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RESEARCH ARTICLE

Characterization of phytochemicals by GC-MS, *in-vitro* biological assays and micronutrient analysis by ICP-MS of *Prunus domestica* L. seeds

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Abstract

In the present study, *Prunus domestica* L. (plum) seeds were used as the source of the oil components. To extract the oil, Soxhlet extraction was carried out using two different solvents petroleum ether and diethyl ether in increasing order of polarity for efficient extraction of secondary metabolites. GC-MS analysis of the obtained oil components was carried out and revealed the identification of 33 and 34 compounds in PE and DEE fractions respectively. 13-Docosenamide, (Z)- was the most abundant compound in PE fraction while the 2nd most abundant compound in DEE fraction. Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester was the most abundant compound in DEE fraction. The oil components were further assessed for *in-vitro* biological assays namely antidiabetic assay, antioxidant assay and antibacterial assay. ICP-MS analysis for the quantification of 12 different metals (K, Mg, Ca, Fe, Mn, Cr, Zn, Ni, Cu, As, Pb, Se) was also carried out. K was the major element in all the tested elements. Lead, arsenic and selenium were present below the limit of quantification (BLQ). Therefore, the current study through the light on therapeutic aspects of the oil components of *P. domestica* seeds.

Keywords

GC-MS, ICP-MS, DPPH, α-amylase, antibacterial activity

Introduction

There has been a long history of the usage of medicinal plants for therapeutic purpose. In modern era, extraction and isolation of the natural products from the different plant species is in trend for the discovery and development of new drugs. The presence of the phenolic compounds (phenolic acids, tannins and flavonoids), nitrogen containing compounds (alkaloids and amines), terpenoids, vitamins and other secondary metabolites is responsible for antioxidant and other activities of the medicinal plants (1). Traditional herbal medicines continue to be interesting despite a huge number of the synthetic pharmaceuticals in the market due to the presence of the high amount of the biologically active compounds causing low toxicity and allergenicity on human health than synthetic counterpart (2). Due to the above-mentioned reasons, demand of the medicinal plants is increasing positively. There is no doubt about the essential service provided by the medicinal plants to the ecosystem. The life without the plants can't be imagined (3).

According to WHO, about 80% of the world population, for their primary health, relies on the traditional healthcare system. There are many traditional systems of medications like Ayurveda, Siddha, Unani, Homeopathy etc., which are primarily based on the plant-based herbal medicines. Ayurveda, whose history goes back 5000 BC is one of the oldest medication systems in India, which alone reports 2000 plant species for their medicinal potentials (4).

The plum (*P. domestica*) is also a medicinal plant belongs to the family *Rosaceae* (19th largest family of the plants) and genus *Prunus* (5-8). The family *Rosaceae* includes about 3000 plants species while the genus *Prunus* reports 400-430 plant species but only 89 are enlisted in the Genetic Resources Information System. In India, about 36 species of *Prunus* have been reported, of which 18 species can be used for the cultivation purposes (9-13). *P. domestica* is a fruit crop ranking fourth after pear, apple and peach. *P. domestica* spread typically temperate northern hemisphere (14). In India, the primary plum-growing states are Jammu and Kashmir, Himachal Pradesh, Uttar Pradesh, Uttarakhand, Arunachal Pradesh, Nagaland, Meghalaya, Manipur and Sikkim (15). Taxonomical classification of *P. domestica* is shown in Table 1.

Table 1. Taxonomical classification of *P. domestica* (16-18)

Kingdom	Plantae
Subkingdom	Tracheobionta (Vascular plants)
Super-divison	Spermatophyta (Seed plants)
Divison	Angiospermae (Flowering plant)
Class	Dicotyledones
Subclass	Rosidae
Order	Rosales
Family	Rosaceae (Rose family)
Subfamily	*Prunoidae* (Spiraeoideae)
Tribe	Amygdaleae
Genus	*Prunus*
Sub-genus	*Prunophora*
Species	*Prunus domestica*

