J. Indian bot. Soc. (2023) Vol. 103 (2): 119-126

e-ISSN:2455-7218, ISSN:0019 - 4468

https://doi: 10.5958/2455-7218.2023.00006.2

www.indianbotsoc.org

ORIGINAL ARTICLE

In-vitro and *in-vivo* antioxidant potential and GC-MS profile of *Argemone mexicana* L.

Mahendra Singh and Vimala Yerramilli*

Abstract

The present study was founded on callus induction, antioxidant activity and thermostable secondary metabolite profiling in the callus and plant parts of an unexplored medicinal plant, *Argemone Mexicana* L. Callus was induced from young stem of *A. mexicana* on MS medium supplemented with different concentrations and combinations of PGRs such as NAA (0.5-1.0 mg/L), KN (0.5-2.0 mg/L) and BAP (0.5-1.5mg/L). The best degree of callus induction was recorded on MS medium containing 1.0mg/L NAA+0.5mg/L BAP and 1.0mg/L NAA+2.0mg/L KN. The free radical scavenging assay (antioxidant activity) using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric ion reducing antioxidant power (FRAP) in various fractions of *A. mexicana* leaves, stem and callus indicated higher content of flavonoids (0.0656±0.0008 mg RUT (Rutin equivalent)/100g-dw) and phenolics (1.63±0.94 mg GAE (Gallic acid equivalent)/100 g-dw conc.) in vitro (callus) compared to different plant parts (explants)advocating strong antioxidant potential of the plant. *In vitro* too, the methanolic extracts obtained from *A. mexicana* callus and stem explants through GC-MS profiling showed richness of various important antioxidants such as1,3-propanediol,2-(hydroxymethyl)-2-nitro-; Squalene;6-alpha-cadina-4, 9-diene,9,(-)-;Stigmast-5-en-3-ol(3-beta);Vitamin-E; 1(2-H)-Naphthalenoneoctahydro-4a-8a-dimethyle-7(1-methyle); 1, 2-benzendicarboxylic acid in callus and Benzene propanoic acid; 3,5-bis (1,1- dimethylethyl)-4-hydrox); Neophytadiene; Di-isooctyles; Hexadecane and Dibutyl phthalate, in stem, respectively. These bioactive compounds may be utilized in treating various ailments such as inflammation, rheumatism, cancer, and jaundice, etc.

Keywords: *Argemone mexicana* L., Callus, DPPH, FRAP, flavonoids, GC-MS, phenolics.

Introduction

Plant tissue culture technique is a powerful tool for large scale production of callus to support commercial industries such as nutraceutical, herbal and pharmaceutical industries (Rahayu *et al*. 2016). Callus formation is an effective way for upscaling secondary metabolites, which may be helpful in the treatment of various diseases. Many factors (light, temperature, and humidity) influence the success of callus initiation and multiplication of woody and spiny plants, including the selection of plant growth regulators (PGRs) and basal medium (Murashige and Skoog and woody plant media). Supplementation of optimized exogenous concentration and combination of PGRs may lead to shoot initiation, root formation or callus development. Such callus may be a good alternative source of antioxidants along with other important secondary metabolites. Though, antioxidant compounds are naturally obtained from plants, fruits, and vegetables as the cheap sources with no or low toxic side effect, yet through tissue culture, if an abundant amount of such secondary metabolites or novel compounds can be harvested then naturally growing plants need not be uprooted or disturbed. The reactive oxygen species such as hydrogen peroxide (H_2O), superoxide anion (O_2^{-}) and hydroxyl radical ($HO^{\cdot}$), attack biomolecules, such as proteins, lipids, and DNA, leading to membrane and DNA damage. In animal cells, disorders due to free radicals like cancer, angiocardiopathy and senility have been reported by Chen *et al*. (2018). Flavonoid consumption has been reported to mitigate cardiovascular disorders, diabetes, obesity, and cancer (Ebegboni *et al*. 2019), and an inverse relationship, to exist between chronic diseases and dietary flavonoids (Hertog *et al*. 1993). Ontiveros *et al*. (2019) have reported that capacity of flavonoids depends on their hydroxyl group,

Department of Botany, Plant Physiology and Biochemistry Lab, C.C.S. University, Meerut, Uttar Pradesh, India.

