Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(4): 664-675
Received: 21-05-2018
Accepted: 25-06-2018

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Phytochemical, antioxidant and a study of bioactive compounds from *Artemisia pallens*

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Abstract
Artemisia pallens has been used in Ayurveda for the treatment of measles, cough, cold, depression, diabetes and high blood pressure. The present investigation is focussed to evaluate the antidiabetic activity of *A.pallens* leaf extracts. The water, methanol, chloroform, N-butanol and diethyl ether extracts of my plant was evaluated. The extracts were analysed for DPPH, phenols, FRAP, antioxidants, SOD and hydrogen peroxide. The extracts were evaluated using enzymatic studies, FTIR, GC-MS and the antimicrobial activity. The water, methanol and chloroform extracts appear to show good results than N-butanol and diethyl ether extracts. The antidiabetic activity of *A.pallens* might be due to presence of many natural compounds which might play a role in controlling diabetes.

Keywords: *Asteraceae*, phytochemicals, quantitative analysis, enzymatic analysis, FTIR, GC-MS, antimicrobial assay

1. Introduction
Artemisia pallens Wall. ex Dc is an aromatic herb belonging to Asteraceae family and is native of India and other tropical countries. Artemisia pallens has been used in Ayurveda for the treatment of measles, cough, cold, depression, diabetes and high blood pressure. Traditionally, the leaf powder is used as an antidiabetic, antihypertensive, and antidepressant medicine. The therapeutic potential is due to the presence of natural bioactive compounds. The antidiabetic activity of the plant has not been explored [1]. Diabetes is a metabolic disorder characterized by the rise or fall of the blood sugar levels. The amount of people suffering from diabetes throughout the world is estimated to be 2.8% and is predicted to double every 20 years up to 2040 [2]. Oral intake of high doses of aqueous or methanolic extract of the aerial parts of the plant was shown to decrease blood glucose level in glucose−fed hyperglycemic and alloxan-treated rabbits and rats [3]. Methyl cinnamate, ethyl cinnamate, bicyclogermacrene, 2-hydroxyisodavanone, farnesol, geranyl acetate, sesquiterpene lactones, germacranolides seems to be the important components which are present [4]. The leaves and flowers are of high value in preparing floral decorations and essential oils. The leaves are very small, bluish green with yellow inconspicuous flowers. It is utilized in traditional Ayurvedic medicinal formulations. Essential oil of Artemisia pallens is used as an antiseptic and disinfectant [5]. Thus, the aim of the study is to investigate the antidiabetic activity of A.pallens and further chemical characterization of the extracts by phytochemical assays, the quantitative, enzymatic studies, FTIR, GC-MS and the antimicrobial analysis.

Fig 1: Photo of raw material used

2. Materials and Methods

2.1 Plant collection and extract preparation

The *A.pallens* was collected from Coimbatore, Tamil Nadu, India. The identity of the plant was confirmed by the Botanical Survey of India, Coimbatore, Tamil Nadu, India. The extract was prepared using dried leaves and stem in water, methanol, chloroform, N-butanol and diethyl ether using a shaker. The extracts were stored in sealed containers.

2.2 Qualitative phytochemical analysis

Phytochemical screening of solvents in extracted samples were carried out according to the standard methods for alkaloids, terpenoids, phenols, sugar, saponins, flavonoids, quinines, protein and steroids as described by [6]. Alkaloids were detected using Mayer's reagent. The test was positive indicating the presence of alkaloids. Terpenoids was detected using concentrated sulphuric acid. The test was positive indicating the presence of terpenoids. Phenols were tested using ferric chloride. Phenols were positive for methanol, diethyl ether and N-butanol extracts. Sugars were tested using Fehling's. Sugars were tested negative indicating the absence of sugars. Saponins were tested using distilled water. Frothing was observed indicating the presence of saponins. Flavonoids were tested using few fragments of magnesium ribbon and few drops of concentrated hydrochloric acid. Flavonoids were tested negative indicating the absence of flavonoids. Quinines were tested using sodium hydroxide reagent. Red colour is indicative of the presence of quinines. Proteins were tested using mercuric chloride or concentrated nitric acid reagent. Yellow colouration was observed which is indicative of the presence of proteins. Steroids were detected by Liebermann-Burchard test. Red colour was observed which is indicative of the presence of steroids.

2.3 Quantitative assays

In vitro DPPH radical scavenging assay was measured by the spectrophotometric method according to [7]. To a solution of DPPH in the tubes, 0.05 ml of extracts were added at different concentrations (250-500µg/ml). Control was also maintained. After 30 mins, the absorbance was read at 517nm. Ascorbic acid was used as a standard. Total amount of DPPH was expressed as µg/ml.

Reducing power of the extract was estimated spectrophotometrically by [7]. About 1 ml of water, methanol and chloroform extracts were mixed with 2.5 ml of phosphate buffer and 2.5 ml of potassium ferricyanide. The mixture was incubated at then rapidly cooled and mixed with 2.5 ml of 10% trichloroacetic acid. 2.5 ml of distilled water and 0.5 ml of ferric chloride was added to it. The absorbance was measured at 700nm. Ascorbic acid was used as a standard. The reducing power was expressed as µg/ml.

The method described by [8] was used to determine the total antioxidant capacity of the extract. The tubes containing 2 ml of water, methanol and chloroform extracts, 1.8 ml of phosphomolybdenum reagent solution (0.6 M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) were incubated at 50°C for 90 mins. After the mixture cooled to room temperature, the absorbance was measured at 695nm. Ascorbic acid was used as the standard. The total antioxidant activity was expressed as mg equivalents of ascorbic acid per gram.

Hydroxyl radical scavenging activity was measured according to [9]. To a solution of hydrogen peroxide, 0.5 ml of water, methanol, and chloroform extracts and 0.9 ml of ethanol were added and incubated at room temperature for 10-15 mins. The absorbance was measured at 230nm. Gallic acid was used as the standard. The hydrogen peroxide assay was expressed as µg/ml.

In the scavenging activity was measured according to [10]. 1 ml of reaction mixture was taken in test tubes. The reaction mixture was prepared by adding 1 ml of 50m phosphate buffer solution, 0.075 ml of EDTA, 20M L-Methionine and 0.04 ml of 10mM Hydroxyamide hydrochloride. The test sample of water, methanol and chloroform extracts were added to all these followed by a brief pre-incubation at 37°C for 10 mins. 50µl of 50mM Riboflavin was later added. The tubes were mixed well and exposed for 5 mins under UV fluorescent light. After the exposure time, 1 ml of Greiss reagent was added and the absorbance of the colour formed was read at 543nm. Greiss reagent was freshly prepared by mixing 1% sulphanilamide in 5% phosphoric acid. Gallic acid was used as the standard. The SOD activity was expressed as µg/ml.

Total phenolics assay was quantified and was expressed as gallic acid equivalent according to [6]. About 1 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent were added to the tubes having water, methanol, chloroform, diethyl ether and N-butanol and was incubated at room temperature for 3 mins. 2 ml of 20% sodium carbonate was added to this and kept at boiling water bath for 1 min. The blue colour formed was read at 650 nm.