The fruit of *P. domestica* is highly nutritious having laxative and digestive properties, generally used for the treatment of jaundice, fever, hypertension and fever. The fruit has antioxidant, anticancer, anti hyperglycemic, antihyperlipidemic, antihypertensive, anti-osteoporosis and hepatoprotective activities (19-21). The fruit constitutes a valuable part of our diet due to the presence of many naturally occurring phytochemicals like anthocyanins, pectin, carotenoids, proanthocyanidin, flavonoids, flavonoid glycosides, cinnamoyl-hexoses, benzoylhexoses, cinnamic acids and coumarins (22, 23). Though, leaves are not widely used in medicine. According to the literature data, leaves contain polyphenols, vitamins, pectin and other biologically active compounds (21). In addition to this, it is a good source of minerals like nitrogen, phosphorus, potassium, calcium, magnesium, iron, manganese, copper, zinc, boron, arsenic, thallium, stannum, chromium, nickel, selenium, lead and titanium (6, 24).

Vegetable oils are the main source of the various phenolic compounds, tocopherols, pigments, vitamins and minerals that have a positive effect on human health. *P. domestica* seeds are the excellent source of oil, which contain about 30% oil. Seeds are also attributed to various bioactive phytochemicals namely lipids, tocols, phenolics and proteins (25). Thus, keeping the above facts in view, the current study is carried out on the seeds of *P. domestica.* Since the fruit of this plant being fleshy and juicy, which is usually eaten fresh by people and seeds are discarded away. There may be numerous health benefits associated with the seeds also. Therefore, the current study is focused on the seeds.

Materials and Methods

Reagents and Chemicals

Petroleum ether (SD fine chem), diethyl ether (SD fine chem), NaOH (Fisher), starch (Merck), NaCl (Hi-media), α-amylase (SRL Pvt. Ltd.), DPPH (Alpha-acer), DMSO (Fisher), acarbose (Merck) Di-nitro salicylic acid (Merck). All the other reagents used were of analytical grade.

Sample Collection and Authentication

For the collection of the seeds, the plums were purchased from the local orchard situated in Haridwar, Uttarakhand (India). After the separation of the seeds from the fleshy part, they were dried under the shade protecting from sunlight to ensure the no loss of the phytochemicals. For the authentication of the plant, two herbarium specimens were prepared, identified and deposited under the accession number 374 in the herbarium of Botanical Survey of India, Dehradun.

Extraction of the Oil Component

For the extraction of the oil components of the seeds, Soxhlet extraction method was employed (29). Briefly, 200 gms of the powdered seeds were put into the thimble of the Soxhlet extractor. PE and DEE were used as the solvent for the extraction of oil component in increasing order of the polarity. Approximately, 70-72 cycles of siphoning were conducted or the extraction was run till the siphoning tube appeared colourless. Then, each fraction was concentrated on the rota evaporator under reduced pressure below the boiling point of the solvent. The obtained oil components were stored in refrigerator till further use.

GC-MS Analysis of the oil Component

GC-MS analysis was performed according to Mahmood et al. (2009) with some modification (7). Shimadzu-GC-MS (TQ8040), HS20 headspace sampler, mobile phase He at 1 ml/min, SH-Rxi-5Sil MS capillary column (Stationary phase: 5% diphenyl-95% dimethyl polysiloxane, length: 30 m, inner diameter ID: 0.25 mm, film thickness df: 0.25 µm), split ratio 1:10, injection temperature 280 ^{0}C, the column oven temperature was raised from 80 ^{0}C to 280 ^{0}C at rate 5 ^{0}C/min. MS conditions were as followed: Ion source was kept at 220 ^{0}C with detector voltage 0.7kV and m/z was in

the range of 40 to 500 and scan time 0.30s.

Identification of the Compounds

Qualitative identification of oil components was based on computer matching of mass spectra fragmentation pattern with NIST14 and FFNSC1.3 mass spectral search program.

Biological Assays

α-Amylase Inhibitory Activity (Antidiabetic Activity)

α-amylase inhibition assay was performed according to the standard method with a little modification (26). 1 ml of the sample at different concentrations in DMSO was mixed with 1 ml of α-amylase solution and the mixture was incubated at 37 ^{0}C for 30 min. Then 1 ml of 1% starch solution was added to the above mixture and again incubated at 37 ^{0}C for 15 min. After that 1 ml di-nitro salicylic acid (DNSA reagent) was added to stop the reaction. The reaction mixture was boiled for 5 min in a water bath and cooled to room temperature. Then, 9 ml distilled water was added. The absorbance of the resultant reaction mixture was measured at 540 nm. The controlled experiments for correction of background absorbance were also carried out similar manner. Experiment with 100% and 0% enzyme activity was also carried out. Acarbose was used as standard antidiabetic reagent.

