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Title Key: FREE RADICAL SCAVENGING POTENTIAL OF …

# FREE RADICAL SCAVENGING POTENTIAL OF *ARGEMONE MEXICANA LINN.* LEAF

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## ABSTRACT:

The free radical scavenging potential of *Argemone Mexicana* Linn. herb was investigated using its leafs ethanol extract by free radical scavenging DPPH assay study. It has been found that effective free radical scavenging potential with $IC_{50}$ value 42.50 µg/ml.

The phytochemical analysis of leafs ethanol extract shows the presence of alkaloids, protein, glycoside, tannins, flavonoids, steroids and phenolic compounds. Therefore, the study clearly indicates that local medicinal plant *Argemone Mexicana* Linn. leafs ethanol extract was rich in phenols, flavonoids and other active components.

The free radical scavenging activity depends on polyphenolic content and other phytochemical constituents present in leafs extract. It could be a potential source of natural free radical scavenger and have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases. Therefore, ethanol extract of *Argemone Mexicana* Linn. leafs reported potent free radical scavenging activity.

**Key words:** *Argemone Mexicana* Linn., ethanol extract, DPPH assay, free radical.

## INTRODUCTION:

The several biochemical reactions occurs in human body generate reactive oxygen species and these are capable of damaging crucial biomolecules. They are not effectively scavenged by cellular constituents, they lead to disease conditions. The use of local traditional medicine from plant sources present in a large scale contains natural antioxidant, which has been showing effective free radical scavenging activity that might serve as leads for development of more active drugs.

Antioxidants are the chemical constituents that neutralize free radicals, otherwise which damages the crucial bio-molecules present in body. Free radicals are chemically active product of metabolism and include reactive oxygen species or reactive nitrogen species. Appearance of radicals originates a number of human neurologic and other metabolic disorders (Nadkarni,1954). These different type of pathological disorders believed to the associated with oxidative stress (Pelicano *et al*, 2004 and Gonçalves *et al*, 2005).

Synthesized antioxidants have been widely used for treatment the pathological conditions. The continuous use of these antioxidants in food preparations have been introduces to potential health risks, toxicity and carcinogenicity (Jeong *et al*, 2004 and Wong *et al*, 2006). Majority of the diseases today are due to the shift

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imbalance of the pro-oxidant and the antioxidant homeostatic phenomenon in the body. Pro-oxidant conditions dominate either due to the increased generation of the free radicals or due to the excessive oxidative stress of the depletion of the dietary antioxidant (Priyesh *et al*, 2007).

Many plants exhaustively studied in the last few years for their antioxidant and radical scavenging activities (Arnous *et al*, 2001). The different parts of the *Argemone Mexicana* Linn. plant is extensively used as traditional medicine for the treatment of numerous diseases. Its chemical investigations reported to have the presence of active phyto constituent's (Sharanappa *et al*, 2014). The aerial parts of *Argemone Mexicana* Linn. showed DPPH scavenging activity (Kushtwar et al, 2016). This activity due to the presence of flavonoids, phenolic and other various constituents in its parts (Oyawaluja *et al*, 2015).

The literature reviewed survey of *Argemone Mexicana* Linn. were summarised for their some important medicinal and pharmacological activities. There is a scope to identify new bioactive compounds and check their claimed pharmacological activities (Husna *et al*, 2017). Therefore, taking into consideration the vast potentiality of *Argemone Mexicana* Linn. plant as source of antioxidants, a systematic investigation of leaf was undertaken to study.

## MATERIAL AND METHODS:

**Plant material:** The leafs of *Argemone Mexicana* Linn. plant were collected from local area, identified and authenticate with the help of our institute botanist.

**Extraction:** The collected leaf of *Argemone Mexicana* Linn. plant was clean and dried in shade. Then dried leaves powdered. 10 gm of powdered material was dissolved in 100 ml of ethanol and kept on a magnetic stirrer for 2 hrs. Thereafter, it was extracted using a soxhlet apparatus sequentially with ethanol solvent. The fractions of extract were collected and the remaining solvent was evaporated out to dryness. The obtained dried sample material was stored in airtight bottle for further studies.