In vitro amylase inhibition was studied by [8]. 1 ml of water, methanol and chloroform extracts were made to react with 0.2 ml of α amylase and 1 ml of 16mM sodium acetate buffer. After 20 mins incubation 1 ml of 0.1 % starch was added. After incubation for 5 mins 500µl of dinitrosalicylic acid was added. They were kept in a boiling water bath for 10-15 mins. The absorbance was recorded at 540nm. The percentage inhibition of α amylase was calculated using the formula

$$\text{Inhibition (\%)} = 100(\text{control} - \text{test})/\text{control}$$

The inhibitory effect of α glucosidase was carried out according to [11]. 1 mg of α glucosidase was dissolved in 100mL of phosphate buffer. The reaction mixture consisting of water, methanol and chloroform extracts were mixed with 1 ml of 2% starch which acts as a substrate. To these, 1 ml of 0.2 M Tris buffer was added. This was later incubated at room temperature for about 30 mins. After incubation 0.2 ml of α glucosidase dissolved in phosphate buffer was added. This was added to initiate the reaction. The reaction was terminated by the addition of 6N hydrochloric acid. The α glucosidase activity was determined spectrophotometrically at 540 nm. The inhibition rate (%) was calculated by the formula

$$\alpha \text{ glucosidase Inhibition (\%)} = 100(\text{control} - \text{test})/\text{control} \times 100$$

2.4 FT-IR

The water and methanol extract of *Artemisia Pallens* was treated with FTIR spectroscopy using Thermo Scientific FTIR. The FTIR analysis was carried out using [12].

2.5 GC-MS

GC-MS analysis of the methanolic extract of *A.pallens* was carried out using Thermo Gas Chromatograph DSQII series fitted with a DB35ms fused and a standard non-polar column

(30mX 0.25mm. film thickness 0.25µm) coupled with 403 series mass detector with an injection volume of 1 µl. Helium was used as a carrier gas with a constant flow mode of 1.0 ml/min. The injector temperature was set at 260°C. The oven temperature of 70°C was raised to 260°C at 6°C/min. The GC-MS was carried out using [13].

2.6. Antimicrobial assay

The agar well diffusion method was used for the antibacterial assay. This was performed according to [14]. Petri dishes were prepared by pouring 15 of Muller Hinton Agar medium and allowed to solidify. The agar medium was solidified and E.coli, MRSA, P.aeruginosa, K.pneumoniae and B.subtilis were swabbed onto the medium uniformly. The inoculum was allowed to dry for 5 mins. The wells were made with sterilized stainless steel cork borer. The wells were labelled and each well was loaded with 300 µL of water, methanol and chloroform extracts. The plates were incubated in an incubator at 37°C for about 24 hrs. Antibacterial activity was determined by measuring the zones of inhibition.

3. Results and discussion

Table 1: Results of the phytochemical analysis

S.NO	Tests	Water	Methanol	Diethyl Ether	N-Butanol	Chloroform
1	Alkaloids	+	+	+	+	+
2	Terpenoids	+	+	-	-	+
3	Phenol & Tannins	-	+	+	+	-
4	Sugar	-	-	-	-	-
5	Saponins	+	-	-	-	-
6	Flavonoids	-	-	-	-	-
7	Quinines	+	+	-	+	+
8	Protein	+	-	-	-	-
9	Steroids	-	+	-	-	+

+ indicates presence and – indicates absence

Phytochemical screening of different solvent fractions of *A. Pallens* showed the presence of alkaloids, steroids, flavonoids, reducing sugars, tannins, saponins, terpenoids, anthraquinone, saponins and phenols as summarised in (Table 1).

In water extract alkaloids, terpenoids, saponins, quinines and proteins were present. In methanol alkaloids, terpenoids, phenols, quinines and steroids were present. In chloroform alkaloids, terpenoids, quinines and steroids were present. In diethyl ether, alkaloids and phenols were present and in N-butanol, alkaloids, phenols and quinines were present. According to [15], the *Artemisia pallens* is an aromatic herb found abundantly in humid habitats in the plains all over India.

3.1 DPPH radical scavenging activity

Artemisia Pallens shows DPPH of water extract to be 50 µg/ml, methanol extract to be 60 µg/ml, chloroform extract to be 30 µg/ml, diethyl ether to be 50 µg/ml and N-butanol to be 60 µg/ml.

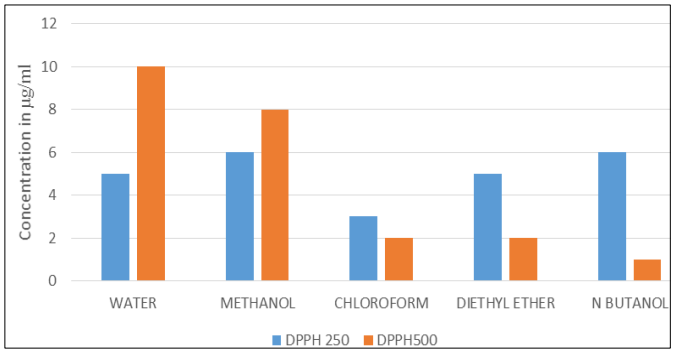


Fig 2: Total DPPH radical scavenging activity of *Artemisia Pallens*

In the present study the water extract of *A.pallens* was 50 µg/ml, methanol extract was 60 µg/ml, chloroform extract was 30 µg/ml, diethyl ether was 50 µg/ml and N-butanol was 60 µg/ml. With respect to [16], the DPPH radical scavenging activity of water, methanol and ethyl acetate extracts of *A.absinthium* were 7.37 µg/ml, 6.77 µg/ml and 41 µg/ml. This suggests that *A.pallens* has very good DPPH radical scavenging activity when compared to [16].

In the present study the water extract of *A.pallens* was 50 µg/ml, methanol extract was 60 µg/ml, chloroform extract was 30 µg/ml, diethyl ether was 50 µg/ml and N-butanol was 60 µg/ml. With respect to [17], the DPPH assay of the ethanol extract of *A. nilagirica* was 70µg/ml and the aqueous extract was 65µg/ml. This indicates that the DPPH activity of [17] was slightly higher than the DPPH assay of *A.pallens.* DPPH assay also plays a role in investigating the radical scavenging activity of several natural compounds.

In the present study the water extract of *A.pallens* was 50 µg/ml, methanol extract was 60 µg/ml, chloroform extract was 30 µg/ml, diethyl ether was 50 µg/ml and N-butanol was 60 µg/ml. In [18], the DPPH assay of the aqueous extract of *A.argyi* was found to be 105µg/ml, ethanol extract was 107 µg/ml and methanol extract was 63 µg/ml. The DPPH radical scavenging activity of the ethanol and methanol extracts of *A.argyi* was slightly higher than *A.pallens* indicating that the nature of the phytochemicals seems to be more complex.

In the present study the water extract of my plant sample was found to be 50 µg/ml, methanol extract was 60 µg/ml, chloroform extract was 30 µg/ml, diethyl ether was 50 µg/ml and N-butanol was found to be 60 µg/ml. The [19] DPPH analysis of the ethanol extract of *A.pallens* was found to be 150 µg/ml. This suggests that the DPPH radical scavenging activity of the ethanol extract appears to be better than all the extracts of *A.pallens*. The DPPH radical scavenging activity depends on the dosage of the plant extract used during the analysis.

