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EVALUATION OF PHYTOCHEMICAL COMPOSITION AND ANTI-OXIDANT POTENTIAL OF *ANDROGRAPHIS SERPYLLIFOLIA* (VAHL) WIGHT: AN ENDEMIC ANTI-OPHIDIC PLANT

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ABSTRACT: In folk medicine, especially in developing countries, several plant species are employed for the treatment of snakebites that lack prompt access to serum therapy. *Andrographis serpyllifolia* (Vahl), belonging to Acanthaceae, has been recorded in ethnobotanical archives as a plant possessing potent anti-ophidic activity for snake bites. The present study emphasis to investigate the phytochemical profile and anti-oxidant properties of various plant extracts of *A. serpyllifolia*. To carry out this work, various cold plant extracts were prepared from shade dried leaves and used. During qualitative analysis, all the plant extracts showed positive results for all phytocompounds. Various quantitative assays exhibited that all plant extracts were having significant level of secondary metabolites with values ranging from 1148 to 286 mg GAE/g for phenol, 1262 to 976 mg LN/g for terpenoids, 172 to 106 mg RE/g for flavonoids and 21.5 to 3.6 mg TA/g for tannins. All the plant extracts tested had higher DPPH, ABTS scavenging activity, and metal chelating ability. The free radical scavenging activity of plant extracts were found to be lower only for aqueous extract during FRAP, phosphomolybdenum, NO_2, and lipid peroxidation assays. During superoxide scavenging activity assay, the lowest percent of inhibition was shown by methanol extract. The benzene extract manifested the lowest hydroxyl radical scavenging activity and reducing power. The investigation proves that *A. serpyllifolia* is a potential anti-ophidic plant due to the presence of the highest level of various secondary metabolites and free radical scavenging activities. Thus, the results confirm its use in the management of snake-bites.

INTRODUCTION: Venomous snakebites represent an important risk for public health worldwide, especially in tropical regions. Snake venom has been the cause of innumerable deaths worldwide [1].

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Snake venoms are a complex mixture of enzymatic and toxic proteins, which include phospholipase A2 (PLA2s), myotoxins, hemorrhagic metalloprotein-ases, proteolytic enzymes, coagulant components, cardiotoxins, cytotoxins, neurotoxins [2, 3, 4]. During snake bite, the venom constituents are mixed with circulatory systems and altered a variety of physiological processes resulting in neurotoxicity, myotoxicity, cardiotoxicity, anticoagulant, hemorrhagic, and edema-inducing effects [5]. Anti-venom immunotherapy is the only specific treatment against snake venom envenomation.

There are various side effects of anti-venom such as anaphylactic shock, pyrogen reaction and serum sickness. Most of these symptoms may be due to the action of high concentrations of non-immunoglobulin proteins present in commercially available hyperimmune anti-venom [6].

India has a rich tradition of the usage of medicinal plants. Plants are used either singly or in combination, as antidotes for snake envenomation by rural populations in India and in many parts of the world. Many Indian medicinal plants are mentioned in the literature, which is used to treat snakebite victims, especially in rural areas. However, only a few species have been scientifically investigated and still less had their active components isolated and characterized both structurally and functionally [1].

According to the literature search performed, a lot of ethnopharmacological studies showing medicinal plants claimed as anti-ophidic [7]. The phytochemicals such as plant phenols, alkaloid, triterpenoid, steroid showed effective anti-snake venom activity [1, 5]. Many plants with potential *in-vitro* antioxidant activities such as *Andrographis paniculata* [8], *Acorus calamus* [9], *Amaranthus spinosus* [10], *Gymnema sylvestre* [11], *Tylophora indica* [12], *Betula alnoides* [13], *Opuntia ficus* [14], *Chenopodium album* [15], *Carrica papaya* [16] *etc*. have been used as popular sources of antidotes for various snake bites [17].

Andrographis serpyllifolia (Vahl) Wight, a member of the family Acanthaceae, is a valuable medicinal endemic plant with significant therapeutic activity and is found incorporated in several ethnobotanical formulations. Though it is included in the genus Andrographis, it has a form, architecture, and structure not shared by any of its 42 or more sister species. *A. serpyllifolia* has evolved into a ground-hugging prostrate, perennial, geophyte that successfully survives multiple geo-ecological challenges and grazing threats year after year. Scientists have gathered information on traditional and folklore usage of leaf paste of *A. serpyllifolia* for the treatment of snake and scorpion bites following extensive interaction with tribal communities in Palani Hills, Tamil Nadu, Andhra Pradesh, Telangana and Karnataka [18]. The objective of this study was to carry out a detailed phytochemical composition analysis, and various antioxidant activities of *A. serpyllifolia* leaves using various solvent extracts.

