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PHYTOCHEMICAL AND PHARMACOLOGICAL PROPERTIES OF *ARTEMISIA PALLENS*

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ABSTRACT

Artemisia pallens Walls. ex DC, commonly known as Davana, is an aromatic herb found abundantly in humid habitats in the plains all over India. *Artemisia pallens* is found in Nilgiri hills, and has been used by the tribal people for various ailments. It has been widely used in Indian folk medicine for the treatment of diabetes mellitus. This plant is accredited with antihelmintic, antipyretic and tonic properties and also considered as a good fodder. The oil possesses antispasmodic, antibacterial, antifungal and stimulant properties. The plant has been screened for the antimicrobial, antidiabetic, antinociceptive and wound healing activity. The current study deals with the Phytochemical and Pharmacological of *Artemisia pallens.*

INTRODUCTION: Genus *Artemisia* (Asteraceae) popularly known as “Sage Brush” or “Worm wood” is bitter aromatics. Artemisia is the largest genus comprising of 400 species widely distributed in South Africa and South America, and 34 species are found in India. This genus is named in honor of Artemis the Greek goddess of chastity. Some of them are sources of volatile oils. Sesquiterpene lactones are known to be present in almost all species [2].

Artemisia species are invariably found as small fragrant shrubs or herbs and most of them yield essential oils. Some of these oils are used as medicine such as vermifuge, stimulant and in perfumery, etc. The leaves of some species are used as culinary herbs. The plants themselves are popular among gardeners as cultivated ornamentals. *Artemisia umbelliformis* is traditionally used to treat loss of appetite and digestive spasms. Some *Artemisia* species are used as stomachic, stimulant, flavoring, antioxidant, antihelmintic, antibacterial, anti-inflammatory, antispasmodic, carminative etc.

India is one of the richest sources of medicinal & aromatic plants. The wealth of India is stored in enormous amount of natural flora, which has been gifted to her. Endowed with a diversity of agro climatic condition, India is virtually a bioethical garden of the world. India possess different ecosystem ranging from temperate in Himalayas to tropical in South India, dry in central India to humid & wet in Assam & Kerala, thus providing conditions favorable for growth of different medicinal & aromatic plants.

The medicinal plant based drugs have the added advantage of being simple, effective & offering a broad spectrum of activity with an emphasis on the preventive actions of drugs. Because of these factors, the demand for plant-based medicines (phytomedicine & phytopharmaceuticals) is increasing worldwide [2]. The flowers of Davanam are racemose panicles bear

numerous small yellow flower heads or capitula, but the silvery white silky covering of down gives the foliage a Grey or white appearance. It has alternate pinnasect leaves (leaf which is divided into opposite pairs of lobes cut almost to the midrib in narrow divisions) or palmatisect leaves (the green tissue is divided into several segments not fully separated at the base) [2].

It is commercially cultivated for its fragrant leaves and flowers. It grows from seeds and cuttings and reaches maturity in four months. The plant is woody in the lower part of the stem, but with yearly branches. Davana is mostly cultivated in the red soil regions in South India. It comes up very well in rich loamy soils. Davana is an annual herb, family compositor, requiring about four months to reach maturity, at which it attains a height of about around one and half feet. Season is very important when the crop is grown for production of oil.

The crop is allowed to grow until it flowers, which take about 4 months from sowing. It is grown as short term crop from November to February/March and as a rotation crop extending upto April/May. The crop does not withstand heavy rains. Total yield of the main crop and the ratoon crop is about 14 tons per hectare, which on shade drying and distillation yields about 10 kg of Davana oil. In large scale distillation, an average yield of 3.2% from a material dried for about 2 days may be considered satisfactory. Oil content in davana is maximum in the flower head and is much less in the leaf and stem.

Vernacular Names: [2]

English	:	Wormwood
Hindi	:	Davana
Kannada	:	Davana
Telugu	:	Davanamu
Malayalam	:	Davanam
Tamil	:	Marikkoluntu

Taxonomy:

Kingdom	:	Plantae
Division	:	Angiosperms
Class	:	Eudicots
(Unranked)	:	Asterids
Order	:	Asterales
Family	:	Asteraceae
Genus	:	Artemisia
Species	:	Pallens
Binomial name	:	Artemisia pallens

Ethno Medical Information: *Artemisia pallens* commonly known as "Davana" has been traditionally used in Indian folk medicine for the treatment of diabetes mellitus, wound healing and immuno-modulating, antihelmintic, antipyretic, antibacterial, antifungal, tonic properties, wound healing and also as stimulant (Drurey and Wallington, 1980). It is also considered a good fodder. The oil possesses antispasmodic, antibacterial, antifungal and stimulant properties [2].