***Corresponding Author:** VimalaYerramilli, Department of Botany, Plant Physiology and Biochemistry Lab, C.C.S. University, Meerut, Uttar Pradesh, India, E-Mail: yvimala17@gmail.cm

How to cite this article: Singh M and Vimala Y (2023). In-vitro and in-vivo antioxidant potential and GC-MS profile of Argemone mexicana L. J. Indian bot. Soc., 103(2):119-126. Doi: 10.5958/2455-7218.2023.00006.2

Source of support: Nil

Conflict of interest: None.

Received: 08/08/2022 **Accepted:** 13/09/2022 **Published online**: 25/01/2023

conjugation, and other substitutions. Zhang *et al.* (2020) have provided theoretical foundation for structural modification of flavonoids as active antioxidant.

Argemone mexicana is a Mexican prickly poppy, belonging to the family Papaveraceae. It is an annual herb and native of tropical America. *A. mexicana* possesses fatty acid, amino acid, phenolics and alkaloids as significant bioactive groups represented by following important phytochemicals viz., Muramine, Coptisine, Berberine, Isoquinoline, Scoulerine, Stylopine, Thalifone, Benzylisoquinolines, Protopine and Tetrahydroberberine, etc. (Charles *et al*. 2012). The present work has been aimed to optimize effective protocol for callus induction and to perform comparative assay for antioxidant activity along with quantification of antioxidants, in mother tissue and the callus, to find an alternative source for antioxidants. Estimation of Total Phenolic Content (TPC), Total Flavonoid Content (TFC), DPPH, FRAP assays and GC-MS profiling of extracted secondary metabolites from plant parts and callus have been carried out for ascertaining a probable drug candidate.

Chemicals and Reagents

All chemicals used for the analyses were of analytical grade. Plant growth regulators (2,4-D, NAA, BAP, KN), labolene detergent, sodium hypochlorite, Polyvinyl pyrrolidone (PVP), Murashige and Skoog's medium, methanol, ethanol, sodium carbonate, Tris-HCl, sodium phosphate(mono- and di-basic), H_2O_2, $AlCl_3$, $NaNO_2$ DPPH (1,1-diphenyl-2-picrylhydrazyl) TPTZ, Gallic acid, Folin-Ciocalteau's (FC) reagent, rutin, and catechol were procured from Sigma Aldrich or Hi-media.

Materials and Methods

Explant Selection and Surface Sterilization

Argemone mexicana L. generally distributed in North America including Mexico and southern Florida is now widely naturalized in many parts of the world. *Argemone mexicana* plants were well acclimatized in Department of Botany, C.C.S University, Meerut, Uttar Pradesh (247amsl; 29.05°N"77.51°E) and specimen was certified by the Botanical Survey of India, Howrah Calcutta, India (BSI, Calcutta, India) and submitted as voucher number CNH/Tech.II/2022/63 (Figure 1A). The stem and leaf explants were collected from the one-month-old, acclimatized plant. The stem and leaves were first surface sterilized with labolene for 5 minutes followed by rinsing with tap water for 10 minutes. Explants were then sterilized with 5% sodium hypochlorite for 6 minutes and rinsed with sterilized distilled water 3 to 4 times. To overcome the problem of yellow latex exudation, stem explants were treated with 0.3% Polyvinylpyrrolidone for 10 minutes (PVP-Himedia: RM854-100G) followed by rinsing once again with sterilized distilled water 3 to 4 times.

Nutrient Media, Culture Establishment and Culture Condition

MS (Murashige and Skoog, 1962) medium with 3% sucrose, 0.8% agar-agar, 0.3% PVP, and essential growth regulators, vitamins, minerals were used as essential medium. The pH of the culture media was adjusted to 5.8 ± 0.01 using 0.3N NaOH or HCl, and autoclaved at 121°C for 15 minutes at 15 psi pressure. Sterilized media was transferred to Erlenmeyer flasks in laminar air flow cabinet. After 48 hours, explants were inoculated under aseptic conditions and incubated in culture room at 25 ± 2°C temperature and 60 to 65% relative humidity.

Callus Induction and Subculturing

For callus induction, stem segments were cultured on MS medium with additive and different concentration and combinations (0.1, 0.2.0.3. 0.4, 0.5 mg/L) of NAA, BAP, and KN. For further subculturing, callus was transferred on optimized concentration and combinations of NAA, BAP, and KN, respectively.