MATERIALS AND METHODS:

Chemicals: All the chemicals used, including the solvents, were of analytical grade. Petroleum ether, benzene, chloroform, acetone and methanol, trichloroacetic acid, hydrogen peroxide, potassium ferric cyanide, sodium carbonate, sodium phosphate, and ammonium molybdate were purchased from Sigma Aldrich (Bangalore, India). Rutin, Gallic acid, Foline-Ciocalteau reagent, ascorbic acid, DPPH, TPTZ, and ABTS were procured from Himedia (India).

Collection and Preparation of Plant Extracts: Leaves of *A. serpyllifolia* were collected from western hills of Dharmapuri district in Tamil Nadu, India, from September to December 2018 and authenticated by Botanical Survey of India, Coimbatore (Cr no; BSI/SRC/23/2019/Tech-3430). After a thorough wash, leaves were dried under shade and ground into a fine powder. The powder was used for cold plant extraction using different solvents such as petroleum ether, benzene, chloroform, acetone, methanol, and water-based on their polarity in 1:10 ratio for 24 h on an orbital shaker at 180 rpm at room temperature.

Determination of Extract Yield: Each extract was filtered using Whatman no. 1 filter paper. Each filtrate was concentrated under reduced pressure at 40 °C using a rotary evaporator. The extracts recovered after 6 successive extractions were weighed and the percentage yield was calculated using the following equation:

Extract yield % = Amount of extract (gm) × 100 / Amount of plant sample (gm)

Evaluation of Phytochemical Composition:

Qualitative Phytochemical Screening: Various plant extracts were examined for the presence of alkaloids, steroids, glycosides, saponins, phenols, tannins, flavonoids, anthroquinones and coumarins by using the standard methods [19, 20].

Quantitative Estimation of Secondary Metabolites:

Estimation of Total Phenols: The Folin-Ciocalteau reagent method has been used for the

estimation of total phenolic content [21]. The gallic acid was used as a standard solution. To carry out this experiment, 0.1 mL of test solution was mixed with 2.5 mL of 1 N folin-Ciocalteu reagent and incubated for 5 min, and then 2 mL of 5% sodium carbonate was added followed by distilled water. After incubation at room temperature for 2 h, the absorbance of the reaction mixture was measured at 760 nm against blank as methanol. The quantification of total phenolic content was calculated using the formula:

Total phenolic content = C × V/M

Where C = Concentration of gallic acid from calibration curve; V = Volume of extract used; M = Mass of extracts used.

The total phenolic content was expressed in ?g of gallic acid equivalent (GAE) of the plant sample.

Estimation of Total Flavonoids: Content of total flavonoids in plant extracts was determined colorimetrically according to the method described by [22] Lamaison and Carret, using quercetin as standard. For this, 5mL of 2% aluminum chloride in methanol was mixed with the same volume of test solution. After incubation of 10 min, absorbance was measured at 415 nm against blank sample. The total flavonoid content was determined using a standard curve of quercetin at 0–50 µg/mL.

The average of three readings was used and then expressed in µg quercetin equivalent flavonoids per mg extract.

Estimation of Total Tannins: Content of total tannins was determined using the Folin-Ciocalteu procedure as mentioned for total phenols, after removal of tannins by their adsorption on an insoluble matrix (polyvinylpolypyrrolidone; PVPP). According to Makkar [23] insoluble, cross-linked PVPP (100 mg) was taken in test tubes, and 500 mL of plant extract added. After 15 min at 4 °C, the tubes were vortexed and centrifuged for 10 min at 4350 g. The resultant supernatant containing non-tannin phenolics was transferred into test tubes and the non-absorbed phenolics were determined as described in the total phenolics estimation. The calculated values were subtracted from the total phenolics contents and the total tannin contents were expressed as milligrams gallic acid/g extract.

Estimation of Total Terpenoids: The total terpenoid content was determined using the colorimetric method. 0.1 ml plant extract was mixed with 3 ml chloroform. The mixture was vortexed and left for 3 min at room temperature. After incubation, 200 µL of H_2SO_4 was added and incubated again for 2 h in dark condition. A reddish-brown precipitate was observed after incubation. Following centrifugation, the supernatant was removed and 3 ml of 95% methanol added to the pellet, and then the OD was taken at 538 nm using Linalool standard.