**DPPH free radical scavenging activity:** The free radical scavenging activity was measured by using stable free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) with the help of UV-spectrophotometer (Blois, 1958 and Shendge *et al*, 2011). A stock solution of 0.1mM DPPH was prepared in ethanol. 1.0ml of this solution was added to 1.0ml of extract solution in water at different concentrations (5-50$\mu$g/ml) and final volume was adjusted to 3 ml by adding distilled water. The absorbance of each concentration was measured at 517 nm after 15 minutes. Ascorbic acid was used as standard radical scavenger. The decrease in absorbance of the reaction mixture at increasing concentrations of sample indicates presence of free radical scavengers. Percentage scavenges of DPPH radical by test samples were determined as

% Scavenged Activity = ($A_{Control} - A_{Test}$/ $A_{Control}$) × 100

$IC_{50}$ value was calculated by using graphical method.

**Phytochemical analysis:**

The freshly prepared *Argemone Mexicana* Linn. leafs ethanol extract was subjected to phytochemical screening tests for the detection of various constituents according to the standard protocol (Tiwari *et al*, 2011).

**GC-MS analysis:** GC-MS analysis was carried out on Shimadzu GC-MS model number QP 2010S. The organic compounds were identified by comparison of mass spectra with the inbuilt libraries NIST–11 and WILEY-8.

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## RESULTS AND DISCUSSION:

In vitro free radical scavenging activity of *Argemone Mexicana* Linn. leafs ethanol extract was studied by DPPH assay method.

**DPPH Free Radical Scavenging Activity:**

The dose response curve of DPPH for ethanol extract of *Argemone Mexicana* Linn. leafs was correlated with reference standard ascorbic acid (Nayak *et al*, 2013 and Goswami *et al*, 2014) (Fig.1).

**Table-1:** DPPH Free Radical Scavenging Activity of Ascorbic Acid.

*Absorbance of the sample at 517nm* *Absorbance of Control = 0.716*

| Sr. No. | Concentration (µg/ml ) | Absorbance | % Scavenged | $IC_{50}$ Value ( µg/ml) |
|---|---|---|---|---|
| 1 | 5 | 0.408 | 43.02 | 15.84 |
| 2 | 10 | 0.388 | 45.81 | |
| 3 | 20 | 0.336 | 53.07 | |
| 4 | 30 | 0.319 | 55.45 | |
| 5 | 40 | 0.288 | 59.78 | |
| 6 | 50 | 0.267 | 62.71 | |

**Table-2:** DPPH Free Radical Scavenging Activity of *Argemone Mexicana* Linn. leafs extract.

*Absorbance of the sample at 517nm* *Absorbance of Control = 0.716*

| Sr. No. | Concentration (µg/ml ) | Absorbance | % Scavenged | $IC_{50}$ Value ( µg/ml) |
|---|---|---|---|---|
| 1 | 5 | 0.624 | 12.85 | 42.50 |
| 2 | 10 | 0.549 | 23.32 | |
| 3 | 20 | 0.476 | 33.52 | |
| 4 | 30 | 0.399 | 44.27 | |
| 5 | 40 | 0.362 | 49.44 | |
| 6 | 50 | 0.344 | 51.95 | |