In the present study the water With respect to [20], the DPPH assay of the methanol extract of *A.indica* was found to be 204 µg/ml. The water extract of *A.pallens* was found to be 50 µg/ml, methanol extract was 60 µg/ml, chloroform extract was 30 µg/ml, diethyl ether was 50 µg/ml and N-butanol was 60 µg/ml. There seems to be very good DPPH radical scavenging assay of *A.indica*. The extract of the plant appears to be a mixture where all the compounds may not possess DPPH radical scavenging activites.

In the present study the water extract of *A.pallens* was found to be 50 µg/ml, methanol extract was 60 µg/ml, chloroform extract was 30 µg/ml, diethyl ether was 50 µg/ml and N-butanol was 60 µg/ml. In [21], the DPPH analysis of the petroleum ether extract of *A.biennis* was found to be 1314 µg/ml, dichloromethane extract was found to be 452µg/ml, ethyl acetate extract was found to be 74 µg/ml, and ethanol extract was found to be 67µg/ml [19]. Apart from the petroleum ether and dichloromethane extracts of *A.biennis*, the ethyl acetate and ethanol extracts were slightly higher when compared to all the extracts of *A.pallens*. This shows that all the extracts of *A.biennis* has good DPPH radical scavenging activity when compared to the DPPH assay of *A.pallens*.

In the present study the water the water extract of *A.pallens* was found to be 50 µg/ml, methanol extract was 60 µg/ml, chloroform extract was 30 µg/ml, diethyl ether was 50 µg/ml and N-butanol was 60 µg/ml. According to [22], the radical scavenging activity of the essential oil *Artemisia amygdalina* was measured by the DPPH assay in-vitro. The highest activity (80.7%) was found at a concentration of 100 µg/mL. The DPPH radical scavenging assay is commonly employed in evaluating the ability of antioxidants to scavenge free radicals. This method has been used extensively to predict the antioxidant activity because of the relatively short time for analysis.

3.2 Antioxidant assay

Artemisia Pallens shows the antioxidant activity of water extract to be 198µg/ml, methanol extract to be 748µg/ml and chloroform extract to be 5µg/ml.

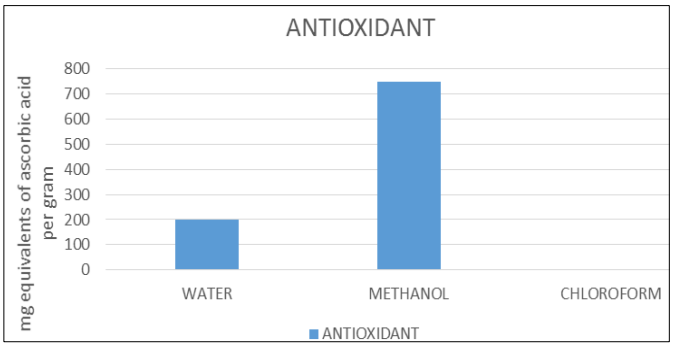


Fig 3: Antioxidant assay of *Artemisia Pallens*

In the present study the water extract of *A.pallens* was 198µg/ml, methanol extract was 748µg/ml and chloroform extract was 5µg/ml. With respect to [20], the methanol extract of *A. indica* was found to be equivalent to 298.80 µg of ascorbic acid. This indicates that *A.pallens* has very good antioxidant assay. The antioxidant assay of the methanol extract of *A.pallens* seems to be very good. This activity may be due to the presence of phytochemicals. Higher antioxidant activity is related to the ability to reduce DPPH.

In the present study the water extract of *A.pallens* was 198µg/ml, methanol extract was 748µg/ml and chloroform extract was 5µg/ml. The [17], states that the total antioxidant activity of the ethanol and aqueous extract of *A.nilagirica* was 61.3 µg/ml and 64.7 µg/ml. These results indicate that antioxidants in *A.pallens* seems to be excellent in water and methanol. These may be attributed to the presence of flavonoids and other specific phytochemicals corresponding to the antioxidant activity.

3.3 FRAP assay

FRAP assay of *Artemisia Pallens* shows the water extract to be 6µg/ml, methanol extract to be 56µg/ml and chloroform extract to be 12 µg/ml.

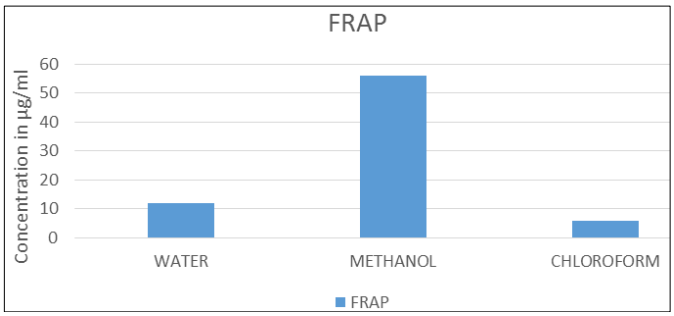


Fig 4: FRAP reducing power assay of *Artemisia Pallens*

In the present study the FRAP assay of the water, methanol and chloroform extract of *A. Pallens* was 6µg/ml, 56µg/ml and 12 µg/ml. In [20], the FRAP value of the methanol extract of *A.indica* was 13 µg/ml. These suggest that the FRAP values of *A.pallens* seems to be excellent. These could be due to the presence of phenols and flavonoids.

In the present study the FRAP assay of the water, methanol and chloroform extract of *A. Pallens* was 6µg/ml, 56µg/ml and 12 µg/ml. With respect to [23], the FRAP assay of *A.selegeneis* of water extract was 46.2 12 µg/ml, ethanol extract was 29.4 12 µg/ml, N-butanol was 5.712 µg/ml, ethyl acetate was 7.1 12 µg/ml and petroleum ether was 0.612 µg/ml. These readings suggest that *A.pallens* has very good reducing power. The FRAP can be applied to all the extracts of the different plants.

In the present study the FRAP assay of the water, methanol and chloroform extract of *A. Pallens* was 6µg/ml, 56µg/ml and 12 µg/ml. In [24], the FRAP assay of the ethanol extract of *A. annua* was 212 µg/ml. These results indicate that FRAP values of *A. annua* seems to be much better than the FRAP values of *A.pallens.*

3.4 Hydrogen peroxide assay

Hydrogen peroxide assay of *Artemisia Pallens* of water extract was found to be 79 µg/ml, methanol extract was 13 µg/ml and chloroform extract was 13 µg/ml.