The % enzyme inhibition was calculated by the following formula:

$$\%\ inhibition = \frac{[(Ac^{+} - Ac^{-}) - (As - Ab)]}{(Ac^{+} - Ac^{-})}$$

Where: Ac^{+} = Absorbance of 100% enzyme activity, Ac^{-} = Absorbance of 0% enzyme activity, As = Absorbance of test sample or extract and Ab = Absorbance blank or control.

DPPH Assay (Antioxidant Activity)

Antioxidant activity of the oil component was assessed according to the method with some modifications (27). 1 ml of extract at different concentration was mixed 0.004% solution of DPPH. Then, the reaction mixture was incubated at room temperature for 1 hr. The absorbance of the resultant reaction mixture was measured at 517 nm by UV-Vis double beam spectrophotometer (Systronics 2205). Ascorbic acid was used as standard reference drug. The change in the colour from pink to orange indicates the effectiveness of the oil components towards antioxidant activity. The % DPPH scavenging activity was calculated by the following formula:

$$\%\ inhibition = \frac{Absorbance\ of\ the\ blank - Absorbance\ of\ the\ sample}{Absorbance\ of\ blank} * 100$$

Antibacterial Activity

The antibacterial activity was measured using the agar well diffusion method with a few alterations (40). Antibacterial activity was evaluated against two Gram(-ve) (*Klebsiella pneumoniae, Pseudomonas aeruginosa*) and one Gram (+ve) (*Streptococcus pyogenes*) bacteria**.** The sterilised Muller Hinton Agar (MHA) was put into Petri plates and allowed to solidify. The MHA plates were swabbed uniformly with overnight cultures of each strain with turbidity equivalent to Mac Farland standard (0.5 CFU). A cork borer was then used to puncture 5 wells. Then 200 µl of each oil component at a concentration of 100 mg/ml in DMSO was put in each well and allowed to incubate at 37 ^{0}C for 18 to 24 hrs and measured the inhibition zone. Standard discs of commercially available antibiotics were used as standard.

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

A Thermo Scientific, iCAP RQ, ICP-MS was used for the metal analysis and the operation was performed following Sun et al. (2022) with some alterations (41). 0.2 gms of the sample was mixed with 3 ml of the Conc. HNO_3 of the EMSURE grade. After that, sample was digested in microwave digester for 1 hr. After cooling at room temperature each sample was diluted with distilled water upto 25 ml. After the dilution, each sample was analysed by iCAP-MS for various elements. For the preparation of the calibration curves, multi-element was used. Standard solutions of each element (K, Mg, Ca, Fe, Mn, Cr, Zn, Ni, Cu, As, Pb, Se) to be tested were prepared at different varying concentrations.

Results and Discussion

GC-MS Analysis

The composition of the oil contents from seeds using PE and DEE were analyzed by GC–MS. A total 33 and 34 compounds were characterized in PE and DEE oil components respectively based on Mass Spectral Library Search Program. Table 2 shows the retention index, molecular mass, and retention time of each compound. The major compounds identified from PE fraction were stigmast-4-en-3-one, 13-docosenamide, (Z)- and gamma.-sitosterol with % composition of 13.34%, 13.16% and 12.46% respectively. Three compounds were also present in significant amount *i.e.,* >2%. Which are tris (2,4-di-tert-butylphenyl) phosphate (6.84%), octadecanoic acid, 2,3-dihydroxypropyl ester (6.33%), hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (5.91%). Two major compounds hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (25.95%) and 13-docosenamide, (Z)- (12.32%) detected in DEE oil component. The compounds with >5% composition are octadecanoic acid, 2, 3-dihydroxypropyl ester (7.41%). Total ion chromatogram (TIC) of the GC-MS instrument is shown in the Fig. 1.