Traditional Uses [3]: The leaves and flowers yield an essential oil known as oil of Davana. Several species yield essential oil and some are used as fodder, some of them are a source of the valuable antihelmintic drug santonin. Davana blossoms are offered to Shiva, the God of Transformation, by the faithful, and decorate his altar throughout the day. Davana has been widely used In Iraqi and Indian medicine for the treatment of diabetes mellitus. Oral administration of an aqueous/methanolic extract from the aerial parts of the plants was observed to reduce diabetes in glucose–fed hyperglycemic and alloxan-treated rabbits and rats.

- Davana oil is emotionally balancing and calming, aids in calming down anxiety.
- Davana oil is used in making perfumes of sweet and fruity fragrances.
- When applied on the skin, Davana is said to smell differently on different persons. This peculiar property is highly valued in high class perfumery to create fragrances with truly individual notes.
- Davana leaves and stalks are used in making bouquets, garlands, fresh or dry flower arrangements.
- Davana is massaged over lower abdominal area to stimulate moon cycle.
- Davana oil is soothing to rough, dry, chapped skin, skin infections and cuts.

- Oral administration led to significant blood glucose lowering effect.
- *Artemisia pallens* is a preferred food for the larvae of a number of butterfly species.

Microscopical Observation:

Anatomy of leaf: The leaf is dorsiventral with isolateral mesophyll tissue **(Figure 2)**. The surface of the leaf is even and uniform. The mid rib is fairly prominent and spindle shaped in cross sectional view, projecting equally on the upper and lower sides **(Figure 2)**. There is a small furrow on the lower side of the mid rib. The epidermal layer of the mid rib is thin and distinct with squarish cells and smooth cuticle. There is a single large vascular bundle which is surrounded by compact parenchymatous tissue; the vascular bundle is collateral with adaxial parallel rows of xylem and abaxial are of phloem **(Figure 2)**. Thick mass of sclerenchyma cells occurs both on the upper and lower sides of the vascular bundle **(Figure 2)**. The mid rib is 550 µm thick.

Lamina: The lamina has even upper and lower surfaces. It has wide; semicircular margin **(Figure 2)**.The lamina is 350 µm thick. The epidermal layers are thin with spindle shaped fairly thick walled cells. The epidermis is stomatiferous, both on the upper and lower sides. The mesophyll consists of a central horizontal layer of two or three rows of cells. On the upper and lower sides of the central row are wide, thin walled palisade cells **(Figure 2)**. The vascular bundles of the lateral veins are located in the median part of the lamina. The bundles become smaller towards the margin. They are collateral with adaxial xylem cluster and abaxial phloem.

Venation: The lateral veins are thin and less and form less distinct vein-islets. The islet is distinct; it is wide and inconsistent in shape. Vein terminations are present sporadically; they are simple, short and thin **(Figure 3).**

Trichomes: Epidermal trichomes are prevalent on the surface of the leaf. There two types of trichomes, both of which are glandular in nature. This type of trichome has short unicellur stalk with two terminal cells placed end to end forming a spindle shape **(Figure 4).**The spindle shaped trichomes are diffusely distributed all over the lamina; they are 50 × 30 µm in size **(Figures 4)**. These types of trichomes are less common. The trichome has a short, unicellular stalk with a circular thin plate of eight or more triangular cells. The cells have prominent nuclei.

The orbicular trichomes are random in distribution. They are 35 µm in diameter **(Figure 4).**

Petiole: In cross sectional view, the petiole of the leaf is boat shaped with wide shallow concavity on the adaxial side and wavy and convex on the abaxial side. The epidermis is prominent and stomatiferous; the epidermal cells are squarish with thick cuticle. The ground tissue consists of a central horizontal band of compact parenchyma tissue and narrow bands of palisade tissue, both on the adaxial and abaxial. These are larger median vascular bundle and three or more small, less prominent vascular strands on either side of the central strand.

