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ANTIOXIDANT POTENTIAL of DIFFERENT SOLVENT EXTRACTS ISOLATED from *ALANGIUM SALVIFOLIUM* FLOWERS

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Abstract:
Background: Medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis. Medicines derived from plants are widely famous due to their safety, easy availability and low cost. Herbal medicines may include whole parts of plant or mostly prepared from leaves, roots, bark, seed and flowers of plants. Present study established the antioxidant potential of *Alangium salvifolium* by studying the efficacy in preventing the *in-vitro* free radical using DPPH and reducing power assay.
Methods: *In vitro* Antioxidant Activity 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity and reducing power assay
Result: Free radicals are highly reactive compounds that can produce oxidative stress on the body contributing to diseases such as atherosclerosis or cancer. The radical-scavenging activity of the pet ether extract, ethyl acetate extract and methanolic extracts of *Alangium salvifolium* was estimated by comparing the percentage inhibition of formation of DPPH radicals with that of ascorbic acid. The DPPH radical scavenging activity of pet ether extracts increased with increasing the concentration. The total antioxidant activity of *Alangium salvifolium* in flower was assessed based on scavenging activity of DPPH free radicals. Conclusion: Therefore, present study concludes that *Alangium salvifolium* is an excellent medicinal plant which holds numerous bioactive phytochemicals and it can be used in prevention of cancer.

Key Words: Herbal medicines, *In vitro* Antioxidant Activity, Reducing power assay, *Alangium salvifolium.*

INTRODUCTION

The antioxidant compound is defined as a compound characterized by the ability to delay or inhibits the oxidation of the substances presented in the medium (**Gutteridge et al 1994)**. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols (**Sies et al 1992).**

Oxygen is absolutely essential for the life of aerobic organism but it may become toxic if supplied at higher concentrations. Dioxygen in its ground state is relatively unreactive; its partial reduction gives rise to active oxygen species (AOS) such as singlet oxygen, super oxide radical anion, hydrogen peroxide etc. This is partly due to the oxidative stress that is basically the adverse effect of oxidant on physiological function. Free oxygen radicals plays cardinal role in the etiology of several diseases (**Cheeseman et al 1993).**

Alangium salviifolium leaves are used as purgative, astringent, anthelmintic, antipyretic, expectorant, anti-inflammatory, emetic, diaphoretic, anticancer, antimicrobial and antitumor agent, also used in treatment of rheumatism, leprosy, stomach ulcers; healing of wounds, arthritis, scabies, gonorrhea, jaundice, hepatitis, diabetes, syphilis and asthma.

Alangium salvifolium leaves, roots and seeds contain alkaloids (such as alangidiol, alangicine, alangimarckine, alamaridine, dimethyl aptaline, isoalamarine, alangimarinone, dimethyl phycotrine, ankorine **(Ramni *et al*., 1693)**. *Alangium salvifolium* leaves contain alkaloids, deoxytubulosine, alangimarckine, dehydroprotoemetine and so forth **(Tran MH *et al*., 2009).** Root bark contains alkaloids, emetine, cephaeline, psychotrine, tubulosine, isotubulosinealangium A, alangium B, marckidine, marckine, and alangine, and also myricylic alcohol, de-Me-psychotrine, alangicin, stigmasterol, and ß-sitosterol. The stem bark includes the alkaloids, alangin, akharkantine, akoline and lamarkine **(Itoh T *et al*., 1994, Itoh A *et al*., 1995).**

Present study established the antioxidant potential of *Alangium salvifolium* by studying the efficacy in preventing the *in-vitro* free radical using DPPH and reducing power assay.

MATERIALS AND METHODS

Authentication of Plant- The plant was identified and authenticated by Dr. Saba Khan, Botanist, Safia college of Science, Bhopal. A voucher specimen number 137/Bot/Safia/19 was kept for future reference.

Collection of Plant- Flowers of *Alangium salvifolium* were collected in the month of March 2019 from Raisen District, Madhya Pradesh, India.

Chemicals- The powdered plant material was extracted with different solvents (Petroleum ether, ethyl acetate, 70% methanol) using soxhlet apparatus. The crude extract obtained was further dried over water bath. Phytochemical screening of the extracts was done using standard procedures (Trease and Evans*.,* 1989 and Kokate *et al.,* 2006).