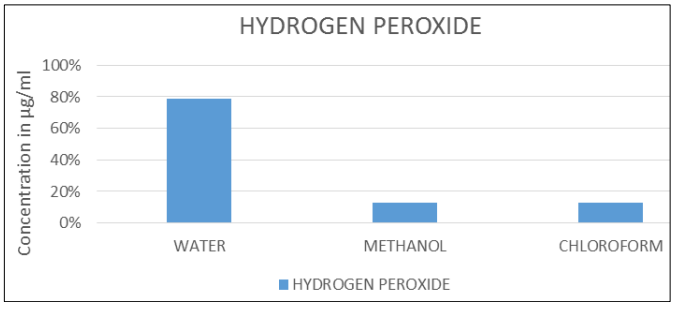


Fig 5: Hydrogen peroxide assay of *Artemisia Pallens*

In the present study the hydrogen peroxide assay of water, methanol and chloroform of my sample extract was 79 µg/ml, 13 µg/ml and 13 µg/ml. From [19], the hydrogen peroxide assay of the ethanol extract of *A.pallens* was 92.0 µg/ml. These results reveals that *A.pallens* may have a potent hydrogen peroxide radical scavenging activity. These plants

may be used as an antioxidant in preventing the damage arising from hydroxyl radicals.
In the present study the hydrogen peroxide assay of *A.pallens* was 79 µg/ml, 13 µg/ml and 13 µg/ml. With respect to [25], the hydrogen peroxide assay of the methanol extract of *A.absinthium* was 109 µg/ml. The results of [25] reveals that hydrogen peroxide assay was higher than that of *A.pallens*. The hydroxyl radicals of [25] has the capacity to modify every molecule of the living cell. The radicals may also damage the DNA leading to cancer.

3.5 SOD assay
SOD assay of *Artemisia pallens* of water extract was found to be 82%, methanol extract was 19% and chloroform extract was 94%.

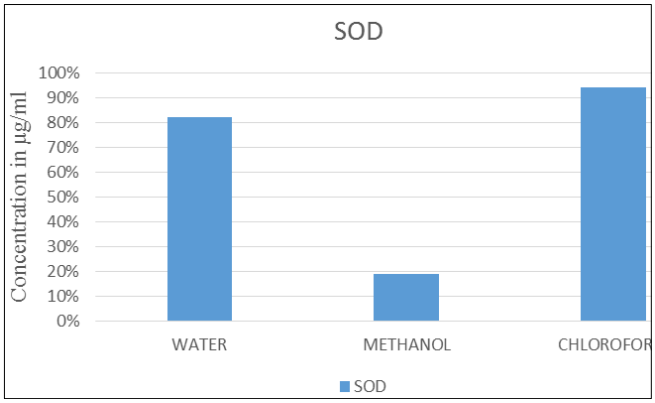


Fig 6: SOD assay of *Artemisia Pallens*

In the present study the SOD analysis of the water, methanol and chloroform extract of *A.pallens* was 82%, 19% and 94%. The [25], states that the SOD assay of the methanol extract of *A.absinthium* was found to be 78%. The above said results shows that *A.pallens* has excellent SOD assay.

3.6 Phenols assay
Artemisia Pallens showed that the phenols present in water extract was found to be 117 GAE, methanol extract was 170 GAE, chloroform extract was 22 GAE, diethyl ether was 69 GAE and N-butanol was 14 GAE.

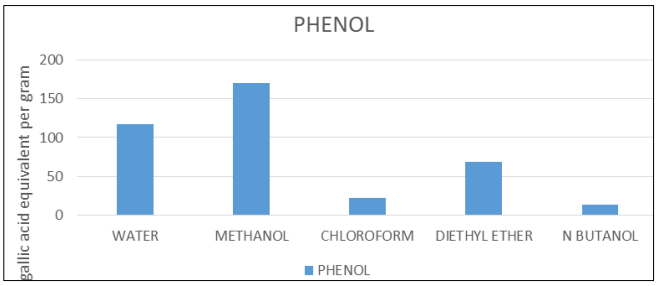


Fig 7: Phenols assay of *Artemisia Pallens*

In the present study the water, methanol, chloroform, diethyl ether and N-butanol extracts of *A.pallens* was 117 GAE, 170 GAE, 22 GAE, 69 GAE and 14 GAE. In accordance to [26], the total phenols in the aqueous extract of *A.vulgaris* was 19GAE. The phenol values of my sample extracts except N-butanol showed a very significant result when compared to the aqueous extract of *A.vulgaris.* Phenols appear to be a very good constituent and plays a role with antioxidants in many plant species. Phenols are very effective hydrogen donors so they are considered to be good antioxidants.

In the present study the water, methanol, chloroform, diethyl ether and N-butanol extracts of *A.pallens* was 117 GAE, 170 GAE, 22 GAE, 69 GAE and 14 GAE. With respect to [27], the total phenol content of ethanolic extract of *A. parviflora* was found out to be 1.09 GAE. These suggests that the phenols present in all the extracts of *A.pallens* seems to be excellent. These components have good antioxidant activities of all plant materials. They are said to inhibit the effects of carcinogen in humans.
In the present study the water, methanol, chloroform, diethyl ether and N-butanol extracts of my sample extract was 117 GAE, 170 GAE, 22 GAE, 69 GAE and 14 GAE. In [28], the ethanol extract of *A.pallens* was 87.2GAE. The water and methanol extract of *A.pallens* has a very high phenol content compared to the ethanol extract of *A.pallens.* These results indicate that this plant may be used to prepare a drug to prevent diabetes.

3.7 Alpha amylase assay
Alpha amylase assay of 0.5 ml water extract of *Artemisia Pallens* was found to be 121% and 1 ml was 11.5%, 0.5 ml methanol extract was 91% and 1 ml was 78%, and 0.5 ml of chloroform extract was 181% and 1 ml was 186%.

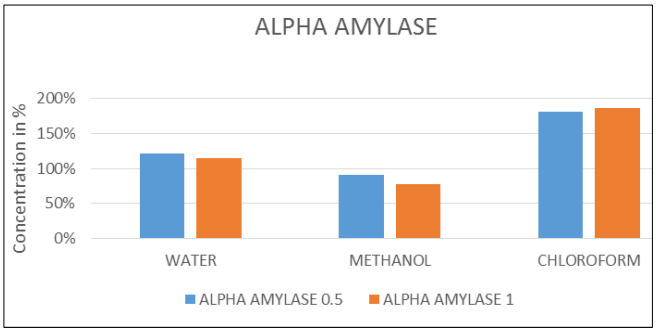


Fig 8: Alpha amylase assay of *Artemisia Pallens*

In the present study the 0.5 ml water extract of *Artemisia Pallens* was found to be 121% and 1 ml was 11.5%, 0.5 ml methanol extract was 91% and 1 ml was 78%, and 0.5 ml of chloroform extract was 181% and 1 ml was 186% respectively. According to [29], the ethanol extract of *Artemisia herba-alba* (Asso.) herbs and mucilage of 70% ethyl alcohol extract of *Artemisia herba-alba* (Asso.) showed the α- amylase assay to be 11% and 2%. The. These indicate that all the extracts of *A.pallens* was found to be higher than [29]. These enzymes seems to play a vital role in carbohydrate digestion.
In the present study the 0.5 ml water extract of *Artemisia Pallens* was found to be 121% and 1 ml was 11.5%, 0.5 ml methanol extract was 91% and 1 ml was 78%, and 0.5 ml of chloroform extract was 181% and 1 ml was 186% respectively. Following [30], the n-butanol and ethyl acetate extract of *A. capillaris* was 68% and 75%. These indicate that all the extracts of *A.pallens* was found to be higher than [30]. These findings suggest that the extracts from *A.pallens* can be used to treat postprandial hyperglycaemia.