The vascular strands are collateral surrounded by a single whole of parenchymatous sheath cells. The central bundle has wide mass of extension both on the adaxial and abaxial sides. Dense epidermal trichomes are spread all along the surface of the petiole. **(Figure 6)**

Stem: The stem is young with primary vascular tissue. The stem surface bears dense trichomes. Epidermis is thin and distinct, comprising of small squarish cells with thick cuticle. Cortex is homogeneous and parenchymatous with small air-chambers. The stele consists of several discrete vascular bundles arranged in elliptical circle. The vascular bundle is collateral with a row of parallel rows of xylem elements. Phloem is in wide mass **(Figure 6)**. Pith consists of a central elliptic cavity, with wide parenchymatous borders **(Figure 6).**

Microscopical observation of *Artemisia pallens* showed that the leaf is dorsiventral with isolateral mesophyll tissue. The midrib is fairly prominent and spindle shaped in cross sectional view, projecting equally on the upper and lower sides. The epidermal layer of the midrib is thin and distinct with squarish cells and smooth cuticle.

There is a single large vascular bundle which is surrounded by compact parenchymatous tissue; the vascular bundle is collateral with adaxial parallel rows

of xylem and abaxial are of phloem. Thick mass of sclerenchyma cells occurs both on the upper and lower sides of the Vascular bundle. The epidermis is stomatiferous both on the upper and lower sides.

Histological Characters:

FIG. 1: SPECIES OF *ARTEMISIA PALLENS*

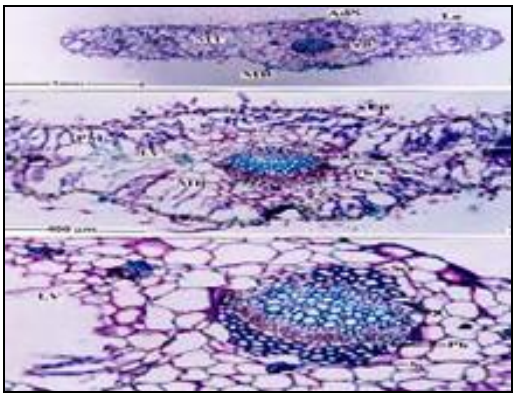

FIG: 2: ANATOMY OF THE LEAF

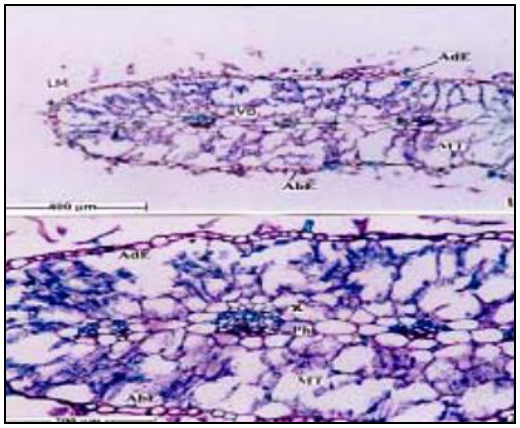

FIG. 3: ANATOMY OF THE LATERAL VEIN WITH LEAF MARGIN

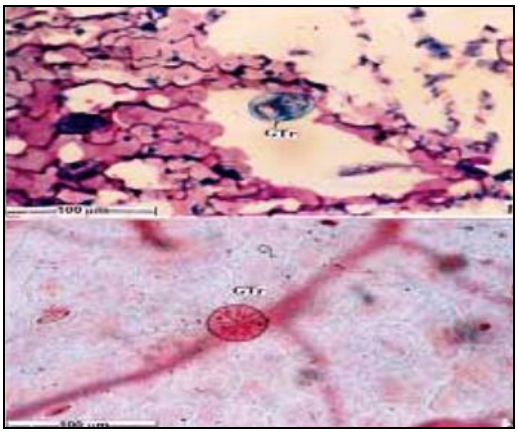

FIG. 4: TRICHOME MORPHOLOGY IN SURFACE VIEW

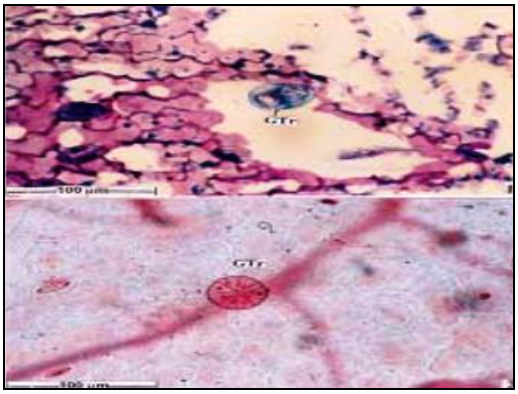

FIG: 5: STRUCTURE OF TRICHOME

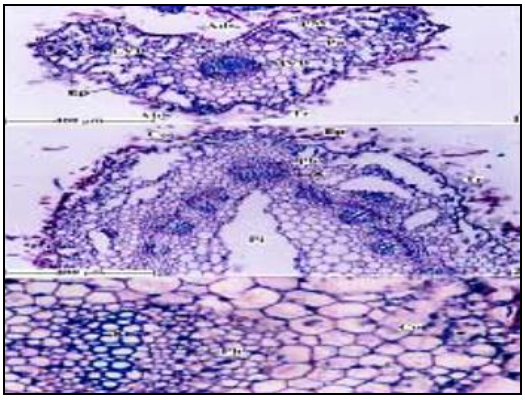

FIG. 6: ANATOMY OF THE PETIOLE AND STEM

LEGENDS FOR FIGURES:

Fig.2: Anatomy of the leaf.
Fig.2.1:T.S.of entire view.
Fig.2.2:T.S. of Midrib enlarged.
Fig.2.3: Midrib vascular bundle enlarged.
(Ads: Adaxial side; Ep: Epidermis, La: Lamina; Lv: Lateral vein; MR: Midrib; MT: Mesophyll tissue; Ph: Phloem; SC: Sclerenchyma; VB: Vascular bundle; X: xylem)
Fig. 3: Anatomy of the lateral vein with leaf margin.
Fig.3.1: T.S. of leaf margin.
Fig.3.2: T.S. of lateral vein enlarged.
(Abe: Abaxial epidermis; Ade: Adaxial epidermis; LM: Leaf margin; MT: Mesophyll tissue; Ph: Phloem; VB: Vascular bundle; X: xylem)
Fig. 4: Trichome Morphology in surface view.

Fig.4.1: Distribution of trichome.
Fig.4.2: One trichome enlarged.
(Gtr: Glandular trichome; MV: Mid vein; VI: Vein-islets; VT: Vein termination)
Fig.5: Structure of trichomes.
Fig.5.1: Two celled spindle shaped trichome.
Fig. 5. 2: Circular peltate trichome. (Gtr : Glandular trichome.)
Fig. 6: Anatomy of the petiole and stem.
Fig.6.1:T.S. of petiole.
Fig.6.2: T.S. of stem.
Fig 6.3:T.S. of stem one vascular bundle enlarged.
(Abs: Abaxial side; Ads: adaxial side; Co: Cortex; Ep: Epidermis; LVB: Lateral vascular bundle; MVB: Median vascular bundle; Pa: Parenchyma; Ph: Phloem; Pi: Pith; PM: Palisade Mesophyll; Tr : Trichome; X:Xylem.)

Habit and Habitat: It shares its habitat with that of the Sandalwood trees of Mysore. It is an aromatic herb found abundantly in humid habitats in the plains all over India. *Artemisia pallens* is found in Nilgiri hills, India [2].

Phytochemical Properties: Davanone, Davana-Ether, Davana Furan and linalol are the major constituents of davana oil. Methyl cinnamate, ethyl cinnamate, bicyclogermacrene, davana ether, 2-hydroxyisodavanone, farnesol, geranyl acetate, sequiterpene lactones, germacranolides etc. are also found. The contents of davanone, the major constituent of davana oil, and linalool decreased while those of (Z) − and (E) − methyl cinnamate, (E) − ethyl cinnamate, bicyclogermacrene, davana ether, 2-hydroxyisodavanone, and farnesol increased from flower heads emergence stage to the initiation of seed set stage. Five compounds, viz., (Z) − and (E) − methyl cinnamates, (Z) − and (E) − ethyl cinnamates, and geranyl acetate, were identified for the first time in davana oil.

Phenol and Flavonoid content: Phytochemical studies of the Hexane, Chloroform, ethanol and chloroform water extracts were conducted and showed the presence of carbohydrate, saponins, phytosterol, proteins and amino acid, tannin and phenolic compounds and flavonoids. It was concluded that the all extracts contain more important chemical constituents for various pharmacological activities [4].

Total phenolic content for *A. pallens* is obtained from the regression equation of calibration curve of pyrocatechol (y = 0.0275x - 0.0278, R^2 = 0.9915) and expressed as pyrocatechol equivalent.

Total flavonoid content for *A. pallens* is obtained from the regression equation of calibration curve of quercetin (y = 0.0307x - 0.0035, R^2 = 0.9978) and expressed as quercetin equivalent. The phenolic and flavonoid content of *A. pallens* in methanol extract is recorded. **(Table 1)**

TABLE 1: TOTAL PHENOLIC AND FLAVONOID CONTENT

Phytoconstituents	Amount
Phenolic content	127.16 / 3.460 mg catechol equivalent/g
Flavonoid content	13.57 / 1.053 mg quercetin equivalent/g

Sesquiterpene ketones related to davanone from *Artemisia pallens*: Extraction of the aerial parts of *Artemisia pallens* was carried out which afforded several sesquiterpene ketones not previously reported from davana oil, including a new 3,4-epoxy derivative of isodavanone, as well as cirsimaritin [5].