In vitro Antioxidant Activity

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity:

The DPPH radical solution (50 µM) was individually combined with a solution of specific plant extract concentrations and normal ascorbic acid. The mixtures of the reaction were thoroughly shaken and held for 30 min at dark. Control solution was prepared by adding 2ml of methanol with 2ml of DPPH solution. The absorbance of all the reaction mixtures and control solution was measured at 517 nm. The percentage inhibition was calculated by following equation:

The % Inhibition was calculated using following formula:

$$\% \text{ Inhibition } = \left[\left(\frac{\text{AC 517 nm} - \text{AS 517 nm}}{\text{AC 517 nm}} \right) \times 100 \right]$$

Where,
AC is absorbance of Control and AS is the absorbance of Sample.
The graph was plotted between % inhibition and different concentrations of plant extracts and ascorbic acid and then IC_{50} value was determined.

Reducing Power Assay

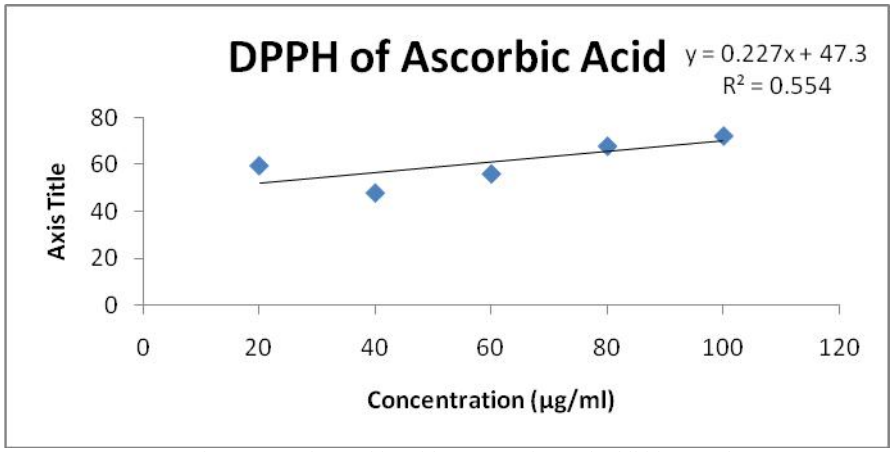
1 mL of various concentrations of extract was mixed with 2.5 ml phosphate buffer solution (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The solution was properly mixed and placed in incubator for 20 min at 50ºC. After incubation, the resulting solution was cooled and 2.5 ml of 10%tri chloro acetic acid was added to reaction mixture, followed by centrifugation at 3000 rpm for 10 min. After centrifugation 2.5 ml of supernatant was mixed with equal volume of distilled water and finally 0.5 ml of 0.1% ferric chloride was added. The reaction mixture was shaken and kept at room temperature for 10 min. The absorbance was measured at 700 nm.

RESULTS

All the extracts of *A. salvifolium* flower observed a good inhibitory activity against DPPH radical. The scavenging activity of *A. salvifolium* flower extract and standard on the DPPH radical expressed as IC_{50} values: AA (11.89) MET (49.23), EA (85.78), PE (94.78). Highest quenching ability was shown by methanol extract while petroleum ether extract showed lowest scavenging activity. The experimental data revealed that polar extracts had stronger free radical scavenging effect than the non polar ones. IC_{50} value of Methanolic extract was close to ascorbic acid which is a well-known antioxidant.