Evaluation of Antioxidant Potential:

DPPH Radical Scavenging Assay: The DPPH (2, 2-diphenyl picrylhydrazyl) scavenging assay has been carried out to quantify the ability of antioxidants to quench the DPPH free radical. The dark purple color of DPPH will be lost when it is reduced to its non-radical form by antioxidants. In the present study, the scavenging effect of various plant extracts on stable radical (DPPH) was studied following the spectrophotometric method described by Blois *et al.* [24] Briefly, 3 mL of DPPH solution (0.1 mM, in methanol) was incubated with 100 µL/µg plant extract. After incubation for 30 min in the dark at ambient temperature, the changes in colour (from deep violet to light yellow) was measured at 517 nm against a blank of methanol to estimate the radical scavenging capacity of each extract, while methanolic DPPH solution was used as a negative control. The positive control was DPPH solution with rutin. The radical scavenging activity of the extracts was expressed as IC_{50} (the concentration of the sample required to inhibit 50% of the DPPH concentration). The ability to scavenge DPPH radical was calculated using the formula:

Scavenging activity (%) = Absorbance of control - Absorbance of test sample × 100 / Absorbance of control

Antioxidant Activity by ABTS Assay: The total antioxidant activity of the samples was measured using 2, 2'azinobis (3-ethylbenzothiozoline-6-sulfonic acid) disodium salt radical ($ABTS^{\cdot +}$) decolorization assay according to the method of Re *et al.* [25] Initially the $ABTS^{+}$ was generated by reacting 7 mM aqueous ABTS solution with 2.4 mM potassium persulfate in the dark for 12 to 16 h at ambient temperature.

Triplicate determinations were made for standard as well as for sample, and the absorbance was read against the blank at 734 nm. Rutin was used as a positive control. A rutin calibration curve was constructed by measuring the reduction in absorbance of the $ABTS^{+}$. Results were expressed as Mm rutin extract equivalent (RE) antioxidant capacity per gram of sample extracts.

Ferric Reducing Power Antioxidant Assay: The ability to reduce ferric ions was measured using the method described by Pulido *et al.* [26] The FRAP reagent was produced just before use by mixing 300 mM sodium acetate buffer (pH 3.6), 20.0 mM TPTZ (tripyridyltriazine) solution and 20.0 mM $FeCl_3.6H_2O$ solution in a ratio of 10:1:1 in volume. Sample was then added to 3 ml of FRAP reagent and the reaction mixture was incubated at 30 °C for 30 min. The increase in absorbance at 593 nm was measured. Fresh working solutions of $FeSO_4$ were used for calibration. The antioxidant capacity based on the ability to reduce ferric ions of the sample was calculated from the linear calibration curve and expressed as mM $FeSO_4$ equivalents per gram of sample.

Phosphomolybdenum Assay: The antioxidant activity of extracts was evaluated by the green phosphomolybdenum complex formation according to the method of Prieto *et al.* [27] A 100 mL aliquot of different extracts was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The test tubes were covered with foil and incubated in a water bath at 95 °C for 90 min. After the samples had been cooled to room temperature, the absorbance of the mixture was measured at 695 nm against the reagent blank.

Percent inhibition was calculated by the following formula:

% inhibition = 1–absorbance of sample × 100 / absorbance of control

Nitric Oxide Scavenging Assay: Nitric oxide generated from aqueous sodium nitroprusside (SNP) solution interacts with oxygen to produce nitrite ions at physiological pH, which may be quantified and determined according to Griess Illosvoy reaction [28]. The reaction mixture contained 10 mM SNP in 0.5M phosphate buffer (pH 7.4) and 100 µg/µL of the plant extracts into a final volume of 3 mL. After incubation for 60 min at 37 °C, Griess reagent (0.1% α-napthyl-ethylenediamine in water and 1% sulphanilic acid in 5% H_3PO_4) was added. The pink chromophore generated during diazotization of nitrite ions with sulfanilamide and subsequent coupling with α-napthyl-ethylene-diamine was measured spectrophotometrically at 540 nm. Ascorbic acid was used as a positive control. The nitric oxide scavenging activity of the extracts was measured using the Trolox standard curve, and results were expressed as mM Trolox equivalent antioxidant capacity (TEAC) per g dried fraction. All determinations were performed in triplicates.