Fragrant components of oil from *Artemisia pallens*: The volatile components of the essential oil of *Artemisia pallens* (davana oil) were investigated. The oil contained more than 50 compounds, of which 34 are identified. Eight components are reported for the first time of which five are new. 11-Hydroxy-8-oxo-9, 10-dehydro-10, 11-dihydronerolidol was also isolated indicating a possible role for this compound in the biogenesis of furano-sesquiterpenes of davana oil [6].

A germacranolide from *Artemisia pallens*: New germacranolide from the aerial parts of *Artemisia pallens* was isolated and the structure was established as 4, 5β-epoxy-10α-hydroxy-1-en-3-one-*trans*-germacran-6α, 12-olide by critical comparison with tagitinin C and spectral analysis [7].

A new germacranolide from *Artemisia pallens*: New germacranolide from the aerial parts of *Artemisia pallens was* isolated and the structure was established as 4, 5β-epoxy-10β-hydroxy-1-en-3-one-*trans*-germacran-6α, 12- olide (1) by comparison with its 10α-epimer isolated from the same plant [8].

Previously isolated classes of constituents: Terpenoids; 4, 5 β-epoxy-10α-hydroxy-1-en-3-one-*trans*- germacran-6α, 12-olidew11x.

New-isolated constituent: 4, 5 β-Epoxy- 10 β -hydroxy-1-en-3-one-*trans*-germacran-6 α,12-olide(1) **(Figure 7).**

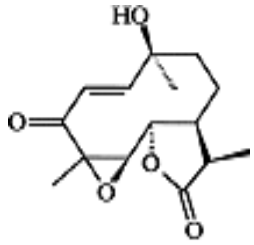

FIG. 7: (4, 5 β- Epoxy-10 β- hydroxy- 1- en- 3-one- *trans*-germacran-6 α, 12-olide)

Influence of Plant Growth Stage on the Essential Oil Content and Composition in Davana (*Artemisia pallens* Wall.): The three plant growth stages influences (full emergence of flower heads, anthesis, and initiation of seed set) the essential oil content and composition in Davana (*Artemisia pallens* Wall) over two successive seasons. The essential oil content was found to be higher at the full emergence of flower heads than at anthesis and initiation of seed set stages. The contents of davanone, the major constituent of davana oil, and linalool decreased while those of (*Z*) - and (*E*)-methyl cinnamate, (*E*)-ethyl cinnamate, bicyclogermacrene, davana ether, 2-hydroxyisodavanone, and farnesol increased from flower heads emergence stage to the initiation of seed set stage.

These results support the general practice of harvesting the crop at full bloom stage. Five compounds, viz., (*Z*) - and (*E*) - methyl cinnamates, (*Z*)- and (*E*)-ethyl cinnamates, and geranyl acetate, were identified for the first time in davana oil [9].

Tissue cultures of *Artemisia pallens*:

Organogenesis, terpenoid production: Germinated seedlings of *Artemisia pallens* gave three types of cultures on MS medium supplemented with different plant growth hormones. Medium containing BA+2,4-D stimulated unorganized callus; BA+IAA medium, semi-organized tissues interspersed with shoot buds; and BA+NAA+IAA medium, multiple shoot cultures. The in vitro shoots developed roots in medium devoid of growth hormones. TLC and GLC analysis of the tissue extracts showed that linalool was present in the cultured tissues, with maximum concentration in the unorganized tissue. Although the TLC profiles of the three culture extracts were similar, the extracts did not contain the major polar compounds of the plant. The plant extracts contained more polar compounds and gave the characteristic fragrance of Davana [10].

***Artemisia pallens* as corrosion inhibitor for mild steel in HCl medium:** Methanolic extract of Artemisia pallens were tested as corrosion inhibitor for mild steel in 4N HCl and conc. HCl. Weight loss and polarization techniques were used for evaluating corrosion inhibition in 4N HCl, whilst weight loss, SEM and FT-IR studies were carried out in conc. HCl. The inhibition efficiency was found to increase with increase of the inhibitor concentrations due to the adsorption of the inhibitor molecules on the metal surface and the adsorption follows Langmuir's adsorption isotherm. The inhibition efficiency was found to be 93% at 1.5 g l−1 in 4N HCl and 96.5% at 40 g l−1 in conc. HCl [11].