Phytochemical Screening for Antioxidant Metabolite

Extraction of Bioactive Compounds

Fresh tissue (leaves, stem) and callus (developed from stem), were sterilized with distilled water and 70% ethanol. The sterilized fresh tissues were dried in oven for 4 days then grounded into a fine powder. 1.0 gm of dried fine powder was extracted in methanol (250 mL) and ethanol, individually, using Soxhlet extraction unit for 48 hours. The extracts were filtered through Whatman no-1 filter paper, concentrated by water bath evaporator, and used directly for the estimation of total flavonoids, phenolics and antioxidant activities through biochemical (preliminary screening, DPPH and FRAP) assays.

Phytochemical screening of methanolic and ethanolic extracts of leaves, stem, and callus of *A. mexicana* using the standard direct tests was carried out (Fardiyah 2020; Wafa Al-Madhagi 2016; Nitika 2020).

Determination of Total Phenolic Content (TPC)

Total phenolic content (TPC) in *Argemone* plant tissue (leaves, stem, and stem callus) extracts was determined using Folin & Ciocalteau's phenol reagent, spectrophotometrically. 0.5 gm

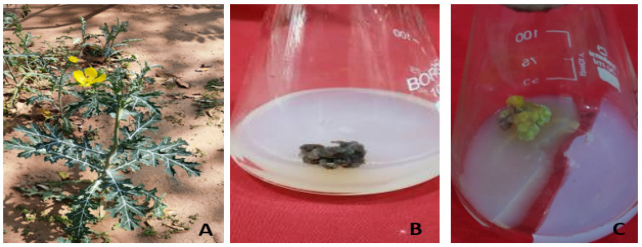

Figure 1: A. Parent plant, **B.** Initiation of callus and **C.** Sub-cultured callus.

dried plant tissue was homogenized in 5 mL 80% methanol, centrifuged at 2000 x g for 10 minutes; clear supernatant was collected, and remaining tissue was recentrifuged in 2 mL 80% methanol. The collected supernatant was kept on water bath until supernatant slightly dried. This dried extract was dissolved in 5ml distilled water and was divided into aliquots (0.2, 0.4, 0.6, 0.8, 1.0 µL). To each aliquot, 3 mL double diluted Folin–Ciocalteau's reagent and 3 mL of 20% Na_2CO_3were added making a final volume of 10 mL. Blue colored solution was formed which was shaken well and incubated for 25 min at 45°C in a water bath. Its absorbance was measured at 760nm against a reference blank using UV-visible spectrophotometer (UV-2600 SHIMADZU). Gallic acid (GAE mg equivalent) was used as standard for calibration (Bray and Thorpe 1954).

Determination of Total Flavonoid Content (TFC)

Total flavonoid content (TFC) of *A. mexicana* plant tissues (leaves, stem, and stem callus) was determined by spectrophotometric method using aluminium chloride (Esterbauer 1977). To 1 ml fresh supernatant, 0.3ml $NaNO_2$, 10% $AlCl_3$ was added; after 5 minutes of incubation 2ml 1M NaOH was added to the mixture and left for 6 min. Final volume of the mixture was made to 10mlwith distilled water. Rutin (Rut mg equivalent) was used as standard for the purpose of calibration. The absorbance was measured at 510 nm against blank using a UV-Vis spectrophotometer (UV-2600, SHIMADZU).

2, 2-diphenyl-1-picrylhydrazyl Radical Scavenging Activity (DPPH) Assay

Free radical scavenging activity estimation of methanolic extracts of *A. mexicana* plant tissue (leaves, stem, and stem callus) was carried out by DPPH assay (Pellegrini, 1999). 10 to 50 µL of aliquots were added to a 2.5 mL of 60 **µM** methanolic solution of DPPH. Reaction mixtures were well shaken and incubated for 12 hours at room temperature in the dark condition and absorbance was recorded at 515 nm by using UV-2600 SHIMADZU Spectrophotometer. The blanks were made in methanol against DPPH at 294 ± 0.2nm instead of the extracts. Ascorbic acid and Catechol were used as positive controls. The IC_{50} values (half maximal inhibitory concentration) was determined from the sample calculation of inhibition using the various concentrations of extracts.