Super Oxide Scavenging Activity: The superoxide anion scavenging activity was determined as described by Mc Cord and Fridovich [29]. The assay was based on the capacity of various extracts to inhibit formazan formation by scavenging the superoxide radicals generated in the riboflavin-light-NBT system. Each 3 mL reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20 mg riboflavin, 12 mM ethylene-diamine tetraacetic acid (EDTA) and 0.1 mg NBT. Briefly, 0.1 to 0.5 mL of the sample solution and standards (rutin) of sample extract at different concentrations and the reaction mixture were mixed and illuminated for 90s. The absorbance was measured at 590 nm against the reaction mixture and distilled water as blank. All measurements were made in triplicate. The abilities to scavenge the superoxide radical were calculated using the following equation:

% inhibition = 1–absorbance of sample × 100 / absorbance of control

Metal Chelating Activity Assay: The extracts were assessed for their ability to compete with ferrozine for iron (II) ions in a free solution. The chelating ability of ferrous ions by various fractions was estimated by the method of Dinis *et al.* [30] Extracts (100 µL/µg), 2.5 ml were added to a solution of 2 mM $FeCl_2.4H_2O$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml); the mixture was shaken vigorously and left standing at room temperature for 10 min. The absorbance of the solution was then measured at 562 nm against the blank performed in

the same way using $FeCl_2$ and water. EDTA served as the positive control, and a sample without extract or EDTA served as the negative control. All tests were run in triplicate and averaged. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated using the formula:

Chelating activity % = 1–absorbance of sample × 100 / absorbance of control

Lipid Peroxidation Inhibition Assay: For this assay, egg yolk homogenate was used as lipid source and free radicals were produced by Fenton reagent ($FeSO_4/H_2O_2$), a modified thiobarbituric acid reactive substances (TBARS) assay [31, 32]. In brief, 1 mL reaction mixture containing 0.5 mL egg yolk homogenate (10% in distilled water, v/v), 0.1 mL of extract was mixed with 0.05 mL $FeSO_4$ (0.07 M) and incubated for 30 min to induce lipid peroxidation. Free radical ruptures the lipid bilayer to form malonaldehyde (MDA) as a secondary product. Two molecules of thiobarbituric acid react with one molecule of MDA to form a pink-colored product showing maximum absorbance at 532 nm called TBARS. When the reaction mixture was mixed with different solvent-based plant extract, it reduces the formation of TBARS products in comparison to control. Inhibition of lipid peroxidation (%) by the sample was calculated according to the following formula:

% inhibition = (1–absorbance of sample/absorbance of control) × 100

Hydroxyl Radical Scavenging Assay: The deoxyribose method is described by (Aruoma and Halliwell [33] was used to determine the hydroxyl radicals trapping capacity of plant extract. $FeCl_3$-EDTA-ascorbic acid was used to generate OH radicals. For this, the reaction was carried out in two conditions, *i.e.* in the presence of EDTA (non-site-specific) to determine its OH trapping capacity and in the absence of EDTA (site-specific) to assess its metal chelation property. This experiment was performed to check the effect of plant extract on hydroxyl ($OH^{\cdot}$) radical's trapping potential. Plant extracts were added to the reaction mixture in a final volume of 1 mL in potassium phosphate buffer (10 mM, pH 7.4). This mixture was incubated at 37 °C for 1 h and then mixed with 1 mL of 2.8% TCA (w/v in water) and 1 mL of 1% thiobarbituric acid (TBA) (w/v). It was then heated in a boiling water bath for 15 min and cooled, and absorbance was taken at 532 nm. Ascorbic acid was used as a positive control. The ability to scavenge the hydroxyl radical was calculated using the following equation;

% hydroxyl radical scavenging activity = (1–absorbance of sample × 100 /absorbance of control

Reducing Power Assay: Reducing power of plant extract was determined according to the method developed by Oyaizu M [34]. A 2.5 mL solution of extract (100µg/100µL) was mixed with an equal volume of phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide and placed in water bath at 50 °C for 20 min. Then it was cooled rapidly and 2.5 mL of 10% trichloroacetic acid was added and vortexed. This incubation mixture was centrifuged at 3,000 rpm for 10 min, and its 5 mL supernatant was mixed with an equal volume of distilled water and 1 mL of 0.1% ferric chloride. It was further incubated at room temperature for 10 min, and absorbance was read at 700 nm. The reducing property of the test sample was standardized against rutin and expressed as a difference in optical density (OD) from control as well as a test as 0.1 and expressed as µg/mL a high degree of absorbance indicate the stronger reducing power.

