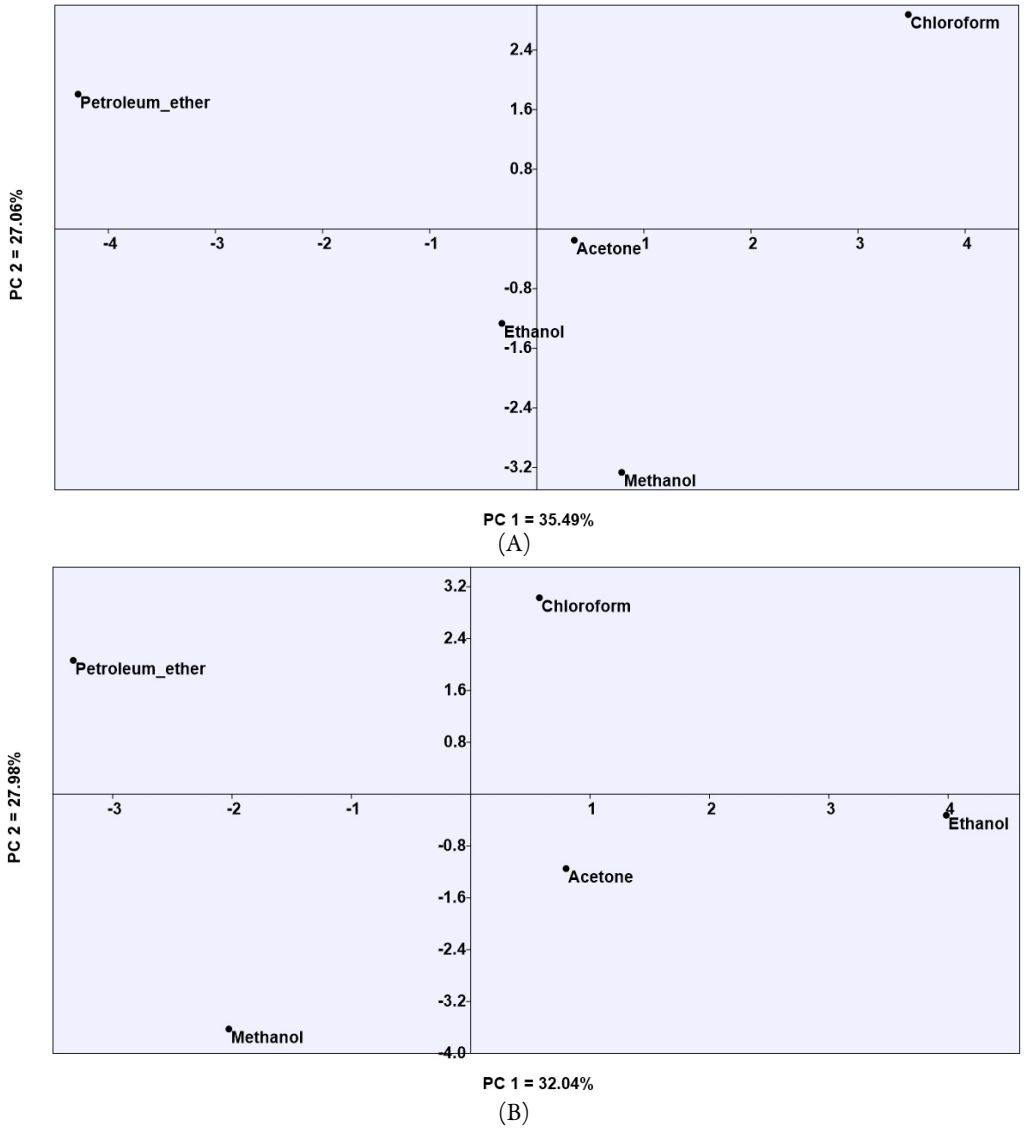

Figure 5. Principal component analysis of metabolites reported in (A) leaves and (B) seeds various solvent extracts