3.8 Alpha glucosidase assay
Alpha glucosidase assay of 0.5 ml of water extract of *Artemisia Pallens* was found to be 55.8%, 1 ml was 58.8%, 0.5 ml of methanol extract was 30.8%, 1 ml was 51.8%, and 0.5 ml of chloroform extract was 27.8% and 1 ml was 56.8%.

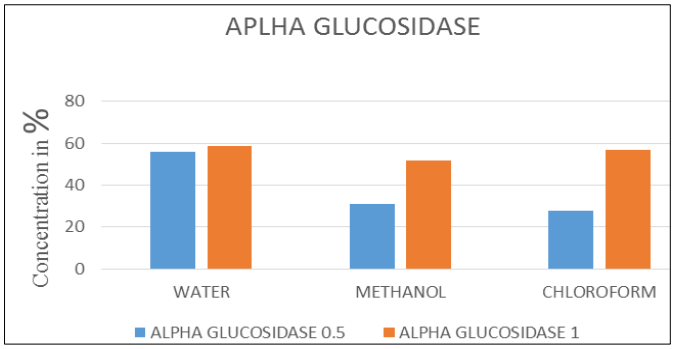


Fig 9: Alpha glucosidase assay of *Artemisia Pallens*

In the present study the α-glucosidase assay of *A.pallens* of 0.5 ml of water extract was 55.8%, 1 ml was 58.8%, 0.5 ml of methanol extract was 30.8%, 1 ml was 51.8%, and 0.5 ml of chloroform extract was 27.8% and 1 ml was 56.8%. With reference to [30], the α-glucosidase assay of N-butanol extract of *A. capillaris* was 71%. These values indicate that all the values of *A.pallens* seems to be slightly lower than [30]. These indicate that the extracts of *A. capillaris* could be used to cause a rise in post prandial blood glucose absorption.

3.9 FTIR

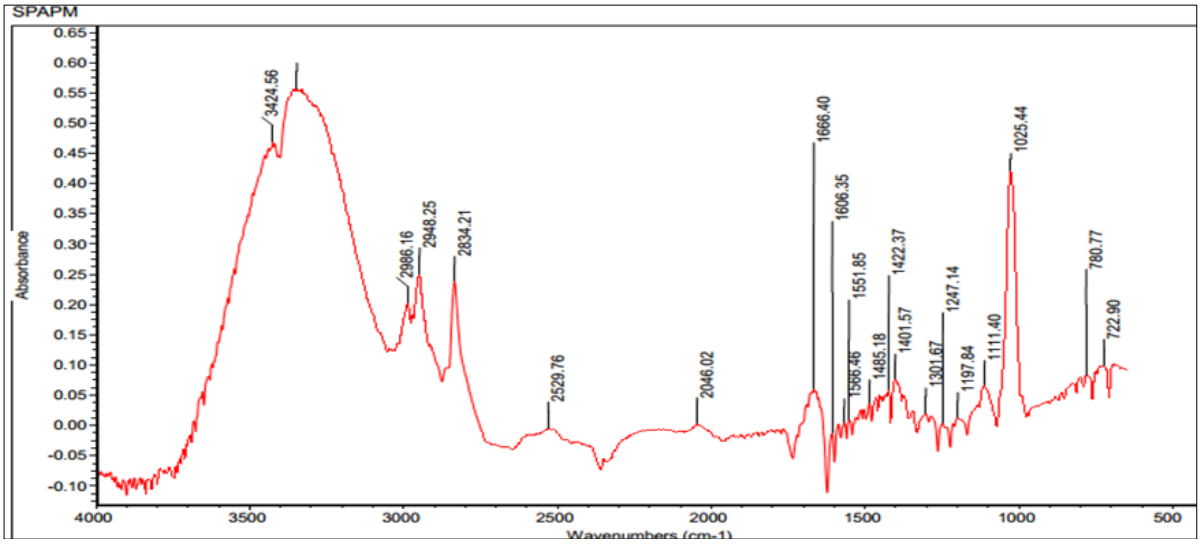


Fig 10: FTIR analysis of methanol extract

Table 2: FTIR activity of methanol extract

S. No	Name of the compound present	Molecular formula	Molecular weight	Activity	Peak value	Intensity	Group assigned
1	Furfuryl alcohol	C5H6O2	98	Igniter of rockets	722.90	0.0979	Alcohol
2	Benzyl alcohol	C7H8O	108	Bacteriostatic preservative	780.77	0.0823	Alcohol
3	R)-(+)-LIMONENE	C10H16	163	Antimicrobial compound	1111.40	0.0650	Cyclic monoterpene
4	Benzyl alcohol	C7H8O	108	Bacteriostatic preservative	1247.14	0.0029	Alcohol
5	4-Ethylbenzyl Alcohol	C7H8O	108	Bacteriostatic preservative	1301.67	0.0187	H-bonded alcohol
6	Phenylphosphonic acid	C6H8PO4	158	Metal chelant	1422.37	0.0576	Organic acid
7	+/-)-beta-Butyrolactone	C4H6O2	86	Recreational drug	1485.18	0.0359	Hydroxycarbocylic acid
8	Alpha-Bromo-p-xylene	C8H9Br	185	Pharmacological intermediate	1551.65	0.0083	Aromatic hydrocarbon
9	triethyl Phosphate	C6H15O4P	182	Industrial catalyst	1566.45	0.0038	Ester
10	Diethyl Ethylmalonate	C7H12O4	160	Flavouring agent	3424.56	0.469	Ether

The FTIR analysis of methanol extract showed the presence of Furfuryl alcohol, benzyl alcohol, Limonene, Ethyl benzyl alcohol, phenyl phosphonic acid, beta butyrlactone, alpha bromo-p-xylene, triethyl phosphate and diethyl malonate which showed major peaks at 722.90, 780.77, 1111.40, 1247.14, 1301.67, 1422.37, 1485.18, 1551.65, 1566.45 and 3424.56.

