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Antioxidant and Anti-inflammatory activities of the flower extracts of Argemone mexicana L.

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ABSTRACT

To assess the antioxidant and anti-inflammatory activities of the powder sample derived from the ethyl acetate fraction of the floral Argemone mexicana L. For antioxidant efficiency, the floral extract was evaluated using 1, 1–diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay and the FRC (Ferric Reduction Capacity) assay. In vitro anti-inflammatory activity was assessed using human peripheral blood mononuclear cells (PBMC) induced by lipopolysaccharide (LPS) and the production method of nitric oxide (NO). The powder sample extracted from ethyl acetate fraction of the floral of Argemone Mexicana L showed good antioxidant activity with the comparative standard sample in scavenging DPPH radicals and in FRC assay. In the cell viability (PBMC influenced by LPS) method and the Nitric oxide (NO) assay, this sample showed even able anti-inflammative activities. Such results indicate a significant antioxidant and anti-inflammatory activity in the powder sample obtained from ethyl acetate fraction in the flower of Argemone mexicana L.

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INTRODUCTION

Most higher plants are real wellsprings of customary things used as pharmaceuticals, flavour, agrochemicals and perfume fixations, and pesticides (Balandrin and Klocke, 1988). Throughout current and future efforts to manage biodiversity conservation and use, the search for new plant metabolites should be necessary. Therapeutic plants commonly used as choice supportive devices for the Countervailing activity of various ailments (Kaur, 2014; Nagavani and Rao, 2010). Herbal medicines remain vital because of availability, in addition to the trendy remedy, specifically low prices and no side effects (Narayanaswamy and Balakrishnan, 2011). Recent times have paid more considerable attention to the field of free radical innovation. Free radicals are responses to various biological processes in the body, oxygen, and nitrogen molecules. Free radicals are responsive oxygen and nitrogen species, which are produced by different physiological procedures in the body. The unregulated era of free radical agitation leads to oxidative anxiety attacks on surface lipids, enzymes, catalysts, and DNA and the ultimate demise of cells. Some ROS are useful for some human degenerative diseases such as diabetes mellitus, tumour, neurodegenerative scatters, Alzheimer's disease, Parkinson's disease, atherosclerosis, ageing, and provocative diseases (Gülçin, 2007). Micronutrients such as vitamin E, beta-carotene, and vitamin C are significant antioxidants in the human body, with differ-

ent chemical structures expecting complimentary radical rummaging. These are to be consumed in fewer calories because the body cannot provide these nutrients (Ramassamy, 2006). Adequate steps of exogenous cell reinforcements will improve protection against free radicals. Cell strengthening is an electron-based steady atom giving a radical free ramping and stopping the chain response before the damage to critical particles. Free radical cancer prevention envoy prevents or impedes damage to the cells (Halliwell, 1995). Inflammation is the key to many physiopathologies in response to tissue damage and as a part of microorganism host protection (Brodsky *et al.*, 2010). Macrophages play a key role in defending against foreign operators in inflammatory processes. Once activated by a lipopolysaccharyde (LPS) inflammatory stimulus, macrophages generate pro-inflammatory mediators, including nitric oxide (NO). Therefore, in recent years, work has been diagnosed with active non-toxic plant compounds with an antioxidant action (Margret *et al.*, 2009). The most active in treating inflammatory diseases are plants with polysaccharide (Chandrika and Chellaram, 2016). Argemone mexicana L (Family: Papaveraceae), generally known as Prickly Poppy in English and Premathandu in Tamil found in Mexico, United States, India, Bangladesh, and Ethiopia yet has broad circulation in numerous tropical and sub-tropical nations. It happens as no man's land, and roadsides weed in practically all aspects of India (Mukherjee and Namhata, 1990; Pk and Misra, 1987; Ibrahim and Ibrahim, 2009). In Mexico, the seeds are considered as a solution for wind venom (Bhattacharjee *et al.*, 2006). In India, the smoke of the seeds is used to reduce toothache. The new yellow, smooth seed isolate contains protein-dissolving substances convincing in the treatment of diuretic, moderating, malarial fever, ailment, scorpion sting, warts, mouth rankles, wound recovering, skin diseases, shivers, jaundice, and a cure to various harmful substances (Chopra *et al.*, 1986; Prusti and Mishra, 2005; Alagesaboopathi, 2009; Dash and Murthy, 2011). Since the antioxidant and anti-inflammatory motion of this plant not logically assessed. Because of this, the current exam has examined to investigate the antioxidant and anti-inflammatory potential of the powder from the portion of ethyl acetate in the Argemone mexicana L flowers.