Ferric Reducing Antioxidant Activity (FRAP) Assay

Ferric reducing activity was estimated by using freshly prepared TPTZ (2, 4, 6-Tri (2-pyridyl)-S-triazine) in 10 mL of 40mM HCl, 30mM acetate buffer (pH-3.6) and 20mM $FeCl_3.6H_2O$, in the ratio of 10:1:1, respectively. To 10 µL plant sample extract, 3.99 mL FRAP reagent was added. After 10 min. of incubation the absorbance was recorded at 593 nm against blank (3.99 mL FRAP+10 µL methanol) using UV-2600 SHIMADZU spectrophotometer. To calculate the concentration of antioxidant having ferric-TPTZ reducing ability, a calibration curve was constructed using vit-C as reference (Polovnikova and Voskresenskaya 2008).

Analysis of Metabolite by Gas Chromatography-Mass Spectrometry (GC-MS)

The analysis was done at the University Science Instrumentation Centre, AIRF, Jawaharlal Nehru University, Delhi. The phytochemical investigation of methanolic extract was performed on a GC-MS equipment (SHIMADZU, Kyoto, Japan) GC-MS-QP2010 ultra, which comprised the auto injector (AOCX-20i) and headspace sampler (AOC-20s) system, equipped with mass selective detector with anion source having temperature 230°C and interface temperature 270°C. Capillary column, Rt × 5MS capillary and 102 column with 30 mm × 0.25mm (length × diameter) and 0.25µm of film thickness was used for MS analysis. The temperature of the injector was adjusted to 260°C, possessing a split injection mode. The initial temperature applied was 50°C (3 minutes), which was further programmed to increase to 280°C at a ramp rate of 15°C/minute. Helium (>99.99%) was used as carrier gas with 39.9 cm/second of linear velocity. The total flow programme was 16.3 mL/minute, with column flow of 1.21 mL/minute. The resulted database of rhizome was compared with database of National Institute of Standard and Technology (NIST, US). NIST has more than 62000 patterns. The resultant compounds name, molecular weight, structure, and functions were analyzed through NIST and WILEY8 library. The structure of novel compounds has been drawn using licensed software Came Draw Ultra 12.0.

Data Analysis

All the measurements of phenolic, flavonoid, enzymes and antioxidant activity of plant and callus extract were conducted in triplicate. All results were performed by SPSS 25 (P value ≤ 0.05) for Windows using ANOVA and post-hoc LSD and Duncan.

Results and discussion

A stimulatory effect of supplementation of KN and BAP in combination with NAA to MS media was noted for Callus formation in *A. mexicana* (Figure 1B & C). KN and BAP could not respond individually with 0.3% PVP, but with NAA, could initiate callus in stem explants of *A. mexicana*. The best callus initiation response was recorded on MS media containing 0.5 mg/L BAP+1.0 mg/L NAA and on 2.0 mg/L KN+1.0 mg/L NAA mg/L (Table 1). Plant tissue culture technique is mainly valuable to produce diverse phytocompounds in bulk quantities for therapeutic uses. Callus shows high quantity of bioactive molecules than parent plant (*A. mexicana*) such as flavonoids, tannin, phenolic, terpenoids, ascorbic acid, cardiac glycosides, and quinine, which are used in many pharmaceutical preparations (Table 2). Hema *et al*. (2020) have reported that PGR supplementation promotes

Table 1: Effect of PGRs on callus induction from stem explants

S. No.	*PGRs concentration (mg/L)*	*Callus colour*	*Callus nature*	*Callus induction (days)*	*Callus induction Response (%)*
1.	MS+1.0NAA+ 0.5BAP	Yellow greenish	Compact	35	50
2.	MS+1.0NAA+1.0BAP	Yellow greenish	Compact	35	40
3.	MS + 1.0 NAA + 1.5BAP	Yellow greenish	Compact	35	40
4.	MS+1.0NAA+2.0KN	Yellow greenish	Compact	35	50