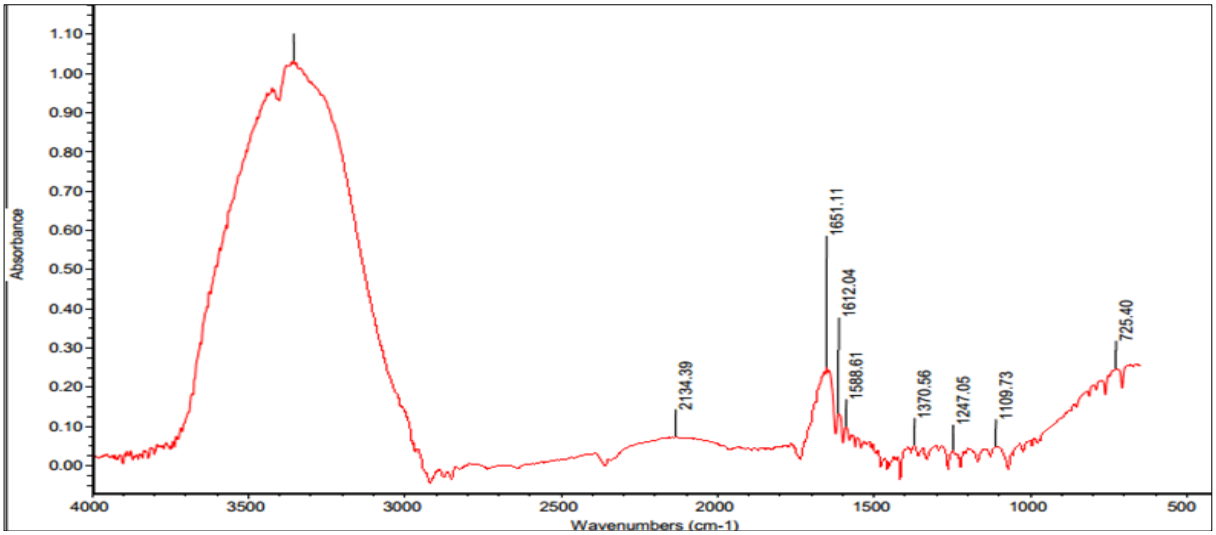


Fig 11: FTIR extract of water extract

Table 3: FTIR activity of water extract

S.NO	Name of the compound	Molecular formula	Molecular weight	Activity	Peak value	Intensity	Nature of compound
1	ACETALDOXIME	C2H5NO	59	Production of pesticides	725.40	0.246	Oxime
2	NONANONE	C9H18O	142	Flavouring agent	1109.73	0.0473	Ketone
3	2-Heptanone	C7H14O	114	Flavouring agent	1247.05	0.0368	Methyl amyl ketone
4	TROPINONE	C8H13NO	139	Cocaine analogues	1370.56	0.0519	Alkaloid
5	2-Octanone	C18H6O	128	Flavour & fragrance ingredient	1588.61	0.0981	Natural ketone
6	3-Octanone	C18H6O	128	Flavour & fragrance ingredient	1612.04	0.132	Natural ketone
7	TRANS-2-HEXENE	C6H12	84	Biochemical for proteomic research	1651.11	0.251	Alkene
8	2-Nonanone	C9H18O	142	Food additives	2134.99	0.0720	Ketone
9	3-Nonanone	C9H18O	142	Flavouring agent	3355.63	1.032	Ketone
10	ACETALDOXIME	C2H5NO	59	Production of pesticides	725.40	0.246	Oxime

In the present study of the FTIR analysis of the methanol and water analysis of *A.pallens* **was** found to contain alcohols, H-bonded alcohol, organic acid, ester, ether, Hydroxycarbocylic acid, Oxime, ketone, alkaloid and natural ketone. This showed the peaks at 722.90, 780.77, 1111.40, 1247.14, 1301.67, 1422.37, 1485.18, 1551.65, 1566.45, 3424.56 725.40, 11.09.73, 1247.05, 1370.56, 1588.61, 1612.04, 1651.11, 2134.99 and 3355.63 respectively. Around three peaks of *A.pallens* seems to be higher than the peaks of *A. annua* and many natural compounds seems to be present in *A. pallens*. These bioactive compounds are supposed to have good antioxidant properties. According to [31], the FTIR assay of the methanol extract of *Artemisia annua* showed the presence of alkenes, aliphatic fluoro components, alcohols, ethers, carboxylic acids, esters, nitro components, alkanes, H-bonded H-X group, Hydrogen bonded Alcohols and Phenols showed major peaks at 777.31, 1028.06, 1155.36, 1315.45, 1417.68, 2848.86, 2918.30, 3273.20 and 3361.93 respectively.