MATERIALS AND METHODS

Collection of flowers

The new flowers of Argemone mexicana L have obtained from Z. Suthamalli, Ariyalur (Dt), Tamil Nadu, India. Dr. S. John Britto officially recognized this plant, President, Rapinate Herbarium, and Center for Molecular Systematics (Authentication No. DP004 dated: 22/01/2016). St. Joseph College (Campus), Tiruchirappalli, Tamil Nadu, India.

Extraction and fractionation

Argemone mexicana L's fresh flowers (3 kg) are soaked and extracted with 90% ethanol (5times500ml). The concentrated alcoholic concentrate collected in a vacuum and the aqueous concentrate w slowly fractionated with petroleum ether (60-80°C) (6times250ml), peroxide-free diethyl ether (4times250ml) and ethyl acetate (8times250ml). The separation of petroleum ether and diethyl ether provided no isolable substances. The concentrating fraction of ethyl acetate yielded a dry powder that was broken up in DMSO to obtain various fixations and used for further analyses.

Phytochemical screening of Ethyl acetate fraction

Chemical test, as described by (Harborne, 1998), was done on the Ethyl acetate fraction utilizing standard strategies.

DPPH scavenging assay

DPPH's radical sample search action was determined, as suggested by (Blois, 1958) comprehensive technique. A 0.5 ml aliquot methanol test solution combined with 2.5 ml 0.5 mM DPPH methanol solution. The blend was shaken energetically and found at room temperature for 30 minutes. Using a UV spectrophotometer, the absorbance measured at 517 nm. Ascorbic acid used as a positive control. This equation was used to measure DPPH radical free scavenging potential (%) (Blois, 1958; Adedapo *et al.*, 2008; Sakat *et al.*, 2010).

% Inhibition=

$$[\{Abs_{control} - Abs_{sample}\}/Abs_{control}] \times 100$$

In which Abs $_{control}$ is the absorbance of the DPPH + ethanol, Abs $_{sample}$ is the absorbance of the DPPH + test separate/standard.

Ferric Reducing Capacity (FRC) assay

Except that it uses O-Phenanthroline rather than TPTZ, the FRC assay is like the

FRAP assay. Phenanthroline forms a Fe^{III}-$(Phen)_3$ complex, which is diminished to an orange-red—shaded Fe^{II}-$(Phen)_3$ complex. This mixture containing 1 ml of 0.05% O-Phenanthroline in methanol, 2 ml ferric chloride (200μM), 20 ml of acetate buffer and 2 ml of different concentrations ranging from 10 to 250μg, it was brooded at room

Table 1: Phyto chemical analysis of Ethyl acetate fraction from ethanolic extract of Argemone mexicana L (Flowers).

Phytochemicals	Ethyl acetate fraction	Standard (quercetin)
Phenols	++	++
Steroids	-	-
Alkaloids	-	-
Flavonoids	+++	+++
Terpenoids	-	-

Table 2: DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging assay

Concentration (μg/ml)	% of Inhibition	
	Sample	Standard (Ascorbic acid)
10	68.07 ± 1.03*	78.98 ± 0.43
50	76.22 ± 0.31*	84.68 ± 0.04
100	77.48 ± 0.26*	86.10 ± 0.34
200	80.27 ± 0.08*	89.82 ± 0.82
250	83.25 ± 0.55*	92.15 ± 0.50
	IC 50< 10 μg/ml	IC 50< 10 μg/ml

Values are shown as for means ±SD of triplicate. *p<0.05 compared with control (one-way ANOVA and t-test)

Table 3: Ferric Reducing Capacity (FRC) assay

Concentration (μg/ml)	% of Reduction	
	Sample	Standard (Ascorbic acid)
10	18.21± 0.48*	25.07±0.98
50	30.77±0.60*	55.20±1.11
100	48.87±1.25*	78.00±0.48
200	70.21±0.40*	92.57±1.02
250	83.95±0.47*	95.73±0.91
	IC 50=88.71± 7.33 μg/ml	IC 50=32.88±2.51

Values are shown as for means ± SD of triplicate. *p<0.05 compared with control (one-way ANOVA and t-test)