α-Amylase Inhibitory Activity (Antidiabetic Activity)

The results of α-amylase inhibitory activity are shown in Table 3. All the results are expressed in terms of IC50. IC50 is the concentration of the extract/oil/drug required for the 50% inhibition of the enzyme. Acarbose was used as standard drug. Lower IC50 value means the higher efficacy of

the extract/oil/drug. DEE fraction of oil found to be most promising in comparison to PE fraction of oil with 3116.014±1.256 µg/ml and 4504.104±1.661 µg/ml IC50 respectively. While the acarbose (standard) has shown an

Table 2. GC-MS analysis of oil component obtained by using petroleum ether and diethyl ether as solvent

S.No.	PE (Oil Component)				DEE (Oil Component)			
	Compound name	RI	Mm	R_t	Compound Name	RI	Mm	R_t
1.	Heneicosane	2109	296	10.175	Docosane <n->	2200	310	10.170
2.	13-Docosenamide, (Z)-	2625	337	39.765	(-)-Epinephrine, 4TMS derivative	2066	471	27.814
3.	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene	1765	262	32.340	13-Docosenamide, (Z)-	2625	337	39.771
4.	1H-Indene, 1-hexadecyl-2,3-dihydro -	2599	342	35.070	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbenzene	1765	262	32.337
5.	2,3-Dihydroxypropyl icosanoate, 2TMS derivative	2979	530	38.200	2,3-Dihydroxypropyl icosanoate, 2TMS derivative	2979	530	38.196
6.	2,4-Di-tert-butylphenol	1555	206	16.035	2,4-Di-tert-butylphenol	1555	206	16.033
7.	2,8,9-Trioxa-5-aza-1-silabicyclo [3.3.3]undecane, 1-ethyl-	1256	203	28.995	4-Bromobutanoic acid, nonyl ester	1777	292	35.640
8.	2-Oxepanone, 5-(1,1-dimethylethyl) -	1340	170	35.560	5,5-Diethylpentadecane	1825	268	35.875
9.	3-Dodecene, 1-(benzyloxy)-4-methyl	2147	288	39.405	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	2081	276	24.685
10.	5-Methyl-Z-5-docosene	2293	322	34.770	Cyclooctasiloxane, hexadecamethyl-	1654	592	18.785
11.	6,6-Diethylhoctadecane	2124	310	37.760	Cyclononasiloxane, octadecamethyl-	1860	666	30.265
12.	7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione	2081	276	24.690	Cyclononasiloxane, octadecamethyl-	1860	666	32.550
13.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	2093	294	28.290	Cyclononasiloxane, octadecamethyl-	1860	666	34.700
14.	9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester	2766	370	39.290	Docosanoic acid, docosyl ester	4562	648	10.825
15.	9-Octadecenoic acid, methyl ester, (E)-	2085	296	28.420	Eicosane	2009	282	16.685
16.	Dotriacontane	3202	450	20.380	Fumaric acid, decyl 2-(diethylamino) ethyl ester	2401	355	23.235
17.	Dotriacontane	3202	450	24.780	Henicosanal			26.980
18	gamma.-Sitosterol	2731	414	38.445	Hexadecanal	1800	240	22.925
19.	Henicosanal	0	310	26.980	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	2498	330	35.370
20.	Hexacontane	5985	842	36.390	l-Norvaline, N-(2-methoxyethoxycarbonyl)-, tetradecyl ester	2852	415	28.995
21.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	2498	330	35.380	Octadecane, 1-bromo-	2107	332	37.735
22.	Hexadecanoic acid, methyl ester	1878	270	25.105	Octadecanoic acid, 2,3 dihydroxypro-pyl ester	2681	358	38.660
23.	N,N-Diethylhexylamine	0	157	23.240	Palmitic Acid, TMS derivative	1987	328	35.060
24.	Octadecanoic acid, 2,3-dihydroxypropyl ester	2681	358	38.670	Pentacosane <n->	2500	352	15.490
25.	Oxalic acid, 3,5-difluorophenyl tetradecyl ester	2569	398	35.205	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	0	646	47.115
26.	Pentacosane <n->	2500	352	15.495	Tetrapentacontane	5389	758	24.780
27.	psi.,.psi.-Carotene, 7,7',8,8',11,11',12,12',15,15'-decahydro-	3878	546	40.470	Tetrapentacontane	5389	758	28.785
28.	Stigmast-4-en-3-one	2714	412	46.940	Tetrapentacontane	5389	758	32.420
29.	Tetrapentacontane	5389	758	28.785	Tetrapentacontane	5389	758	35.200
30.	Tetrapentacontane	5398	758	32.425	Tetrapentacontane	5389	758	35.745
31.	Tetrapentacontane	5398	758	35.750	Tetrapentacontane	5389	758	39.080
32.	Tetrapentacontane	5398	758	39.075	Triacontane <n->	3000	422	20.290