4. GCMS

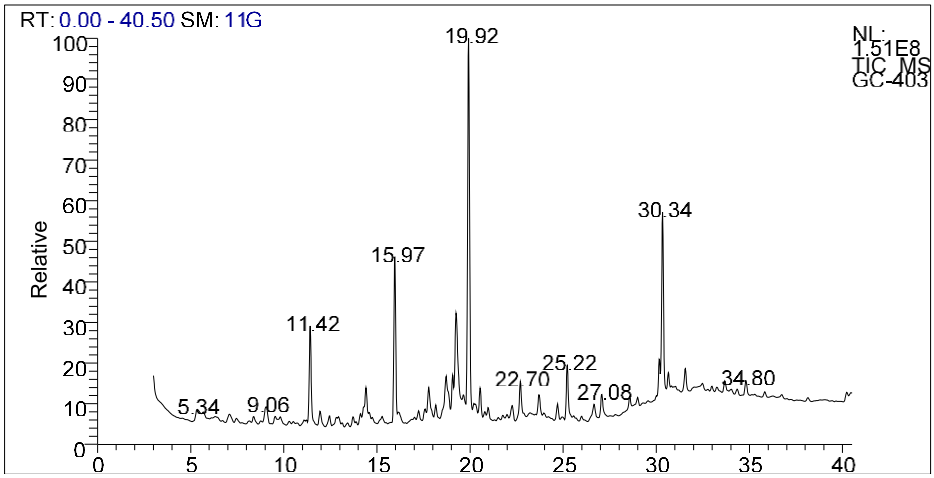
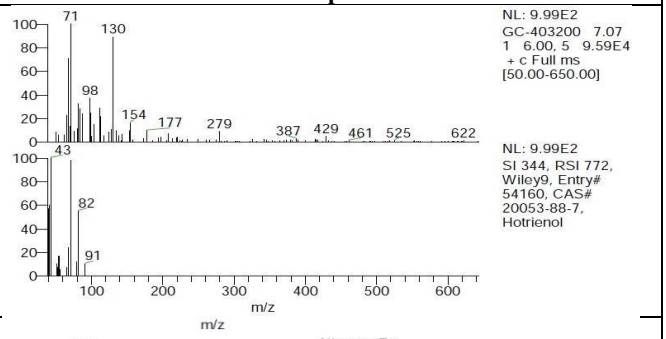
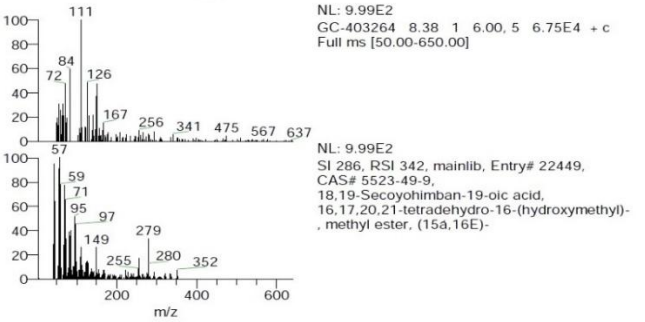
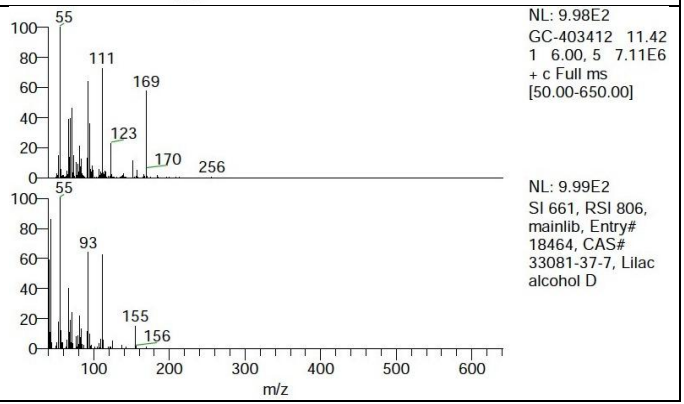
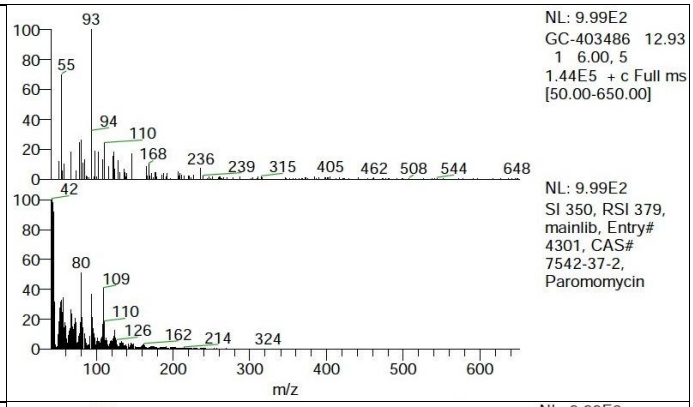
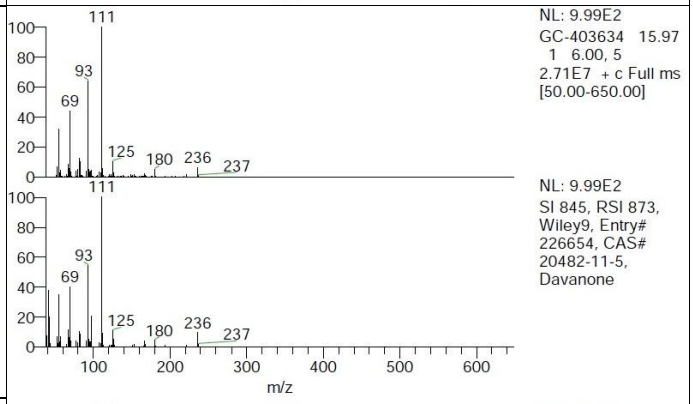
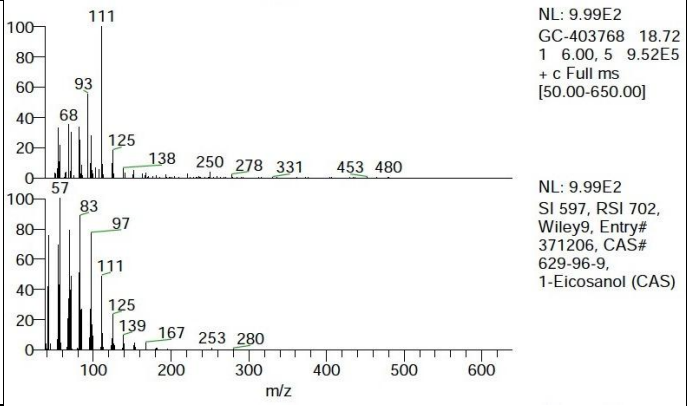
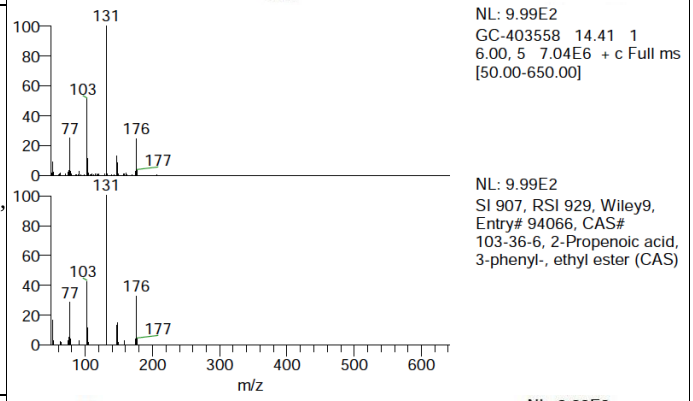
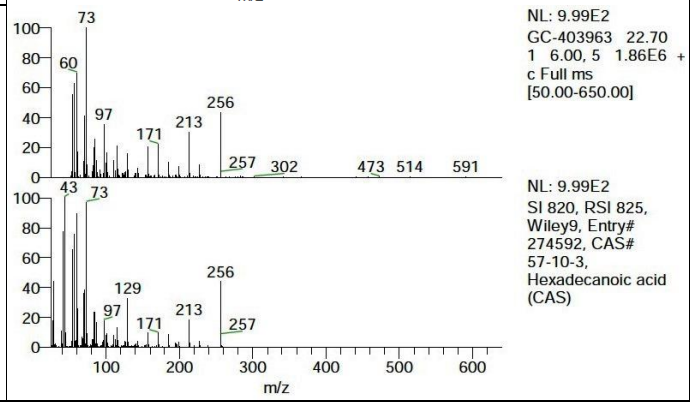


Fig 12: GCMS assay of *A.pallens*

Table 4: GCMS analysis of *Artemisia* pallens

S.No	Name of the compound	Molecular formula	Molecular weight	Compound nature	Pharmacological action
1	Hotrienol	C10H16O	152	Alkaloid	Flavour and fragrance agent
2	18,19-Secoyohimban-19-oic acid,	C21H24N2O3	352	Essential oil	Anti-Microbial and antiviral
3	Lilac alcohol	C10H18O2	170	Alkaloid	Flavour and fragrance agent
4	Paromomycin	C23H45N5O14	615	Antibiotic	Including amoebiasis, giardiasis, Leishmaniasis, and tapeworm infection
5	Davanone	C15H24O2	236	Essential oil	Perfumes, flavours and cosmetics, anti-insects
6	1-Eicosanol (CAS)	C20H42O	295	Straight chain fatty acid	Anti-inflammatory
7	2-Propenoic acid, 3-(2,3-dimethoxyphenyl)-,	C11H12O4	208	Unsaturated carboxylic acid	Anti tumor and anti-microbial
8	n-Hexadecanoic acid	C16H32O2	256	Palmitic acid	Antioxidant 5 Alpha reductase inhibitor Pesticide Nematicide Lubricant Hypocholesterolemic Antiandrogenic
9	Ascorbic acid	C38H68O8	652	Essential acid	Vitamin C
10	Formic acid	C15H24O3	252	Essential acid	A major use of formic acid is as a preservative and antibacterial agent in livestock feed. Formic acid arrests certain decay processes and causes the feed to retain its nutritive value longer, and so it is widely used to preserve winter feed for cattle
11	Isophytol	C20H40O	296	Essential oil	Perfumes, flavours and cosmetics, anti-insect and anti-bacterial
12	Gamolenic Acid	C18H30O2	278	Essential oil	Used for rheumatoid arthritis (RA), polyps in the mouth, high cholesterol and other blood fats, heart disease, metabolic syndrome (Syndrome-X), diabetic nerve pain, attention deficit-hyperactivity disorder (ADHD), depression, depression after childbirth, chronic fatigue syndrome (CFS), and hay fever (allergic rhinitis). Some people use it to prevent cancer and to help breast cancer patients respond faster to treatment with the drug tamoxifen.