Microwave assisted extraction of *Artemisia pallens* for Tyrosinase inhibitory activity: Microwave was reported as a tool for extraction of phytoconstituents present in *Artemisia pallens* wall (compositae) and determination of tyrosinase inhibitory activity which serves as a useful target in the treatment of hyper pigmentation skin disorder. The percentage yield of extract obtained by microwave assisted extraction (MAE) of plant was found to be highly significant when compared to Soxhlet extraction (SE) method.

The tyrosinase inhibitory activity of MAE was found to be highly significant ($p < 0.0001$) when compared with that obtained by SE method. *Artemisia pallens* was found to be rich in phenolic compounds consisting of hydrophobic part which would have acted as competitive inhibitor on the enzyme tyrosinase and thereby on melanin synthesis. Hence the determination of tyrosinase inhibitory potential of *Artemisia pallens* paves a way for development of skin whitening agents [12].

Quantification of Santonin from *Artemisia pallens* Wall by HPTLC: A simple High Performance Thin Layer Chromatographic (HPTLC) method was developed for the analysis of santonin in different extracts of *Artemisia pallens* Wall. The quantification of analytes was carried out using a mobile phase hexane: ethyl acetate (3:2) on pre-coated aluminium plates (Silica gel Merck 60 F254) and densitometric determination was carried out. The spots were located at 258 nm. The amount of santonin in the extracts A, B, C, D was estimated by comparing the peak area using the standard.

Linearity of the standard was found in the concentration range of 1 to 5 µg/spot. The correlation coefficient value was 0.9835. Different compositions of the mobile phase were tested and the desired resolution was achieved by hexane: ethyl acetate (3:2). Spectral characteristics of the peak of standard and that of the extracts were compared for identification of santonin. Calibration curve of santonin was obtained by plotting peak areas versus concentration applied. It was found to be linear in the range of 1 µg to 5 µg per spot. Equation of the calibration curve is y = 5083x + 4343.8. The correlation coefficient was found to be 0.9835 and thus exhibits good linearity between concentration and area. The amount of santonin in the extract A and B was found to be 31.29 mg/g and 40.71 mg/g respectively. Santonin content of extracts C and D were found to be 1.940 and 20.87 mg/g of acetone extract respectively. The proposed HPTLC method was found to be simple, faster and reliable for analysis of santonin [13].

Determination of Artemisinin in *Artemisia pallens* by LC/MS Method: A simple, selective, rapid and precise reverse phase LC/MS method was developed for the standardization of *Artemisia pallens* (Family: Asteraceae) using Artemisinin as an analytical marker. The method was carried out on a Princeton SPHER C18 (150 x 4.6 mm i.d. 5µ) column with a mobile phase consisting of Acetonitrile: Water (60:40 v/v) at a flow rate of 0.5 ml/min.

Detection was carried out at 283 nm. The calibration curve was linear in the range of 10 µg/ml to 140 µg/ml of Artemisinin and the correlation coefficient was 0.9992, indicating good linear dependence of peak area on concentration. The developed method was validated in terms of accuracy, precision, linearity, limit of detection and limit of quantitation. The proposed method can be used for the standardization of Artemisinin in the ethanolic extracts *Artemisia pallens* extract [14] **(Table. 2)**

TABLE 2: ESTIMATION OF ARTEMISININ CONTENT

Extract	Amount of Artemisinin (%w/w)	RSD (%) (n=6)
Atremisia pallens	0.1031	4.91

Pharmacological Studies:

Effects of *Artemisia pallens* Wall. on blood glucose levels in normal and alloxan-induced diabetic rats: Oral administration of the methanol extract of the aerial parts of *Artemisia pallens* Wall (used in Indian folk medicine for the treatment of diabetes mellitus) was carried out on glucose-fed hyperglycaemic and alloxan-induced diabetic rats which led to significant blood glucose lowering effect in the rats. This effect of the extract was dose dependent and significant at 100 mg/kg level in glucose-fed rats. In fasted normal rats, the extract caused a moderate hypoglycaemic effect at a higher dose (1000 mg/kg). The water extract (1000 mg/kg) was inactive [15].