Table 2: Preliminary screening of phytochemical constituents

S. No	*Test type*	*Methanolic extract of leaves*	*Methanolic extract of stem*	*Methanolic extract of stem callus*	*Ethanolic extract of leaves*	*Ethanolic extract of stem*	*Ethanolic extract of stem callus*
1.	Phenol	+	++	+++	+	++	++
2.	Terpenoid	+	+++	+++	++	+++	+++
3.	Flavonoids	+++	++	+++	+++	++	+++
4.	Saponin	+	-	-	+	+	+
5.	Cardiac glycoside	+	++	++	+	+	+
6.	Quinine	++	++	+++	++	+	++
7.	Tannin	-	-	-	+	+	++
8.	Ascorbic test	-	-	+	-	-	-

production of secondary metabolites in plant tissue culture. In the present investigation too, PGR supplementation (NAA+BAP, IAA+KN) to the basal medium led to enhanced biosynthesis and accumulation of flavonoid, tannin, phenolic, sesquiterpenes in *Argemone* callus compared to parent plant tissue. NAA+BAP greatly enhanced Isoflavonoid accumulation and showed significant effect on callus growth. Downey *et al*. (2013) have reported Isoflavonoid to be a large group of flavones which have good antioxidant properties in various types of foods such as soy products. Most valuable phytochemicals were identified such as terpenoids, flavonoids, phenol, cardiac glycoside, saponin, tannins, ascorbic acid and quinines were detected in *Argemone mexicana* species (Table 2). Each bioactive compound showed high potency toward biological action such as antioxidant, anti-microbial, anti-inflammatory, anticancer and hepatoprotective potential (Pub chem.). Each Phytochemical has novel biological behavior which may increase the chance of exploring new activity of the compounds such as antibiotics. Flavonoids are good polyphenols having the capacity to produce antibiotics because flavonoids make complexes with bacterial cell wall, protein, and other ingredients (Edge 1997, Cowan1999). Terpenoid, saponin and tannins also communicate the plant potential against bacterial and fungal infection. The phenol, quinine, flavonoid and terpenoid contents were abundant in methanolic and ethanolic extracts of callus than the parent plant. Quinines, saponins, tannins, cardiac glycosides and ascorbic acid were recorded to be higher in methanolic extracts of calli compared to the mother tissue. Tannins are high molecular weight polyphenolic compounds with antioxidant property. Total phenolic and flavonoid contents were determined by the Folin & Ciocalteau's phenol red and Aluminium chloride methods spectrophotometrically. Phenolic and flavonoid contents increased in callus making it a potential source of antioxidant to effectively act against various ailments. Flavonoids being polyphenolic (Hassan *et al*. 2020) have several Hydroxyl groups, with free radical scavenging properties (antioxidant property). Total phenolic content was highest in stem callus in terms of mg Gallic Acid equivalents per gram dry matter (0.42 ± 0.003 mg GAE/g DM) of *Argemone mexicana* than in other parts of parent plant such as stem (0.20 ± 0.003 mgGAE/g DM) and leaves (0.12 ± 0.005 mgGAE/g DM) as shown in Figure 2A. Total flavonoid content was highest in stem callus in terms of mg Rutin equivalents per gram dry matter (0.065 ± 0.008 mgRUT/g DM) of *Argemone mexicana* than in the parent plant parts (leaves 0.042 ± 0.0006 mgRUT/g DM) and (stem 0.039 ± 0.0006 mgRUT/g DM) as shown in Figure 2B. In addition, through GC-MS analysis of methanolic extracts of *A. mexicana* callus and stem explant, forty-seven compounds such as aromatic hydrocarbons, phenolics, flavonoids, terpenoids, alcohols, fatty acids, esters, and aldehydes, etc. were detected. Their % peak area, retention time and chromatogram of phytocompounds are shown in Tables 3&4 and Figures 3&5. In all the test samples (callus and stem), most of the phytochemicals were different but some phytochemicals were same in low or high amounts. The compounds present in high quantity in callus extract according to the GC-MS library identification were 3A, 4, 5, 5B,7A,12A,12B,13,14,14A,14B-Dodecahydro (38.20%) and 1,3-propanediol,2-(hydroxymethyl)-2-nitro- (25.88%) (Table 3). In the extract of parent plant part (stem) high quantity of 10-Nonadecanol (70.25%) and Gamma-sitosterol (9.11%)

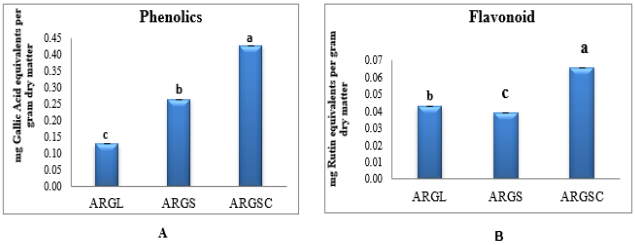

Figure 2: A. Total phenolic content **B.** Total flavonoid content (significance level is α = 0.05).