Statistical Analysis: Data from all the assays were subjected to one-way ANOVA followed by DMART test for post-hoc analysis. The level of statistical significance was set at $p < 0.05$. For all statistical analyses, SPSS software, version 13.0 (SPSS Inc., Chicago, IL, USA) was used. Data are presented as means ± SEM.

RESULTS:

Extract Recovery Percentage: In the present investigation, the highest yield percentage was observed in aqueous extract and lowest in benzene extract **Fig. 1**.

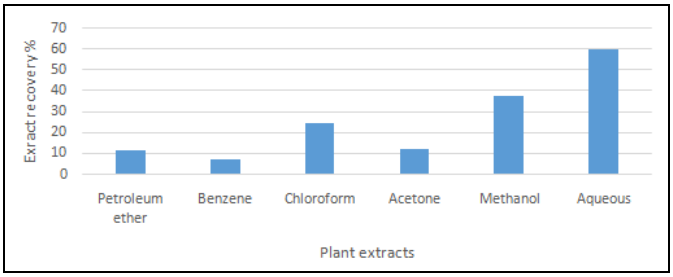

FIG. 1: EXTRACT RECOVERY PERCENTAGE OF *A. SERPYLLIFOLIA* LEAF EXTRACTS

The decreasing order of recovery percentage was: aqueous (60%) > methanol (37.5%) > Chloroform (25%) > Acetone (12.5%) > petroleum ether (12%) > and Benzene (7.5%).

TABLE 1: PHYTOCHEMICAL ANALYSIS OF VARIOUS SOLVENT EXTRACTS OF *A. SERPYLLIFOLIA*

S. no.	Phytochemicals	Petroleum ether	Benzene	Chloroform	Acetone	Methanol	Aqueous
1	Alkaloids	++	++	++	-	-	+
2	Steroids	+	+	+	+	+	+
3	Glycosides	+	-	-	+	+	+
4	Saponins	-	+	+	-	-	-
5	Phenols	+++	+++	+++	+++	+++	+++
6	Tannins	++	++	++	++	++	++
7	Flavonoids	-	+	-	+	+	+
8	Coumarins	+	+	+	+	+	+

Symbols denotes +: present, ++: present at moderate intensity, +++: present at higher intensity, and -: absent

Quantitative Estimation of Secondary Metabolites: Various quantitative assays exhibited that all the plant extracts were having a significant level of secondary metabolites with values ranging from 1148 to 286 mg GAE/g for phenol, 1262 to 976 mg LN/g for terpenoids, 172 to 106 mg RE/g for flavonoids and 21.5 to 3.6 mg TA/g for tannins **Table 2**. Phenolic content estimation resulted that all extracts are showing a higher quantity of gallic acid equivalence in 500 µg/µL plant extract concentration. Of this, methanol (1148 ± 11 mg/g GAE) shows higher phenolic content. Acetone and chloroform extracts show a similar level of phenolic contents (Acetone 730 ± .083, chloroform 709 ± 0.65 mg/g GAE). Aqueous (677 ± 11 mg/g GAE), benzene (370 ± 0.30mg/g GAE) and petroleum ether (286 ± 0.12 mg/g GAE) showed its phenol content at descending order. All values are expressed in mean± standard deviation (n=3).

TABLE 2: DETERMINATION OF TOTAL PHENOLICS, FLAVONOIDS, TANNINS AND TERPANOIDS

A. serpellifolia plant extract fractions	Total Phenolics (mg GAE*/g extract)	Total Flavonoids (mg RE€/g extract)	Total Tannins (mg TA¥/g extract)	Terpenoid (mgLN§/g extract)
Petroleum ether	286 ± 0.12^{e}	$172 \pm .07^{a}$	$12.5 \pm .038^{b}$	$1019 \pm .078^{c}$
Benzene	370 ± 0.30^{d}	$131 \pm .008^{c}$	$3.6 \pm .031^{d}$	$976 \pm .106^{d}$
Chloroform	709 ± 0.65^{b}	118 ± 0.8^{d}	$11.1 \pm .025^{b}$	$1254 \pm .968^{b}$
Acetone	730 ± 4.0^{b}	$106 \pm .015^{e}$	$21.5 \pm .039^{a}$	$1363 \pm .116^{a}$
Methanol	1148 ± 11^{a}	$106 \pm .016^{e}$	$6.2 \pm .77^{c}$	$1262 \pm .968^{b}$
Aqueous	677 ± 11^{c}	$156 \pm .009^{b}$	$0.067 \pm .007^{e}$	$1015 \pm .108^{c}$

*Gallic acid equivalents; €Rutin equivalents; ¥Tannic acid equivalence; §Linalool equivalence, values which are statistically significant at $p<0.05$ where a > b > c > d>e. Where all values are represented as mean ± SD (n = 3).