33.	Triacontane <n->	3000	422	16.585	Triacontane <n->	3000	422	20.380
34.	-	-	-	-	Triarachine	6721	974	29.405
35.	-	-	-	-	Triarachine	6721	974	32.890

Where: RI =Retention index, Mm= Molecular mass of compound and R_t = Retention time (in minutes)

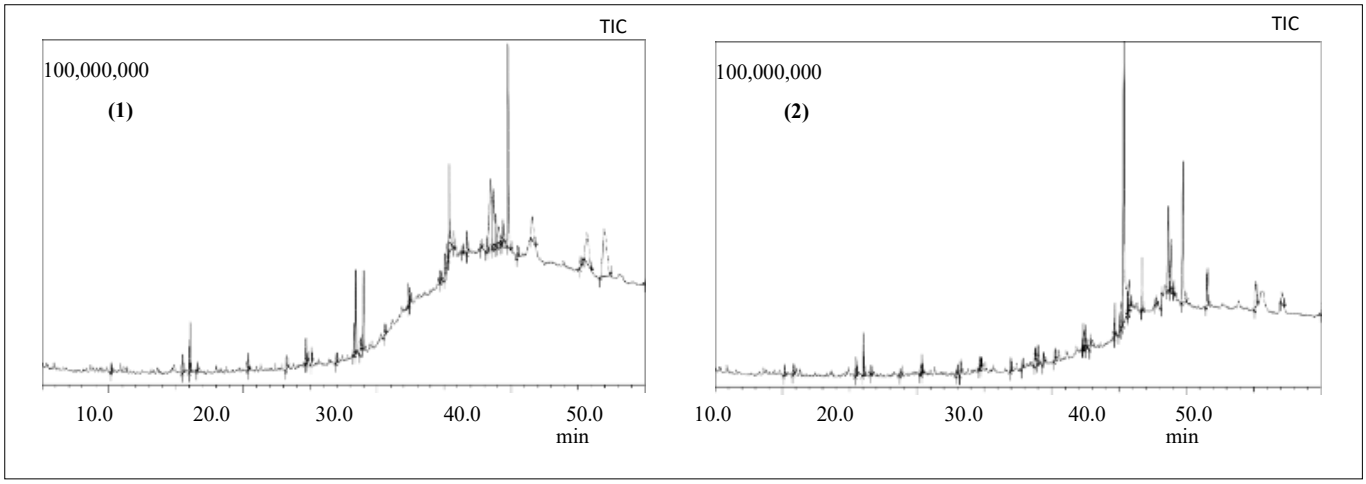


Fig. 1. Total ion chromatogram (TIC) of oil components (1): Petroleum ether fraction (2): Diethyl ether fraction.

Table 3. IC50 value of oil components and standard (acarbose) for α-amylase inhibition assay

Oil component/Standard	IC50 (µg/ml)
Petroleum ether	4504.104[a]±1.661
Diethyl ether	3116.014[a]±1.256
Acarbose (standard)	86.409[a]±0.232

Each experiment was performed in triplicate and results are expressed as mean ±SD. Anova analysis was performed using Graphpad prism software. The difference between the means is significant when p<0.05, when compared with the mean of standard, denoted by superscript.

IC50 value 86.409±0.232 µg/ml. The variation in the % inhibition of amylase with concentration is shown in Fig. 2. Both the oil components of the seeds of the *P. domestica* have shown the dose dependent α- amylase inhibitory activity. The α-amylase inhibitory activity of the oil components is due to the presence of the major phytoconstituents present in the oil components screened by GC-MS. The literature survey also suggests that terpenoids, glycosides, alkaloids, phenolics and flavonoids compounds to show alpha amylase inhibitory activity (28).