Table 4: PBMCs with LPS of cell viability

Concentration (μg/ml)	Cell Viability (%)
Control	100
Positive control(LPS)	60.79 ± 0.74
25	60.17 ± 0.62*
50	72.33 ± 0.94*
100	79.65 ± 0.99*
200	86.72 ± 0.37*

Values are shown as for means ±SD of triplicate. *p<0.05 compared with control (one-way ANOVA and t-test)

Table 5: Nitric oxide (NO) inhibition assay

Concentration (μg/ml)	NO production (%)
Control(LPS)	100
25	94.35 $\pm$ 0.89*
50	86.51$\pm$ 0.75*
100	78.57 $\pm$ 1.36*
200	71.53 $\pm$ 0.62*

Values are shown as for means $\pm$SD of triplicate. *p<0.05 compared with control (one-way ANOVA and t-test)

temperature for 10 min and the absorbance of the same that was measured at 510 nm. The newly prepared FRC reagent clear vision, it was taken at 510 nm. Freshly prepared FRC reagent, the blank reading was taken at 510 nm. The capability of the plant extracts tested and compared to ascorbic acid in this assay. All tests were taken three times, and the mean was taken (Benzie and Strain, 1996; Kumar *et al.*, 2013; Lim and Lim, 2013).

$$\% \; Reduction \; = \; A_{510} sample \; / \; A_{510} blank \; reagent$$

Isolation of Human PBMC and Culture

Ficoll-Hypaque gradient centrifugation separated PBMCs from healthy contributors from the EDTA tube blood. In short, peripheral blood from the donors was finally diluted and overlayed with a Ficoll-Hypaca solution by a sterile phosphate buffer, and the centrifugation took place at room temperature at 350x g for 10 minutes. The recovered PBMC incubated for 30 minutes at RPMI-1640, and 95% O_2 + 5% CO_2 was produced at 37 $^\circ$ C before the tests were carried out (Jenny *et al.*, 2011).

The cell viability of PBMCs with LPS Stimulation

Following the PBMC isolation and pre-incubation period, a final concentration of 100 ng /ml and different concentrations of a sample in dimethyl sulfoxide (DMSO) were grown in a 96-well polypropylene plate in serum-free RPMI-1640 medium with LPS. The cells were incubated at 37 $^\circ$ C, 95% O_2 + 5% CO_2 for 24 hours under all conditions. Supernatants are removed from the treated cells to examine the scavenging activity of nitrous oxide (NO). Cell viability was tested using the MTT (3-(4.5-dimethylthiazol-2-yl)-2.5 diphenyltetrazolium bromide) method to determine the cytotoxicity of a fraction. The cells incubated with LPS and DMSO vehicles in the positive control wells. By measuring absorption at 570 nm by a microplate reader, cell viability calculated (Jenny *et al.*, 2011).

% Cell viability =

$$(OD \; of \; the \; sample / OD \; of \; the \; control) \times 100$$

Nitric oxide (NO) inhibition assay

Exploring the use of Griess reagent was nitric oxide produced in the supernatants. The supernatants (100 μl) were mixed in 5 % phosphoric acid with 20 μl of 1 % sulfanilamide, hatched at room temperature for 10 min, contained 20 μl of 0.1 % naphthyl-ethylenediamine dihydrochloride and brooded at room temperature for 10 min. After that, the response blend's absorbance perused at 540 nm. For the modification bend, sodium nitrite was used (Green *et al.*, 1982; Saravanan *et al.*, 2014; Ignácio *et al.*, 2001).

% NO Production =

$$(OD \; of \; the \; sample \; / \; OD \; of \; the \; control) \times 100$$

Statistical analysis

All the data reported as the mean $\pm$ standard deviation (S.D.). All statistical analysis performed by methods for one-way analysis of variance (ANOVA) and Student's t-test utilizing Graph Pad Prism statistical software package version7.02. The IC_{50} esteem was processed from nonlinear backslide examination using the Graph Pad Prism programming with the condition: Y = 100[1 + $10^{(X'' Log IC_{50})}$]. Only a value of p < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening

The consequences of different qualitative tests performed in the research centre are illustrated in Table 1. The nearness of flavonoids was affirmed by screening tests, which showed a positive test for flavonoids and negative for steroids, terpenoids, and alkaloids.