Table 1: DPPH radical scavenging activity of Std. Ascorbic acid

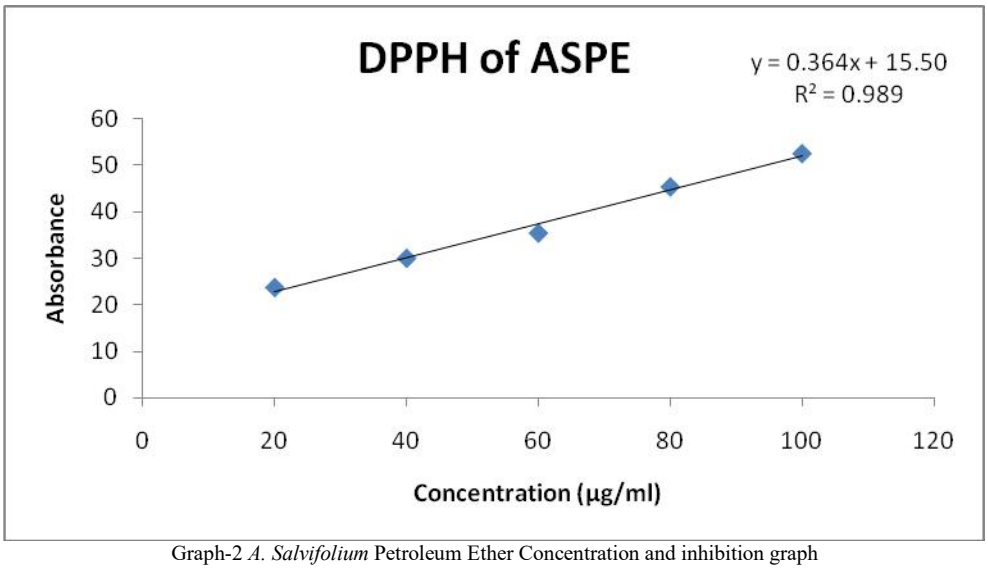
Ascorbic acid (std.)			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	**20 µg/ml**	0.231	59.7561
2.	**40 µg/ml**	0.298	48.08362
3.	**60µg/ml**	0.251	56.27178
4.	**80µg/ml**	0.183	68.11847
5.	**100µg/ml**	0.158	72.47387
IC_{50}		**11.89**	



Graph-1 DPPH of Ascorbic acid concentration and Inhibition graph

Table 2: DPPH radical scavenging activity of Petroleum ether extract of *A. salvifolium*

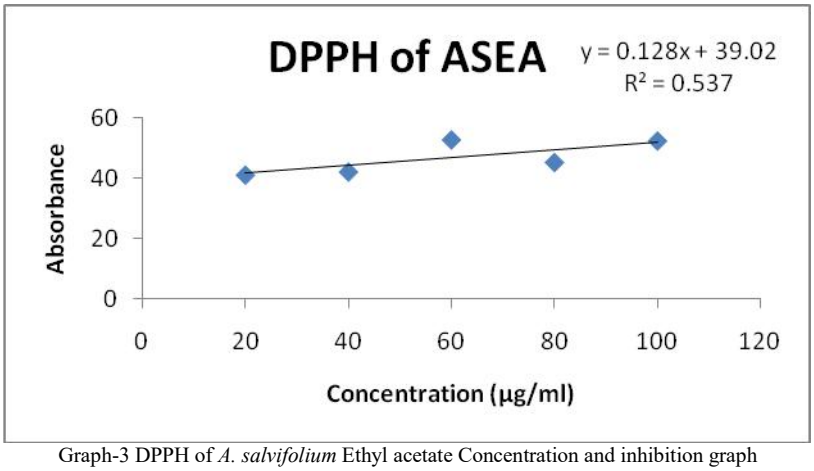
Petroleum ether extracts of *A. Salvifolium*			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	**20 µg/ml**	0.438	23.69338
2.	**40 µg/ml**	0.402	29.96516
3.	**60µg/ml**	0.371	35.36585
4.	**80µg/ml**	0.314	45.29617
5.	**100µg/ml**	0.273	52.43902
IC_{50}		**94.78**	



Graph-2 *A. Salvifolium* Petroleum Ether Concentration and inhibition graph

Table 3: DPPH radical scavenging activity of Ethyl acetate extract of *A. Salvifolium*