Antioxidant activity of methanol extract: Antioxidant activity for methanol extract of aerial parts of *Artemisia pallens* Wall was evaluated, by employing radical scavenging assays; 2,2 -diphenyl, 1-picryl hydrazyl (DPPH) and nitric oxide. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 516 nm induced by antioxidants. DPPH free radical scavenging activity of methanol extract of *A. pallens* is depicted in **(Figure 8).**

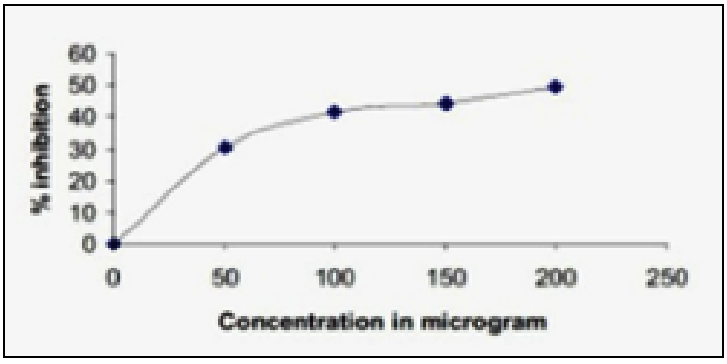

FIG. 8: DPPH-FREE RADICAL SCAVENGING ACTIVITY OF *ARTEMISIA PALLENS* METHANOL EXTRACT

IC_{50} values of DPPH free radical scavenging activity for methanol extract and ascorbic acid are recorded in **(Table 3)**. Nitric oxide free radical scavenging activity of methanol extract of *A. pallens* is depicted in **(Figure 9)**. IC_{50} values of nitric oxide free radical scavenging activity for methanol extract and ascorbic acid are recorded. It was seen that the DPPH and nitric oxide free radical scavenging activity for the methanol extract of *A. pallens* was found to be significant. Presence of major phytocompounds i.e. phenolics, flavonoids may have been responsible for the observed antioxidant activity [16] **(Table 4).**

TABLE 3: DPPH RADICAL SCAVENGING ACTIVITY

Material	IC_{50} Concentration
Ascorbic acid	9.025mcg
Artemisia pallens methanol extract	292.7mcg

TABLE 4: NITRIC OXIDE RADICAL SCAVENGING ACTIVITY

Material	IC_{50} Concentration
Ascorbic acid	148.92 mcg
Artemisia pallens methanol extract	204.61mcg

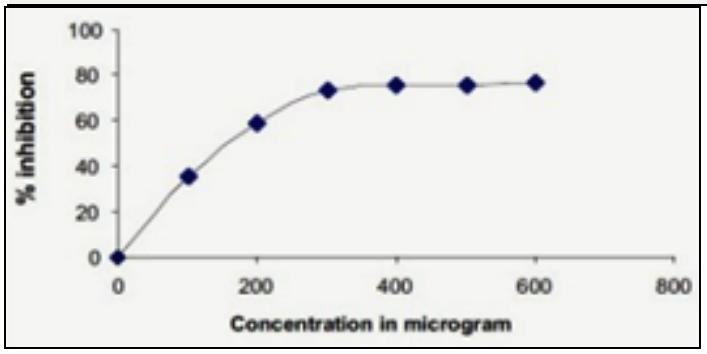

FIG. 9: NITRIC OXIDE-FREE RADICAL SCAVENGING ACTIVITY OF *A. PALLENS* METHANOL EXTRACT

The ethanolic extract of *Artemisia pallens* was screened for their anti-oxidant activity by Diphenyl picryl hydrazine and Hydroxyl radical scavenging methods. The DPPH radical scavenging activity of MEBR was determined from the reduction in absorbance at 517 nm due to scavenging of stable DPPH free radical.

The positive DPPH test suggests that the samples are free radical scavengers. Artemisia pallens shows scavenged DPPH radical with the IC_{50} value 150.33±1.5 µg/ml. The scavenging activity was found to be dose dependent [17]. Where ascorbic acid used as standard shown IC_{50} value of 6.1±0.35 µg/ml and also the scavenging activity was dose dependent as shown in **(Table 5).**

TABLE 5: FREE RADICAL SCAVENGING ACTIVITY OF ARTEMISIA PALLENS AND ASCORBIC ACID IN DPPH METHOD

Concentration in µg/ml	% Free radical scavenging of *Artemisia pallens*	Concentration of Ascorbic acid in µg/ml	% Free radical scavenging of Ascorbic acid
1.95	3.53±0.22	1.0	37.23±0.49
3.9	5.76±0.37	2.0	42.29±0.67
7.75	9.42±0.10	5.0	49.92±0.12
15.5	14.32±0.90	10.0	75.93±0.11
31.5	23.79±1.03	15.0	94.70±0.23
62.5	40.60±1.06	25.0	95.41±0.32
125	49.32±0.37	50.0	96.13±0.37
250	75.93±0.86	--	--
500	94.70±0.33	--	--
1000	95.41±0.39	--	--
IC50	**150.33±1.05 ug/ml**	--	**6.1 ± 0.35µg/ml**