Anti-oxidant activity

DPPH assay is a fast, easy, and practical approach for quantifying an antioxidant effect, including the use of free radical, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) (Upadhyay *et al.*, 2014). DPPH is used

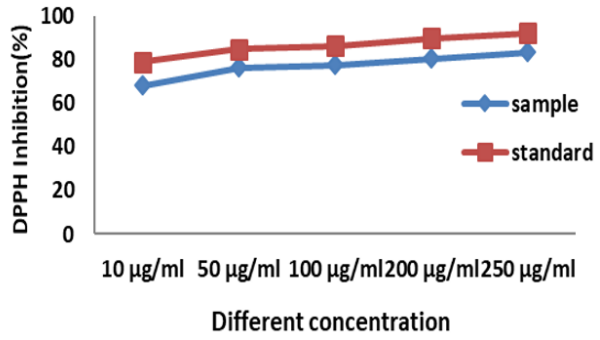

Figure 1: DPPH inhibition (%) activity of the sample (Solid powder of Argemone mexicana L flower fraction) and standard (Ascorbic acid) at different concentration.

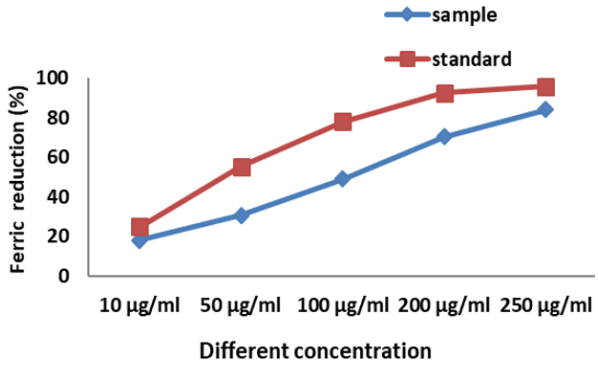

Figure 2: Ferric reduction (%) of the sample (Solid powder of Argemone mexicana L flower fraction) and standard (Ascorbic acid) at different concentration.

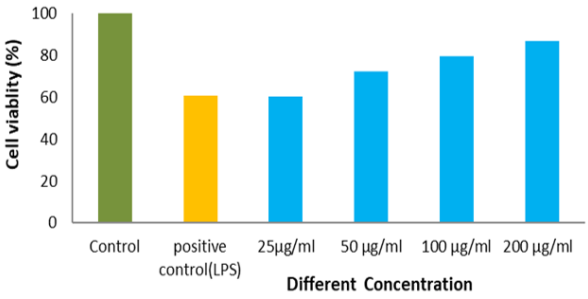

Figure 3: The cell (PBMC) viability percentage of without LPS (control), with LPS (positive control) and the sold powder (Argemone mexicana L flower fraction) at different concentrations

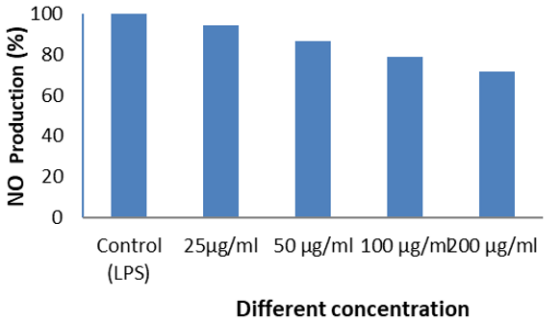

Figure 4: The effect of solid powder (Argemone mexicana L flower fraction) different concentrations on NO production by human PBMC stimulated with LPS

to assess the ability of mixtures to act as freely foragers or contributors of hydrogens and relies on DPPH's ability to colour with an antioxidant in mind (Sumanya *et al.*, 2015; Saha *et al.*, 2008). The concentration-dependent inhibition of DPPH radical was powder from the fraction of ethyl acetate of the Argemone mexicana L flower (Figure 1). The solid powder inhibition rate ranged from 68.07 $\pm$ 1.03 % to 83.25$\pm$0.55 % at different concentrations (10, 50,100,200 and 250 μg / ml). This powder fraction was able to reduce the radical 50% DPPH with IC_{50}< 10 μg / ml compared to the standard ascorbic acid with an IC_{50} < 10 μg / ml. The solid powder obtained from the Argemone mexicana L flower fraction of ethyl acetate displayed an excellent anti-radical efficacy in scavenging DPPH radicals and showed a peak inhibition of 83.25$\pm$0.55 % at a concentration of 250μg / ml. Argemone mexicana L's DPPH scavenging method shown in Table 2.