Total flavonoid content in large quantities was showed by petroleum ether extract (172 ± .07 mg/g Rutin eqv.). Whereas aqueous (156 ± .009 mg/g Ru eqv), benzene (156 ± .009mg/g Ru eqv), chloroform (156 ± .009 mg/g Ru eqv), Methanol (106 ±.016 mg/g Ru eqv) and Acetone (106 ± .016 mg/g Ru eqv) showed flavonoid content in considerable amount. Total tannin content is found to be higher in acetone extracts (21.5 ± .039 mg/g tannic acid eqv). The aqueous extract showed very lower level (0.067 ± .007) of this content.

Terpenoid content was found at a higher level in all the extracts. Among them, acetone showed the highest terpene content (1363 ± .116 mg/g linalool eqv). Benzene had comparatively low terpenoid content (976 ± .106 mg/g linalool eqv).

***In-vitro* Antioxidant Assays:**

DPPH Radical Scavenging Activity: The radical scavenging activity is expressed as a percentage of reduction of initial DPPH absence in plant extracts **Fig. 2**.

Among the various extracts, petroleum ether showed the highest percentage of radical scavenging activity (PI-78.98%). Acetone extract showed the lowest percentage of DPPH radical scavenging activity (53%).

ABTS Radical Scavenging Activity: Amongst all extracts tested highest anti-oxidant potential was shown by petroleum ether (99.12 ± .009 µg/mL Ru eqv) when compared to standard rutin (47 ± .034 µg/mL Ru eqv) **Table 3**.

TABLE 3: FREE RADICAL SCAVENGING ASSAY OF *A. SERPYLLIFOLIA* LEAF EXTRACTS

A. serpellifolia plant extract fractions	ABTS Radical Scavenging assay (µg/mL)	FRAP Radical Scavenging assay (µg/mL)	NO_2 Radical Scavenging assay (µg/mL)	Reducing Power assay (µg/mL)
Petroleum ether	92.12 ± .009^{d}	81.42 ± .005^{d}	220.29 ± .12^{c}	67 ± .02^{d}
Benzene	97.23 ± .004^{b}	92.3 ± .017^{a}	243 ± .17^{b}	56 ± .12^{e}
Chloroform	97.87 ±.003^{a}	90.3 ± .005^{b}	255.81 ± .02^{a}	76 ± .15^{c}
Acetone	96.91 ± .018^{c}	92.13 ± .017^{a}	222 ± .034^{c}	161 ± .002^{a}
Methanol	97.27 ± .017^{b}	88.03 ± 0.08^{c}	221 ± .026^{c}	145 ± .023^{b}
Aqueous	86.98 ± .016^{e}	61.77 ± 0.17^{e}	145 ± .002^{d}	76 ± .16^{c}

Values which are statistically significant at $p< 0.05$ where a > b > c > d>e. Where all values are represented as mean ± SD (n = 3).

FRAP: The ferric reducing ability of the all extracts revealed that percent inhibition of petroleum ether (81.42 ± .005 mM $FeSO_4$ eqv) benzene (92.13 ± .017 mM $FeSO_4$ eqv), chloroform (90.3 ± .005 mM $FeSO_4$ eqv) acetone (92.13 ± .017 mM $FeSO_4$ eqv) and methanol (88.03 ± 0.08 mM $FeSO_4$ eqv) except aqueous extracts (61.77 ± 0.17 mM $FeSO_4$ eqv).

Phosphomolybdenum Assay: Petroleum ether extract had strongest effect (101%) on reducing Mo radical compared to other extracts.

Nitric Oxide Scavenging Activity: Highest activity was expressed by chloroform (255.18 ± .02 mM Trolox eqv) and lowest activity was showed by aqueous extract (145 ± .002 mM Trolox eqv).

Super Oxide Radical Scavenging Activity: For scavenging superoxide radicals, chloroform extract showed higher percentage of inhibition (155.0%) followed by acetone (81.58%) and benzene (80.42%). Methanol extract exhibited a very lower percentage of inhibition (17.44%).

Metal Chelating Activity: Almost all fractions of *A. serpyllifolia* showed a very good ability to chelate Fe ions in an effective manner. However, chloroform extract displayed the highest chelation ability for this metal (percentage inhibition- 214%).