DPPH Assay (Antioxidant Activity)

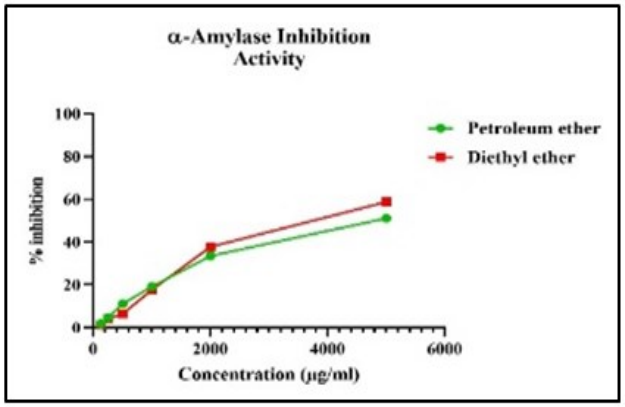
The results of the DPPH free radical scavenging assay are shown in the Table 4. Here again, the results are expressed in terms of IC50 value. Ascorbic acid has shown an IC50 13.296±0.075 µg/ml and DEE oil component has an IC50

Table 4. IC50 value of oil component and standard (acarbose) for DPPH free radical scavenging assay

Oil component/Standard	IC50 (µg/ml)
Petroleum ether	9677.964[a]±0.935
Diethyl ether	3730.567[a]±0.914*
Ascorbic acid (standard)	13.296[a]±0.075*

* Reported in our previously published study[29]. Each experiment was performed in triplicate and results are expressed as mean ±SD. Anova analysis was performed using Graphpad prism software. The difference between the means is significant when p<0.05, when compared with the mean of standard, denoted by superscript.

value 3730±0.914 µg/ml. Which is reported in our previous study (29). PE oil component has an IC50 value 9677.964±0.935 µg/ml. When activated form of oxygen *i.e.,* superoxide is formed, the damage of the human cells is initiated. This activated form combines with human cells and continues till the introduction of an antioxidant agent to scavenge this activated oxygen or free radicals. Thus,



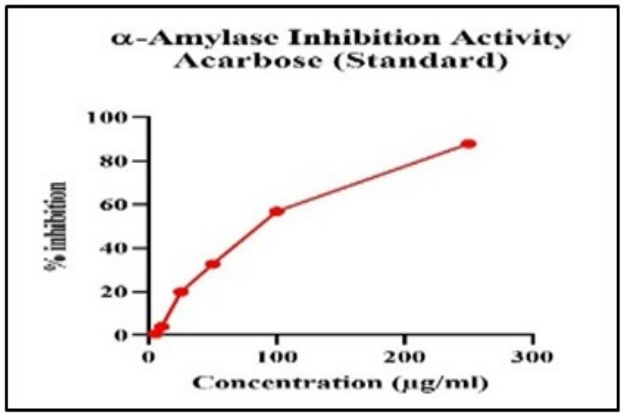


Fig. 2. Variation of % inhibition of α-amylase against concentration.

protection of the cells from the damaging effects of superoxide, singlet oxygen, hydroxyl radical, peroxy radicals and peroxynitrite is done by antioxidants (30). The results of DPPH assay show that seeds of *P. domestica* are the good source of antioxidant.

Antibacterial Activity

Results of the antibacterial activity are shown in the Table 5. Results are expressed in term of zone of inhibition, which are measured in millimetre (mm). PE oil component showed the highest activity against *P. aeruginosa* with inhibition zone of 12.00±0.87 mm followed by *K. pneumoniae* and *S. pyogenes* with inhibition zone of 3.17±0.29 mm and 3.67±0.58 mm respectively. Same pattern is also observed in DEE oil component. Highest activity is observed against *P. aeruginosa* followed by *K. pneumoniae* and *S. pyogenes* with inhibition zone 11.17±0.76 mm, 2.33±0.50 mm and 2.17±0.29 mm respectively. Antibacterial study of the seeds oil of *P. domestica* is suggestive that the seeds are good contributor towards the inhibition of bacterial diseases. Gentamicin and Chloramphenicol were used as the standard antibiotics.