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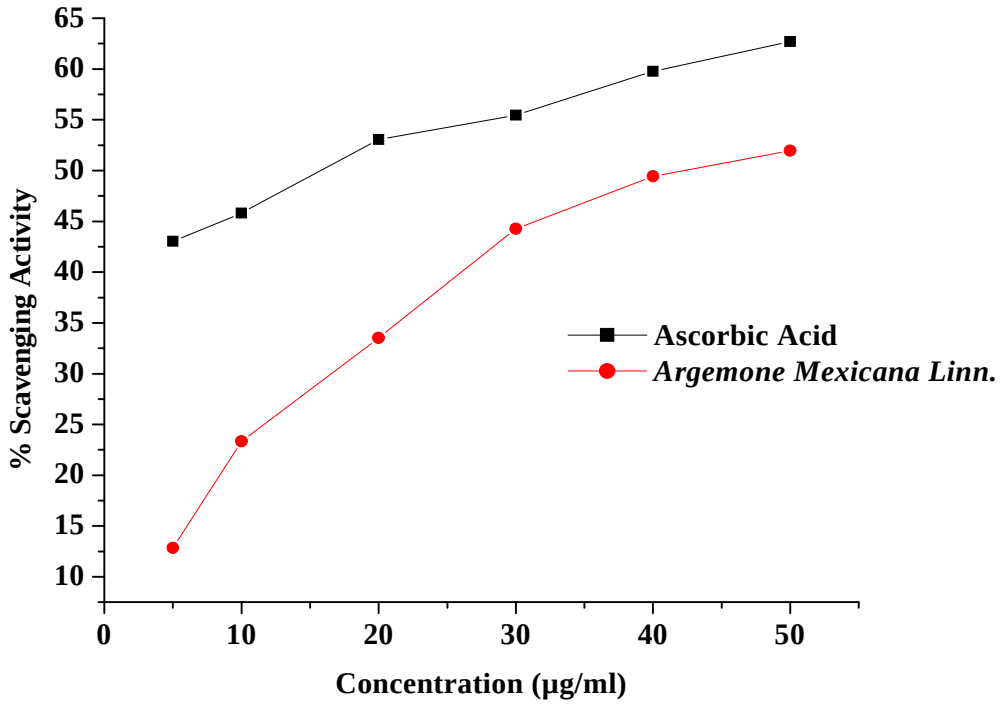


**Fig.1.** Free radical scavenging activity of *Argemone Mexicana Linn.* leafs ethanol extract.

The control ascorbic acid as a reference compound shows the highest activity at all concentrations with $IC_{50}$ value of 15.84µg/ml in DPPH assay (Table.-1) and test samples have their $IC_{50}$ value 42.50 µg/ml (Table.-2). This indicates that ethanol extract of *Argemone Mexicana* Linn. leafs has good potential as a source for natural antioxidants (Duhan *et al*, 2011).

**Phytochemical analysis:**

The phytochemical analysis of the leafs ethanol extract shows the presence of alkaloids, protein, glycoside, tannins, flavonoids, steroids and phenols as shown in Table 3.2 (Dash *et al*, 2011).

**Table-3:** Phytochemical analysis of *Argemone Mexicana* Linn. leafs extract.

| Phytochemicals | Result |
|---|---|
| 1. Alkaloid | + |
| 2. Carbohydrate | - |
| 3. Protein and amino acids | + |
| 4. Glycoside | + |
| 5. Tannin | + |
| 6. Saponin | - |
| 7. Flavonoids | + |
| 8. Steroids | + |
| 9. Triterpenoids | - |
| 10. Phenolic compounds | ++ |

(+) for present, (++) more intense, (+++) highly intense and (-) for absent

**GC-MS analysis:**

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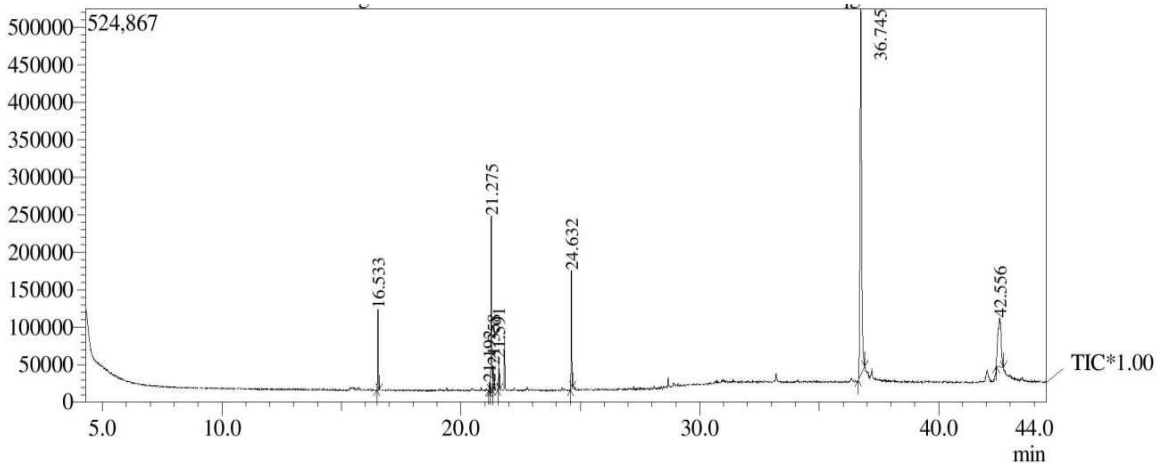