Lipid Peroxidation Assay: The percentage of inhibition of lipid peroxidation was significantly higher ($p < 0.01$) in acetone extract compared to other extracts.

Hydroxyl Radical Scavenging Activity: Petroleum ether, benzene, chloroform, acetone, methanol and aqueous extracts scavenged hydroxyl radical as follows: PI-85%, 54%, 63%, 71%, 109% and 112%, respectively. The scavenging effect of ascorbic acid (43%) were significantly lower against various extracts of *A. serpyllifolia.*

Reducing Power Assay: Among all extracts tested, the reducing capacity was found for acetone (145 ± .002 µg/mL) was found to be higher than rutin (standard- 39 ± .67 µg/mL).

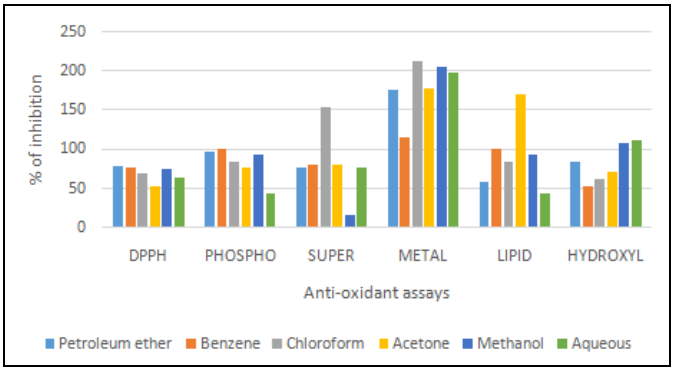

FIG. 2: FREE RADICAL SCAVENGING ACTIVITY OF *A. SERPYLLIFOLIA* LEAF EXTRACTS

DISCUSSION: Snake envenomation is a serious medical problem. Pre or post-synaptic neurotoxicity, myotoxicity, cardiac toxicity, hemolysis, hypotension, edema *etc*. are some effects caused by snake venoms [35]. However that anti-venom has certain drawbacks; it may not be readily available in the rural areas, the composition and antigenic reactivity of the venom may vary due to geographic and taxonomic diversities of the snakes, and some patients may suffer from adverse allergic reactions to the anti-venom [36]. Herbal medicine and its derived products have been the mainstay of traditional medicines around the world. The aim of this study was to carry out the qualitative, quantitative, and anti-oxidant potential of different solvent extracts of *A. serpyllifolia* leaves. Generally, the extract yield percentage tended to increase with the increasing polarity of the solvents used as extraction medium [37]. Accordingly, in the present research, the highest yield percentage was observed in aqueous extract and lowest in benzene extract.

The use of different solvents with increasing polarity led to a primary by metabolite oriented purification [38].

Phenols and tannins are known due to the important biological activities attributed to this class of compounds. All the six extracts analyzed revealed the presence of these phytocompounds in various intensities. Plant secondary metabolites also play a crucial role in envenomation. Phenolic compounds are widely distributed in plants, and in recent years they have gained much attention due to their antioxidant activity and free radical-scavenging ability with potential beneficial implications in human health [39]. Of various extracts, polar based extracts such as methanol .and aqueous showed higher phenolic contents. Phenolic compounds are more soluble in polar organic solvents due to the presence of a hydroxyl group; therefore, methanol was selected as the extracting solvent [40]. Lee *et al*., [41] showed that water is the most suitable solvent for the extraction of phenolic compounds from *Pleurotus citrinopileatus*.

Flavonoids are one of the most diverse and widespread groups of natural compounds and are one of the most important natural phenolics. These compounds possess a broad spectrum of activities, including radical scavenging properties [42]. Stankovic *et al.,* [43] found that the highest concentration of flavonoids in the extract obtained using non-polar solvents, as evidenced in the present investigation using petroleum ether. Present investigation results showed a higher level of tannin content in extracts obtained using mid-polar and moderate polar solvents. Phenolics, specifically polyphenols, like a few tannins bind proteins, acting upon components of venom without delay and disabling them to act on receptors. They may also act by competitive blocking of the receptors[44]. Tannic acid has been observed to be a potent inhibitor of hyaluronidase [45].

Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical scavengers. Phenolic compounds such as flavonoids, phenolics acid, and tannins possess diverse biological activities, including anti-inflammatory, anti-carcinogenic, and anti-atherosclerotic activities. These activities might be related to their antioxidant activity [46].