Table 5. Antibacterial activity of the oil component of *P. domestica*

Type of bacteria	Name of bacteria	Zone of Inhibition (mm)			
		PE (Oil component)	DEE (Oil component)	Chloramphenicol	Gentamicin
Gram (-ve)	*K. pneumoniae*	$3.17^{a,b}$±0.289	$2.33^{a,b}$±0.50	20.50^{a} ±0.50	27.13^{b}±0.23
	P. aeruginosa	$12.00^{c,d}$±0.87	$11.17^{c,d}$±0.76	26.43^{c}±0.40	22.83^{d}±0.76
Gram (+ve)	*S. pyogenes*	$3.67^{e,f}$±0.58	$2.17^{e,f}$±0.29	19.60^{e}±0.53	26.50^{f}±0.50

Each experiment was performed in triplicate and results are expressed as mean ±SD. Anova analysis was performed using Graphpad prism software. The difference between the means is significant when $p<0.05$, when compared with the mean of standard, denoted by superscripts.

Inductively Coupled Plasma Mass Spectrometry

Results of the ICP-MS analysis are shown in the Table 6. A total of 12 metals were analysed. In all the metals studied, potassium was the most abundant element, followed by magnesium, calcium and iron. K is required for the proper functioning of the body's cells, tissues and other organs. It is crucial for the digestive system and has an important role in cardiac and muscle function (31). It is highly beneficial for bone strength since it has a high calcium content. Magnesium is necessary for muscle growth and energy release. The presence of iron confirms that it is a good source of assistance in the production of haemoglobin and red blood cells (6). Mn is a necessary component of biological activities such as bone health, macronutrient metabolism and reactive oxygen species defence (32). The data also show that the seeds contain considerable amounts of Cr, Zn, Ni and Cu. In living creatures, Cr is also favourable to the circulation of glucose, lipids and insulin (33). Zinc is required for several physiological and metabolic functions. Increased zinc concentrations, on the other hand, can be hazardous. Inadequate Zn intake or insufficiency can also lead to crohn's disease and irritable bowel syndrome. Zinc plays an important role in protein synthesis as well (34, 35). Nickel is an essential micronutrient for hormone function and lipid metabolism (36). Cu functions as a stimulant for the immune system to combat parasite infections, the digestive system by enhancing the manufacturing of digestive enzymes and acts as a catalyst for enzymes within the cells (37). However, the levels of lead and arsenic were well below the acceptable limit (38). Selenium deficiency can cause keshan illnesses and it's also needed to boost the activity of the selenium-containing enzyme glutathione peroxidase in the blood (39).

Table 6. ICP-MS analysis of the seeds (*P. domestica*) for metal detection

S. NO.	Metal	Concentration(ppm)
1.	Potassium (as K)	7868.30
2.	Magnesium (as Mg)	2884.45
3.	Calcium (as Ca)	383.02
4.	Iron (as Fe)	41.40
5.	Manganese (as Mn)	10.51
6.	Chromium (Cr)	0.13
7.	Zinc (as Zn)	19.04
8.	Nickel (as Ni)	0.36
9.	Copper (as Cu)	14.33
10.	Arsenic (as As)	BLQ
11.	Lead (as Pb)	BLQ
12.	Selenium (as Se)	BLQ

Where: BLQ = below the limit of quantification.

Statistical Analysis

Each experiment was performed in triplicate and results are expressed as mean ±SD. Anova analysis was performed using *Graphpad prism* software. The difference between the means is significant when $p<0.05$ when compared with the mean of standard.

Conclusion

GC-MS analysis of the oil components of the seeds done for the phytochemical screening, revealed it to be a good source naturally occurring secondary metabolites. Antidiabetic activity by *in-vitro* method showed that the seeds have a good antidiabetic potential towards inhibition of the alpha-amylase enzyme. Antioxidant activity was carried out by DPPH free radical scavenging assay showed that seeds of *P. domestica* have good free radical scavenging potential. Another important activity *i.e.*, the antibacte-

rial activity is also carried out on three different bacteria *P. aeruginosa, K. pneumoniae* and *S. Pyogenes* revealed it to be good antibacterial agent. Micronutrients are also investigated in the seeds by ICP-MS analysis. Overall, this study through the light on phytochemistry, biological activities and micronutrients using modern techniques.

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Authors contributions

Concept and planning of the work were supervised by RKS and AS. Experimental work was done by K. SK with K has participated in the design and study of the antibacterial activity. Manuscript writing and statistical calculations were done the K. All the authors have read the manuscript and given their approval for the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors declare that they don't have any conflict of interest..

Ethical issues: None.

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