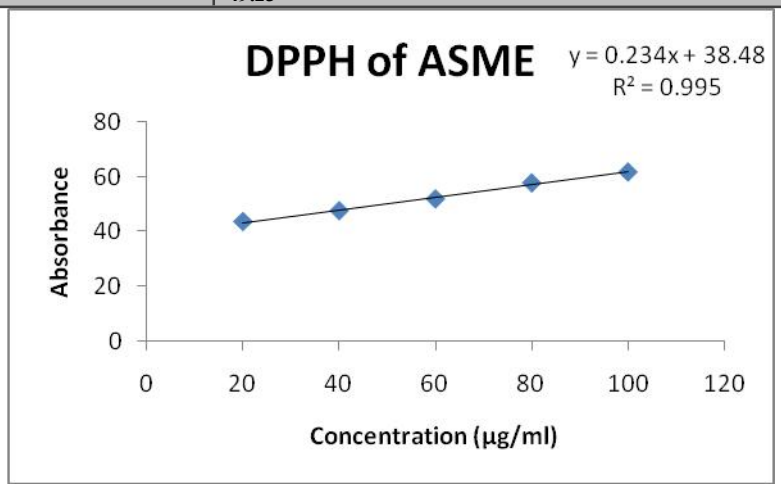
Ethyl acetate extract of *A. Salvifolium*			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	**20 µg/ml**	0.338	41.11498
2.	**40 µg/ml**	0.332	42.16028
3.	**60µg/ml**	0.271	52.78746
4.	**80µg/ml**	0.314	45.29617
5.	**100µg/ml**	0.273	52.43902
IC_{50}		**85.78**	



Graph-3 DPPH of *A. salvifolium* Ethyl acetate Concentration and inhibition graph

Table 4: DPPH radical scavenging activity of Methanolic extract of *A. Salvifolium*

MeOH extract of *A. Salvifolium*			
S. No.	**Concentration**	**Absorbance of Sample**	**% Inhibition**
1.	**20 µg/ml**	0.324	43.55401
2.	**40 µg/ml**	0.301	47.56098
3.	**60µg/ml**	0.276	51.91638
4.	**80µg/ml**	0.242	57.83972
5.	**100µg/ml**	0.219	61.84669
IC_{50}		**49.23**	

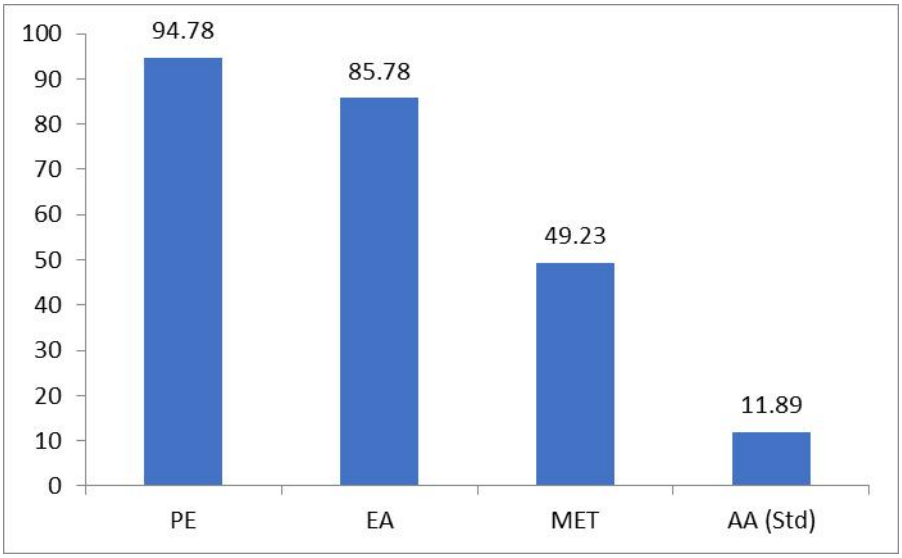


Graph – 4 DPPH of *A. Salvifolium* MeOH Concentration and Inhibition graph

IC_{50}values of plant extracts of *A. salvifolium*

***A. salvifolium* extracts**	**IC_{50}**
PE	94.78
EA	85.78
MET	49.23
AA (Std)	11.89

IC_{50} values of extract, PE=Pet Ether, EA= Ethyl acetate, MET=Methanol, AA=Ascorbic acid