An important group of compounds found in the plants considered to have antiophidic activity is terpenes and terpenoids [47]. Free radicals of 1, 1-diphenyl1-2-picrylhydrazyl (DPPH) are widely used for screening of medicinal plants to investigate their antioxidant potential. In these procedures free, DPPH radicals, when dissolved in methanol, give violet color in methanol solution. The results existed clearly indicate that in the screening of various extracts of AS, petroleum ether extract had marked scavenging effect with 78.98% when compared to other investigations [48]. The ABTS radical cation decolorization test is another widely established approach adopted to evaluate antioxidant activity. Colour reduction shows a decrease of ABTS radical [49]. The ABTS radical scavenging capability of AS extracts can be ranked as follows: *PE* > chloroform>Benzene >methanol >acetone>aqueous extracts.

The transformation ability of compounds from Fe^{3+}/ferricyanide complex to Fe^{2+}/ferrous form acts as a potential indicator for antioxidant activity [50]. The reducing power of Fe^{2+} by six extracts was evaluated in **Table 3,** and the greatest reducing antioxidant power was recorded for benzene extracts compared to standard $FeSO_4$. The phosphomolybdate method has been routinely used to evaluate the total antioxidant capacity of extracts [51]. The results showed petroleum ether extract of AS 101% radical scavenging activity indicated significant antioxidant activity. It is well known that nitric oxide has an important role in various inflammatory processes. Sustained levels of production of this radical are directly toxic to tissues [52]. The toxicity of NO increases greatly when it reacts with superoxide radicals, forming the highly reactive peroxynitrite anion [53] (ONOO-). The nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite. The extract inhibits nitrite formation by directly competing with oxygen in the reaction with nitric oxide. The present study proved that the chloroform extract has more potent nitric oxide scavenging activity than other extracts.

The decrease in absorbance at 560 nm with antioxidants indicates the consumption of the superoxide anion in the reaction mixture [54]. Superoxide free radicals showed maximum inhibition of 155.0% at a concentration of

100 µg/µL chloroform extract. The antioxidant ability of plants depends on both the composition and test system. The ferrous ion-chelating activity of different plant extracts of *A. serpyllifolia* can be attributed to their total phenolic content [55]. Oxidative stress was characterized by increased lipids peroxidation and altered non-enzymatic and enzymatic antioxidant [56]. Acetone extract of *A. serpyllifolia* markedly reduced lipid peroxidation compared to other fractions and reference compounds.

Scavenging of hydroxyl radical is an important antioxidant activity because of the very high reactivity of the OH radical, enabling it to react with a wide range of molecules found in living cells, such as sugars, amino acids, lipids, and nucleotides [57]. Thus, removing $OH^{\cdot}$ is very important for the protection of living systems. The hydroxyl radical scavenging potential of various solvent extracts of AS leaves is shown in **Fig. 2**. In the present investigation, the hydroxyl radical scavenging activity observed was in the range of 54% to 112% at the concentration of 100 µg/µl. While scavenging hydroxyl radicals, the ability of aqueous extract (112%) was found to be higher than other sample extracts.

In the reducing power assay, the presence of reductants (antioxidants) in the extracts would bring about the reduction of Fe^{3+}/ferricyanide complex to the ferrous form by giving away an electron. Increasing the absorbance at 700 nm implies an increase in its ability to reduce. The sequence for this reducing power was as follows methanol>acetone>aqueous>chloroform>petroleum ether>benzene. Some degrees of electron-donating capacity were found in all examined extracts. Among the examined extracts the methanol extract of *A. serpyllifolia* showed the highest reducing power with 145 ± .002 µg/mL RE at 700 nm, so this extract could act as electron donors and also could convert free radicals to more stable products [58], although, in comparison with the positive control (rutin), it is significantly lower **Table 3**.

CONCLUSION: In India, snakebite is a major health problem that leads to several deaths annually. Snake anti-venom remains the only specific treatment against envenomation. Several Indian medicinal plants are employed by folk medicine for the treatment of snakebites. In the present study, we examined the phytochemical composition and antioxidant potential of various leaf extracts of *A. serpyllifolia*. The results obtained showed that the very important secondary metabolites such as phenols, flavonoids, tannins, and terpenoids were present in the reasonable amount in all the extracts. And also noted that antioxidant potential of various *A. serpyllifolia* leaf extracts was found to be significant. Thus, the study provides scientific validation for its ethnobotanical use against snake bites through an elaborate study on the assessment of phytochemicals and antioxidants of this plant. In the future, the inhibitory activities of these extracts against some of the snake venoms will be studied by using *in-vitro* models.

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