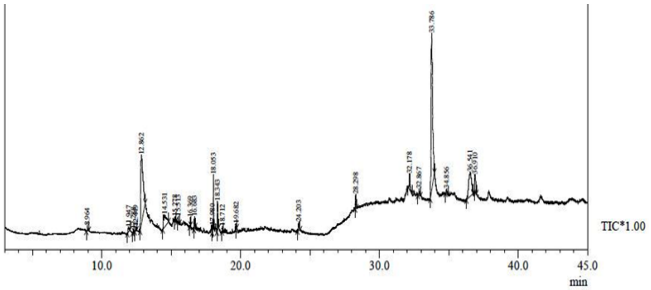

Figure 3: GC-MS Chromatogram of methanolic stem callus extract of *A. mexicana*

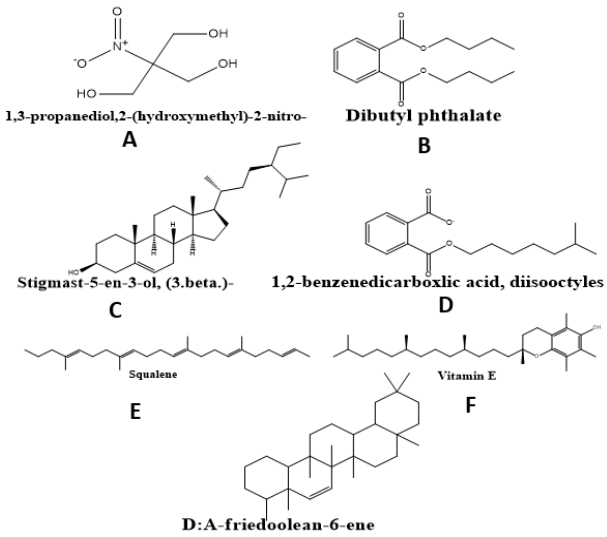

Figure 4: Structure of some valuable novel bioactive compounds from calli of *Argemone mexicana* stem (NIST and PUBCHEM); **4A:** 1,3 propanediol,2-(hydroxymethyl)-2nitro-; **4B:** Dibutyl phthalate; **4C:** Stigmast-5-en-3ol, (3- beta); **4D:**1,2-benzenedicarboxlic acid, diisooctyles; **4E:** Squalene; **4F:** Vitamin E; **4G:** D: A-friedoolean-6-ene

Table 3: *In vitro* GC-MS analysis of *A. mexicana* callus

S. No.	*R. Time (Min.)*	*Compound name*	*Area %*	*Molecular mass(mg/mol)*	*Molecular structure*
1.	8.964	Tridecane	0.44	184.36	$C_{13}H_{28}$
2.	11,947	Oxalic acid,2-ethyle hexyl ester	0.75	286.41	$C_{16}H_{30}O_4$
3.	12.288	Cyclohexaneethanol,4ethenyl-4-methyl-	0.18	220	$C_{15}H_{24}O$
4.	12.449	*6-alpha-cadina-4,9-diene,9,(-)-	0.27	204	$C_{15}H_{24}$
5.	12.862	*1,3-propanediol,2-(hydroxymethyl)-2-nitro-	25.88	151	$C_4H_9NO_5$
6.	14.531	Oxalic acid, monoamide, N-allyl-, dodecyl ester	5.40	297	$C_{17}H_{31}NO_3$
7.	15.278	1-(4-isopropylphenyl)-2-methylpropyle acetate	0.30	234	$C_{15}H_{22}O_2$
8.	15.513	*1(2H) Naphthalenone,octahydro-4a,8adimethyle7(1methyle)/Valeranone	0.20	222.37\	$C_{15}H_{26}O$
9.	16.369	1-Iodo-2-methylundeane	0.48	296.23	$C_{12}H_{25}I$
10.	16.683	Sulfurous acid, 2-ethyl hexyl-hexyl ester	0.82	278.45	$C_{14}H_{30}O_3S$
11.	17.980	Undecanoic acid, methyl ester	0.40	200.31	$C_{12}H_{24}O_2$
12.	18.053	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydrox	2.83	292.4	$C_{18}H_{28}O_3$
13.	18.343	*Dibutyl phthalate	2.55	278.34	$C_{16}H_{22}O_4$
14.	18.712	2Bromododecane	0.30	249.23	$C_{12}H_{25}Br$
15.	19.682	13, 16-Octadedienoic acid, methyl ester	0.24	294	$C_{19}H_{34}O_2$
16.	24.203	*1,2-benzenedicarboxlic acid, diisooctyles	0.98	390.6	$C_{24}H_{38}O_4$
17.	28.298	*Squalene	1.29	410.7	$C_{30}H_{50}$
18	32.178	*Stigmast-5-en-3-ol, (3.beta.)-	2.58	414	$C_{29}H_{50}O$
19.	32.867	*Vitamin E	0.66	430	$C_{29}H_{50}O_2$
20.	33.786	3A, 4, 5, 5B, 7A, 12A, 12B, 13, 14, 14B-Dodecahydro	38.20	335	$C_{20}H_{17}NO_4$
21.	34.856	Unknown compound	0.80	Unknown	Unknown
22.	36.541	*D:A-friedoolean-6-ene	11.5	410	$C_{30}H_{50}$
23.	36.910	*Stigmast-5-en-3-ol, (3.beta.)-	3.12	414	$C_{29}H_{50}O$