As a primary point of its potential antioxidant activity, the decreasing furthest reaches of a compound that fill in. The science of iron-based assays could be stuffed with the condition of reaction:

Fe (III)–L + antioxidant alike (II)–L + oxidized antioxidant

Where L is the ferrous-specific chromogenic ligand and that delivers the shaded species Fe(II)–L due to the redox response. Longer wavelengths are quite often an essential favoured point of view in deciding spectrophotometric strategy because most plant pigments and, besides, a few antioxidants exhibit tremendous assimilation at shorter wavelengths near the unmistakable UV spectrum. This reasoning applies to L: O-Phenanthroline for modern FRAP assay instead of TPTZ ligand. The redox response of a polyphenolic compound Ar(OH)n with tris(phen)Fe(III) is as follows:

$$\mathbf{Fe(phen)_3^{3+} + Ar(OH)}\boldsymbol{n} \leftrightarrow \boldsymbol{n}\mathbf{Fe(phen)_3^{2+}} + \mathbf{Ar(=O)}\boldsymbol{n} + \boldsymbol{n}\mathbf{H^+}$$

Table 3 displays the reduction values of the solid powder obtained from the ethyl acetate fraction of the Argemone mexicana L flower at different concentrations (10, 50,100,200&250 μg / ml) ranging from 18.21$\pm$ 1.03% to83.95$\pm$0.47%. Compared to standard ascorbic acid with a 32.88$\pm$0.47 μg / ml, the IC_{50} values of this solid powder are found to be88.71$\pm$7.33 μg / ml. All solid powder displayed reduction capacity in the concentration extent tested, which increased directly with concentration (Figure 2). Therefore, the powder obtained from the Argemone mexicana L flower fraction of ethyl acetate showed excellent antiradical activity in the FRC assay and showed a peak 83.95$\pm$0.47 % reduction at 250 μg / ml.

Anti-inflammatory activity

PBMCs were the perfect state for the development of pro-inflammatory cytokines by LPS. LPS is a gram-negative microbe with endotoxin and an external layer portion. By controlling the development of inflammatory mediators like TNF-α in PBMCs, LPS enhances innate immunity. The results showed (Figure 3) that the dose-dependent demonstrated by the solid powder obtained from the ethyl acetate fraction of the Argemone mexicana L flower. Examination of the fraction's cell viability in PBMCs using the MTT assay showed that the fraction at (25 to 200) μg / ml did not affect PBMC's viability. Therefore, the inhibition by the solid powder of LPS-induced mediator inflammation was not the result of a potential cytotoxic effect on these cells. Thus, these results suggest the anti-inflammatory effect of solid powder (Table 4), focusing on the generation of pro-inflammatory cytokines, and the use of this solid powder may not have had adverse effects.

Nitric oxide (NO) is a biological carrier of fundamental importance. As an inflammatory arbiter, NO takes part in a wide range of physiological and pathophysiological procedures, such as cytotoxicity, vein dilation, smooth muscle unwinding, and neurotransmission (Forstermann and Kleinert, 1995). PBMCs refined with LPS (100 ng / ml), and the proven nitric oxide (NO) substance calculated as a component of macrophage actuation in the supernatants. The impact of nitric oxide (NO) observed in the PBMC cell culture medium containing various concentrations (25 to 200) μg / ml of solid powder from the Argemone mexicana L flower ethyl acetate fraction as shown in Table 4. Table 5 shows the percentage of NO output of solid powder at different concentrations (25 to 200) μg / ml ranging from 94.35$\pm$0.89 % to 71.53$\pm$0.62 %. This study showed that the fraction of solid powder ethyl acetate from the Argemone mexicana L flower significantly reduced nitric oxide (NO) development. The data show a significant decline in solid powder NO production compared to the control group (Figure 4). It is therefore clear that the solid powder obtained from the Argemone mexicana L flowers ethyl acetate fraction was anti-inflammatory in NO assay and showed a maximum reduction in NO output of 71.53$\pm$0.62% at 200 μg / ml.

CONCLUSIONS

In conclusion, screening tests confirmed the proximity of flavonoids and show contemplate showed the in-vitro antioxidant and anti-inflammatory activity of the solid powder extracted from the Argemone mexicana L ethyl acetate fraction of the flowers. Additional work is needed to determine the chemical component responsible for antioxidants and anti-inflammatory drugs. In our laboratory, experiments are underway to elucidate the compound's molecular structure to leads to potent antioxidant and anti-inflammatory drug development.

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