Peak Report TIC

| Peak# | R.Time | Area | Area% | Height | Height% | Name | Base m/z |
|---|---|---|---|---|---|---|---|
| 1 | 16.533 | 178670 | 4.45 | 107727 | 9.49 | PHENOL, 2,4-BIS(1,1-DIMETHYLETHYL)- | 191.10 |
| 2 | 21.192 | 18168 | 0.45 | 10418 | 0.92 | BUTANAL, 3-HYDROXY- | 70.05 |
| 3 | 21.275 | 493338 | 12.29 | 232730 | 20.50 | Neophytadiene | 68.05 |
| 4 | 21.358 | 79370 | 1.98 | 32211 | 2.84 | 2-UNDECENE, 9-METHYL-, (E)- | 70.10 |
| 5 | 21.591 | 68998 | 1.72 | 40975 | 3.61 | 16-Heptadecenal | 82.10 |
| 6 | 24.632 | 322263 | 8.03 | 157666 | 13.89 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 71.05 |
| 7 | 36.745 | 2238028 | 55.78 | 488528 | 43.03 | 1-EICOSANOL | 83.10 |
| 8 | 42.556 | 613751 | 15.30 | 65129 | 5.74 | Oxirane, hexadecyl- | 43.00 |
| | | 4012586 | 100.00 | 1135384 | 100.00 | | |

**Fig.2.** GC-MS Chromatogram of *Argemone Mexicana* Linn. leafs ethanol extract.

Phytochemical screening and GC-MS analysis (Fig. 2) of *Argemone Mexicana* Linn. leafs extract revealed the presence of various bioactive components (Apu *et al*, 2012). The plant is used in different parts of the world for the treatment of several ailments. Chemical constituents isolated from this plant are mostly belong to the class of alkaloids, besides, terpenoids, flavonoids, phenolics, long chain aliphatic compounds and few aromatic compounds (Brahmachari *et al*, 2013 and More *et al*, 2017). Therefore *Argemone Mexicana* Linn. is an important source of various types of compounds, which are responsible for many pharmacological activities (Verma, 2017).

Different extracts of *Argemone Mexicana* Linn. leafs were also reported to exhibit superoxide anion scavenging activity (Bhardwaj *et al*, 2011). This activity is due to the presence of total phenolic and flavonoidal content in different parts of plant (Sharma *et al*, 2013). As comparable with ascorbic acid used as a standard the ethanol extract exhibit significant antioxidant activity (Devakumar *et al*, 2014). Phenolic compounds are responsible for antioxidant activity, because they are effective hydrogen donors, which make them antioxidant (Rice-Evans *et al*, 1995 and Vijay *et al*, 2002).

This overall discussion of results indicates that leafs of *Argemone Mexicana* Linn. have potent free radical scavenging activity.

**CONCLUSION:**

The present investigation was indicated that extract of *Argemone Mexicana* Linn. leafs has been denoted free radical scavenging activity. The overall antioxidant activity depends on polyphenolic content and other phytochemical constituents present in

leafs. It could be a potential source of natural antioxidant and have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases. Therefore, it was concluded that ethanol extract of *Argemone Mexicana* Linn. leafs showed potent free radical scavenging activity.

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