were detected (Table 4). Interestingly, these compounds were found in only stem callus, which may be useful for pharmaceutical industries at commercial level. In the extract of callus, one unknown compound was also detected but the structure, function, formula could not be deciphered. It has retention time of 34.856 min. and retention area% of 0.80 % after screening of libraries which may possibly be a novel bioactive compound. The major bioactive compounds with high antioxidant potential as depicted by asterix (*in the Table 3&4) identified in the methanolic extracts of callus and stem of *Argemone mexicana* are useful in the treatment as antimicrobial, antioxidant, anti-inflammatory, anti-arthritic, antiasthma, diuretic, analgesic, anti-diabetics, anti-dermatitis, anti-leukemic, anti-tumor, anticancer, hepatoprotective, anti-spasmodic (Farina *et al*. 2014, Alqahtani *et al*. 2019, Pathak *et al.* 2021). All the compounds of callus were pharmacologically very important not only due to antioxidant nature but also for drug development. Structures of some valuable novel bioactive compounds used in the treatment of various acute and chronic diseases (liver injury and cancer), recorded from *A. mexicana* stem callus in GC-MS report (not recorded in the parent plant tissue) are as follows Figure 4. These compounds were not detected in methanolic extract of stem and exhibited more area (%), indicating availability of high amounts in calli. 10-Nonadecanol is a major alcoholic bioactive compound which has maximum area 71.08% but their biological activity not found in literature. Nonane, 3, 7-dimethyl-; Bicyclo (7.2.0) undec-4-ene,4,11,11-trimethyl-8-/ Beta-caryophyllene,1(2H)-nepthhalenone, octahydro-4a,8a-dimethyle-7(1-methyle)/valeranone;Gamma-sitosterol / Clionasterol, stigmast-5en-3ol, (3-beta.); Phytol;stigmast-5en-3ol, (3-beta.);Neophytadiene, benzenepropanoic acid, 3,5-bis(1,1- Dimethylethyl)-4-hydrox and dioctyl phthalate are important bioactive compounds that have significant role in various pharmaceutical activities like neuroprotective effect, nephroprotective, anti-microbial, immune modulatory activity, hepatoprotective, gastro protective, anticancer, cardio protective, anti-inflammatory and antioxidant (Bizimenyera *et al*.2007; Arjun *et al*. 2010; Shettima *et al*.2013; Machado *et al*. 2018;). Gamma-sitosterol /Clionasterol, stigmast-5en-3ol, (3-beta.) and Phytol