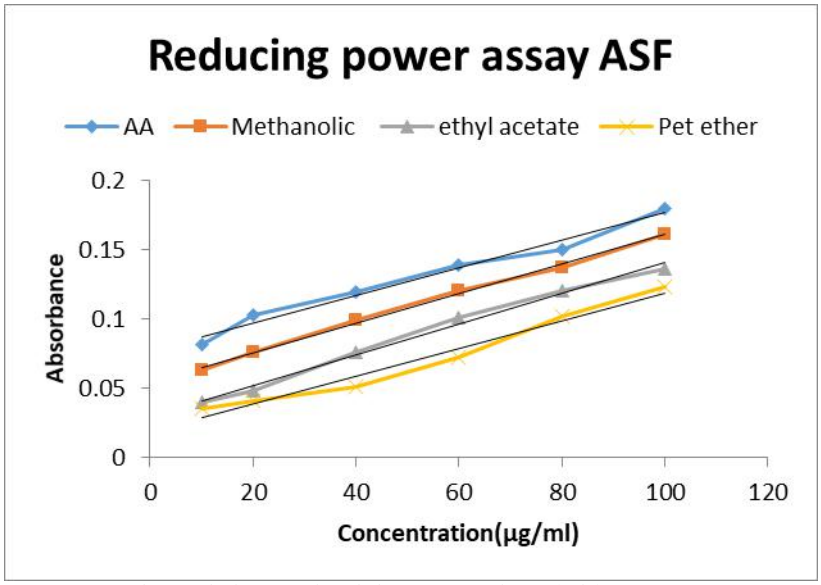
Graph- 5 IC_{50} values of different plant extracts of *A. salvifolium*

Reducing power assay

The reducing power of extracts is shown graphically by depicting absorbance as a function of concentration. The reducing power of all the extracts increased with increase in concentration. Reducing power of methanol extract is highest which is comparable to standard compound ascorbic acid.

Table 5: Reducing Power Assay of Absorbance of different extracts with different Concentration

Reducing Power Assay					
S. No.	**Concentration**	**Absorbance of Ascorbic acid**	**Absorbance of Petroleum ether extract**	**Absorbance of Ethyl acetate extract**	**Absorbance of Methanolic extract**
1.	**20 µg/ml**	0.082	0.035	0.04	0.063
2.	**40 µg/ml**	0.103	0.041	0.048	0.076
3.	**60µg/ml**	0.12	0.051	0.076	0.099
4.	**80µg/ml**	0.139	0.072	0.101	0.121
5.	**100µg/ml**	0.15	0.102	0.121	0.137



Graph 6: Reducing capacity of plant extracts of *A. Salvifolium*

DISCUSSION

Medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis (**Davidson-Hunt, 2000).** There are nearly 2000 ethnic groups in the world, and almost every group has its own traditional medical knowledge and experiences (**Liu *et al.,* 2009; Kebriaee-zadeh, 2003**).

Present study established the antioxidant potential of *Alangium salvifolium* by studying the efficacy in preventing the *in-vitro* free radical using DPPH and reducing power assay.

The radical-scavenging activity of the pet ether extract, ethyl acetate extract and methanolic extracts of *Alangium salvifolium* was estimated by comparing the percentage inhibition of formation of DPPH radicals with that of ascorbic acid. The DPPH radical scavenging activity of pet ether extracts increased with increasing the concentration.

The total antioxidant activity of *Alangium salvifolium* in flower was assessed based on scavenging activity of DPPH free radicals. Among them methanolic extract shows highest % inhibition in all type of extract of *Alangium salvifolium*. The IC_{50} value is another parameter introduced for the interpretation of the results from the DPPH method. Its value for ascorbic acid was found to be 11.89. The IC_{50} value obtained for petroleum ether extract was 94.78; ethyl acetate extract was 85.78 and methanolic extract was 49.23.

These results indicated that methanolic flower extracts of *Alangium salvifolium* have a noticeable effect on scavenging free radical. Antioxidant activities due to the presence of some bioactive compounds like phenolics including flavonoids. In general, phenolic compounds were commonly found in plants and have reported several biological activities including potent antioxidants and free radical scavengers apart from the primary defense role **(Kähkönen *et al*., 1999).** Epidemiological studies suggest that the consumption of flavonoids is effective in lowering the risk of coronary heart diseases and in raising the hepatoprotective activity **(Rice-Evans *et al*., 1996; Elsayed *et al*., 2012).**

CONCLUSION

Therefore, present study concludes that *Alangium salvifolium* is an excellent medicinal plant which holds numerous bioactive phytochemicals and it can be used in prevention of cancer. Almost every part of this plant has been used in the Ayurveda, Siddha and various other traditional systems of medicines for treatment of various diseases. In modern scientific literatures, plant extracts have been reported to have potential efficacy against hypertension, diabetes, epilepsy, cancer, inflammation, ulcer, etc. Various plant parts have been found to possess biological activity more specifically towards overcoming metabolic ailments.

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