Hydroxyl radical scavenging activity was estimated by generating the hydroxyl radicals using Phenylhydrazine. Phenylhydrazine in solution has been shown to produce hydroxyl radicals. Hydroxyl radical scavenging was measured by studying the competition between deoxy-d-ribose and sample extracts for hydroxyl radicals produced by Phenyl hydrazine[17]. The extent of deoxy-d-ribose degradation is measured as TBARS by method of Ohkawa et al. Artemisia pallens scavenged the hydroxyl radicals strongly with an IC50 value 92.0 µg/ml. IC50 value of GA is 3.3±0.2 µg/ml. **(Table 6).**

TABLE 6: HYDROXYL RADICAL SCAVENGING ACTIVITY OF *ARTEMISIA PALLENS* AND GALLIC ACID HYDROXYL RADICAL SCAVENGING ACTIVITY OF *ARTEMISIA PALLENS* AND GALLIC ACID

Concentration in µg	% OH. radical scavenging of *Artemisia pallens*	Concentration in µg/ml	% OH. radical scavenging of Gallic acid
25.0	8.17±4.06	0.5	20.91±6.51
37.5	29.34±4.08	1.0	25.81±5.65
50.0	40.20±4.51	2.0	36.54±3.97
75.0	44.14±3.61	3.0	46.41±2.81
100.0	53.68±3.58	4.0	58.39±2.62
IC_{50}	92.0 ±3.2µg/ml	IC_{50}	3.3±0.2 µg/ml

Antimicrobial Screening: The ethanolic extract of *Artemisia pallens was* screened for their antibacterial activity. The ethanolic extracts of *Artemisia pallens* was found to be effective against various bacteria as

indicated by zone of inhibition. Maximum inhibition was obtained against *Bacillus stearothermophilus* (27.6 mm) followed by *Klebsiella pneumonia* (27.4 mm), *Micrococcus luteus* (26.2 mm), *Pseudomonas cepacia* (22.2 mm), *Salmonella typhi* (19 mm) at a concentration of 30mg/ml. The results indicate that, ethanolic extract of *Artemisia pallens* possess antimicrobial activity and presence of flavonoids are found to be responsible for this activity [17] **(Table 7).**

TABLE 7: ANTIBACTERIAL ACTIVITIES OF THE ETHANOLIC EXTRACTS OF *ARTEMISIA PALLENS* BY CUP PLATE METHOD

S. no	Organism	Diameter of Zone of Inhibition in mm			
		Artemisia pallens Conc. (mg/ml)			
		10	20	30	STD
GRAM POSITIVE STRAINS					
1	*Bacillus subtilis*	00	00	00	15
2	*Bacillus stearothermophilus*	21.0	24.5	27.6	30.0
3	*Micrococcus luteus*	22.2	24.2	26.2	31.4
GRAM NEGATIVE STRAINS					
1	*Klebsiella pneumonia*	21.1	24.3	27.4	32.3
2	*Pseudomonas cepacia*	10.1	17.3	22.2	24.8
3	*Salmonella typhi*	10.9	20.1	19.0	20.1

The Extracts of *Artemisia pallens* were analyzed for their antibacterial capacity against six bacterial strains and a yeast strain. The antibacterial activity of methanolic extract of *Artemisia pallens* i.e., Non-polar and semi polar extract does not show any activity against the test organisms. Only methanolic extract showed activity against *Bacillus cereus*. Antimicrobial activity of fractions of this extract against *Bacillus cereus* is represented in **(Table 8)** and **(Table 9).** Chloroform: methanol (8:2) was found to be more active [18].

TABLE 8: ANTIMICROBIAL ACTIVITY OF PLANT EXTRACTS

Microorganism	Gram	Diameter of zone of inhibition (mm)[a]	
		Methanolic extract	Standard
Escherichia coli	-ve	--	20
Salmonella typhimurium	-ve	--	20
Staphylococcus aureus	+ve	--	20
Pseudomonas aeruginosa	-ve	--	13
Proteus vulgaris	-ve	--	22
Bacillus cereus	+ve	10	40
Saccharomyces cerevisiae	--	--	25

TABLE 9: ANTIMICROBIAL ACTIVITY OF FRACTIONS OF METHANOL EXTRACT

Fractions	Diameter of zone of inhibition (mm)a
A	09
B	10
C	13
D	07

a= Zone of inhibition including the diameter of filter paper disc (5 mm)

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