S.No	Name of the compound	Graph
1	Hotrienol	
2	18,19-Secoyohimban-19-oic acid,	
3	Lilac alcohol	

4	Paromomycin	
5	Davanone	
6	1-Eicosanol (CAS)	
7	2-Propenoic acid, 3-(2,3-dimethoxyphenyl)-,	
8	n-Hexadecanoic acid	

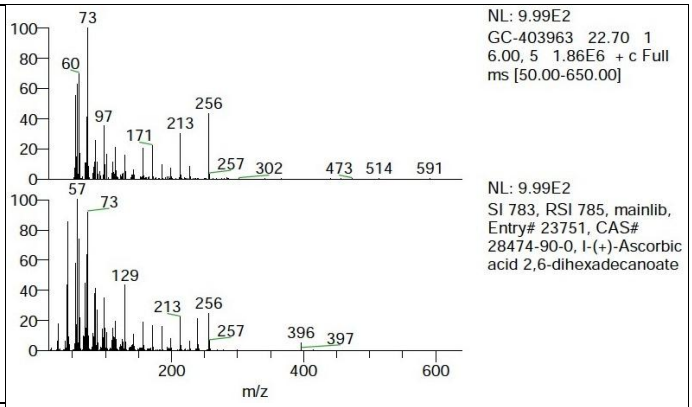
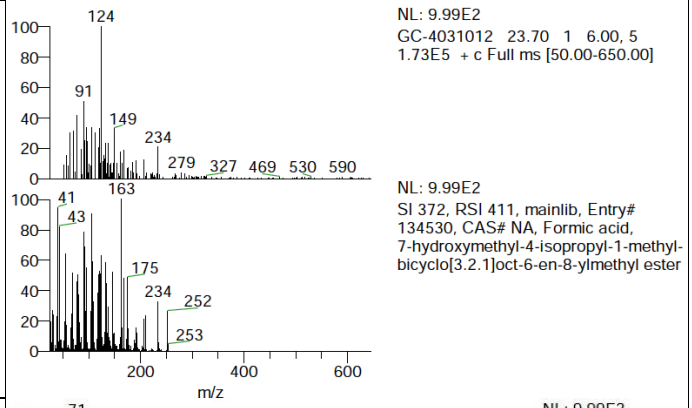
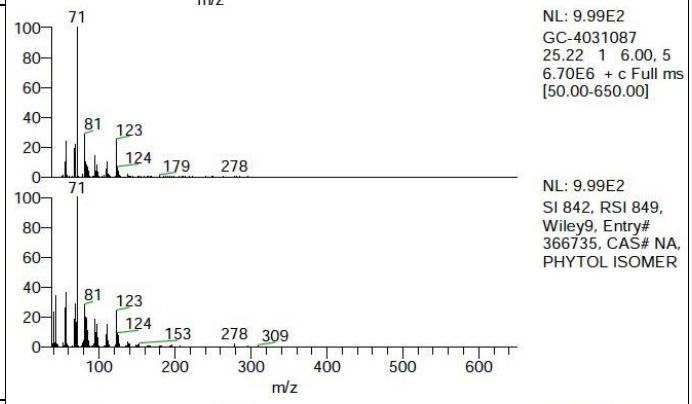
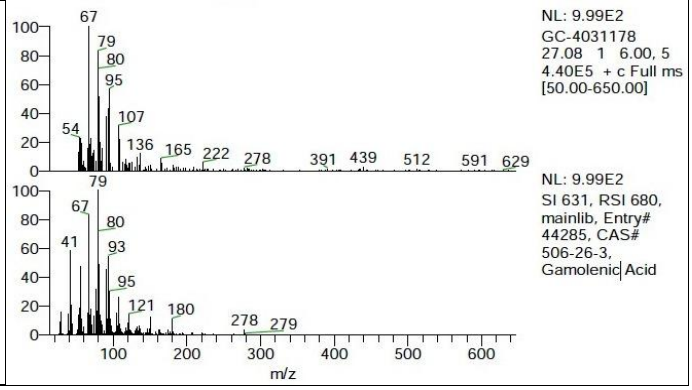
9	Ascorbic acid	
10	Formic acid	
11	Isophytol	
12	Gamolenic Acid	

Fig 13: GCMS analysis of *Artemisia* pallens

In the present study of the methanol extract of *A.pallens* showed the presence of many essential compounds like ascorbic acid, formic acid, lilac alcohol, Paromomycin, Hotrienol, Isophytol, 18,19-Secoyohimban-19-oic acid, Davanone, 1-eicasonol, 2-Propenoic acid, 3-(2,3-dimethoxyphenyl), n-Hexadecanoic acid and Gamolenic acid. Some of these compounds are used in pharmaceuticals. According to [22], the essential oil of *A.amygdalina*, showed the presence of Sabinene (14.3%), p-cymene (12.5%), Eucalyptol (16.7%), and L-borneol (12%).

4.1 Antibacterial activity

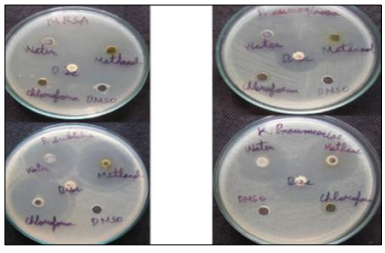


Fig 14: Photo of Microorganism used

Table 5: Microorganisms used to study the antimicrobial activity of *A.pallens*

Organism name	Disc	Water	Methanol	Chloroform	DMSO
Bacillus subtilis	4 mm	14 mm	15 mm	18 mm	Nil
E.coli	10 mm	8 mm	9 mm	2 mm	Nil
K.pneumoniae	12 mm	11 mm	12 mm	3 mm	Nil
Pseudomonas aeruginosa	8 mm	10 mm	5 mm	8 mm	Nil
Staphylococcus aureus	Nil	5 mm	5 mm	Nil	Nil

According to the present study of *Artemisia Pallens*, the water, methanol and chloroform extract was found to be very effective against various bacteria as indicated by the zone of inhibition. Maximum inhibition was obtained by the chloroform extract of *Artemisia Pallens* against *Bacillus subtilis* (18mm), followed by methanol extract and the water extract, methanol extract of *K.pneumoniae* (12mm) and water extract of *K.pneumoniae* (11mm).

According to [15], the ethanolic extract of *A.pallens* revealed the tested organisms like *Bacillus lentus* and *salmonella paratyphi* showed sensitivity response towards plant extract containing saponin subsequently. Other organism such as *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumonia* were considered as moderate sensitivity in response to the saponin content in the plant sample extract when compared with ciprofloxacin. The results showed the zone of inhibition of the100 µg of sample extract when compared with 10 µg of standard were susceptible to antibiotic which shows the sample sensitivity against antibiotic (ciprofloxacin disc).

5. Conclusion

Artemisia Pallens showed the presence of alkaloids, steroids, flavonoids, reducing sugars, tannins, saponins, terpenoids, anthraquinine, and phenols. The *Artemisia Pallens* leaves showed significant antioxidant activity against the standard. It also revealed the significant DPPH radical scavenging assay, total phenols assay, FRAP reducing power, Hydroxyl radical scavenging activity and also showed significant Super oxide radical scavenging activity, alpha amylase, alpha glucosidase, GCMS, FTIR and antibacterial assays.

6. Acknowledgement

The author is thankful to the director and also to the staff of CBNR for providing the necessary support to carry out this work.

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