The seeds petroleum ether extract shows presence of 9, 12-octadecadienoic acid [zz] with 39.08% having antioxidant anti-cancerous and hypocholesterolemic potential as earlier recorded in GC-MS analysis of *Cassia italica* leaf methanol extract by Sermakkani and Thangarpandia (Sermakkani and Thangarpandia, 2012). Oxalic acid cyclohexyl pentyl ester (13.37%) shows antioxidant and antibacterial properties, η-Hexadecanoic acid constituted 11.28% possess antibacterial and antifungal activity (Hema *et al.*, 2011). Moreover, seeds chloroform extracts contain 5.71%, with antioxidant, antifungal, hypocholesterolemic, antimicrobial, anti-malarial, haemolytic and nematicidal properties (Hema *et al.*, 2011; Pietro *et al.*, 2010). Decanamide, N-(2-hydroxyethyl) obtained from petroleum ether extracts was found to be 7.92% with anticonvulsant potential; Pentadecanoic acid, 2,6,10,14-tetramethyl- methyl ester was obtained from seeds ethanolic extract contained 6.93% and 8.45% respectively with antioxidant, antimicrobial and antifungal properties (Chandrasekaran *et al.* 2011). 1, 2-Benzene dicarboxylic acid, mono (2-ethylhexyl) ester (4.44%) identified in seeds ethanolic extracts paying crucial role in therapeutic treatments as antifungal, anti-retroviral, antidiabetic, anti cancerous, antioxidant, antimicrobial and anti-inflammatory agents (Bagavathi and Ramasamy, 2012; Balachandran *et al.*,

2012; Phaniendra *et al.*, 2015; Airaodion *et al.*, 2019). Tridecanoic acid methyl ester (5.27%) reported as antibacterial, antifungal and antioxidant potential, Octadecanoic acid methyl ester (1.77%) derived from seeds methanolic extract shows antimicrobial, antifungal and antifungal agent (Chandrasekaran *et al.*, 2011). Azuline (3.37) and beta-tocopherol (3.39) belong to seed chloroform extract showing antioxidant and antifungal properties (Venkata Raman *et al.* 2012). The seeds methanolic extracts showed presence of Benzoic acid,3,4-dimethoxy-4-[ethyl[2-[4-methoxyphenyl]-1-methylethyl]amino]butyl (4.67%) having antimicrobial and preservative potential (Revathi *et al.*, 2014). The Benzenepropanoic acid, 3,5-bis (1, 1-dimethylethyl)-4-hydroxy-methyl ester (9.11%) compound also reported in seed methanolic extract has antioxidant, antibacterial and antifungal potential which were also reported in *Azadirachta indica* leaves extracted in hexane (Akpuaka *et al.*, 2013; Airaodion *et al.*, 2019).

Principal component analyses were performed to visualize the metabolites variation in leaves and seeds under various solvents (Figure 5). PCA analysis revealed that PC 1 accounted 35.49% and 32.04% variance and the PC 2 27.06% and 27.98% variance in leaves and seeds respectively. Each solvent lies apart from each other on PCA plot of leaves and seeds revealed solvent dependent metabolites variations. However, different metabolites were reported in various solvent extracts showed solvent specific extraction of metabolites (Dhawan and Gupta, 2017). In the present investigation; majority of promising phytoconstituents was been identified in leaves and seeds; the biological properties of which could not be ascertained they are 2-2-Dimethyl butanedioic acid (35.52%), 4-Methyl [trimethylene] silyloxyoctane (3.94%), 2-Pentanone.4-methoxy-4-methyl (47.12%), Octanamide, N-[2-hydroxy ethyl] (7.12%), Oxalic acid, allyldodecyl ester (4.32%), Oxalic acid, allyltridecyl ester (4.29%), Valeric acid,3-tridecyl ester (9.35%), 1-octadecyne (19.08%), 2-Cyclohexen-1-one, 3,5-dimethyl (10.65%), 1H-Pyrazole,4,5-dihydro-5,5-dimethyl-4-isopropylidene (7.09%), Propane,1,1,3-triethoxy (2.89%), 2-Cyclohexen-1-one, 3,5-dimethyl (9.90%), Oxalic acid, allyldodecyl ester (6.62%), 2-Buten-1-one,1-(2,2,5a-trimethylperhydro-1-benzoxiren-1-yl) (3.58%), Oxalic acid, allyltridecyl ester (6.88%), Fumaric acid, 2dimethylaminoethyl heptyl ester (1.33%). However, these compounds were also reported in various plants (Dhawan and Gupta, 2017; Casagrande *et al.*, 2018; Airaodion *et al.*, 2019).

Conclusions

The phytoconstituents derived from leaves and seeds extracts showed various biological properties. From the present study it was revealed that the leaves and seeds of *Mucuna pruriens* (L). DC. are one of the key sources for nutraceutical and various bioactive compounds. The phytochemical profiling revealed bioactive potential of *Mucuna pruriens* (L). DC. leaves and seeds. However, further purification of compounds and compound specific biological activity study is in need to explore its medicinal applicability for mankind. It is also necessary to develop a proper cultivation protocol for their better utilization.

Authors' Contributions

KCM: Designed the experiments, analysed the data and finalized the manuscript, DBS: Analysed data and prepared draft manuscript, ST: Performed the experiments, collected and analysed the data, PG: Analysed data and prepared draft manuscript, HBS: Analysed data and prepared draft manuscript: All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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