Table 4: *In vivo* GC-MS profile of *A. mexicana* stem

S. No.	*R. Time*	*Compound name*	*Area %*	*Molecular mass(mg/mol)*	*Molecular structure*
1.	5.509	Decane,3-methyl-	0.22	156	$C_{11}H_{24}$
2.	12.021	Nonane,3,7-dimethyl-	0.78	156	$C_{11}H_{24}$
3.	12.295	*Bicyclo(7.2.0)undec-4-ene,4,11,11-trimethyl-8-/ Beta-caryophyllene	0.43	204	$C_{15}H_{24}$
4.	14.400	3,5-Dodecadiene, 2-methyl-	0.25	180	$C_{13}H_{24}$
5.	15.513	*1(2H)nepthhalenone,octahydro-4a,8a-dimethyle-7(1-methyle)/Valeranone	0.21	222	$C_{15}H_{26}O$
6.	16.710	Decane, 2,3,5-trimethyl-	0.43	184	$C_{13}H_{28}$
7.	17.075	*Neophytadiene	0.42	278	$C_{20}H_{38}$
8.	18.053	Benzenepropanoic acid, 3,5-bis(1,1- dimethylethyl)-4-hydrox	2.83	292.4	$C_{18}H_{28}O_3$
9.	18.343	*Dibutyl phthalate	2.55	278.34	$C_{16}H_{22}O_4$
10.	17.980	Undecanoic acid, methyl ester	0.40	200.31	$C_{13}H_{26}O_2$
11.	18.722	Decane, 1-bromo-2-methyl-	0.16	235	$C_{11}H_{23}Br$
12	19.791	*Phytol	0.82	296	$C_{20}H_{40}O$
13.	21.320	Octanoic acid,2-dimethylaminoethyl ester	0.18	215	$C_{12}H_{25}NO_2$
14.	22.008	3,7-dimethyl-1-octylmethyphosphonofluoridate	0.32	238	$C_{11}H_{24}FO_2P$
15.	24.193	*Dioctyl phthalate	0.53	390	$C_{24}H_{38}O_4$
16.	29.327	*Hexadecane	0.13	226	$C_{16}H_{34}$
17	31.168	3,4-Dimethoxy-6-methyl-5,7,8,15-tetrahydrob	1.58	405	$C_{21}H_{24}ClNO_5$
18.	32.185	*Stigmast-5en-3ol, (3-beta.)-	4.08	414	$C_{29}H_{50}O$
19.	32.493	10-Nonadecanol	71.08	284	$C_{19}H_{40}O$
20.	35.418	26,27-Dinorcholesta-5,22-Dien-3-ol,(3.beta., 22E	1.21	356	$C_{25}H_{40}O$
21.	36.902	*Clionasterol	9.11	414	$C_{29}H_{50}O$
22.	37.456	Oxirane, hexadecyl-	4.01	268	$C_{18}H_{36}O$
23.	45.411	Phytyl decanoate	2.14	450	$C_{30}H_{58}O_2$

exhibit important role as antibacterial, anti-inflammatory, antihypercholesterolemic, anti-protozoal (Leishmania), Lipid metabolism regulator, Antiparasitic, Antihelmintic (Adnan *et al*.2019).

In DPPH activity, any substance which can donate a hydrogen atom to DPPH reduces it to a stable molecule with deep violet colour. The earlier researchers reported that the hydrogen donating phenolic compounds inhibit lipid peroxidation and cause discoloration of DPPH from purple to yellow (Tauheeda *et al*. 2012). Methanolic extract of calli exhibited higher IC_{50} (20.92 ± 0.07µg/mL) value than other explants (leaves and stem). DPPH assay shows lower IC_{50} value with higher scavenging activity (Figure 6A). FRAP is a strong antioxidant assay because it measures the reducing potential with a ferric tripyridyltriazine complex and producing a blue colored ferrous tripyridyltriazine (Fe^{3+}-TPTZ→ Fe^{2+}-TPTZ). The reducing properties of FRAP are directly associated with the presence of compounds using their action by breaking the free radical chain through donating H^+ atom. Methanolic extract of *Argemone mexicana* callus showed high FRAP value as 0.11 ± 0.002 mg equivalents of ascorbic acid/ g dry weight of plant material than other plant extract materials as shown in Figure 6B. The highest total amount of flavonoid (0.065 ± 0.008 mg/ RUT/g DM) and phenolic (0.42 ± 0.003 mg/GAE/g DM) compounds were detected in methanolic extract of callus. The IC_{50} value of DPPH and FRAP activities of the methanolic extracts were recorded to be highest in calli (20.92 ± 0.073 µg/mL and 0.11 ± 0.002 mg equivalents of ascorbic acid/g dry weight, respectively) which is higher than reported in *Grewia carpinifolia* by Mahdi-Pour (2012). The GC-MS analysis has thus revealed various valuable bioactive compounds like fatty acid methyl esters, phenolics, flavonoids, terpenoids, alcohols and aromatic compounds that can be postulated for antioxidant activity. The identified secondary metabolites may be used in nutraceutical, herbal and pharmaceutical industries for drug development.

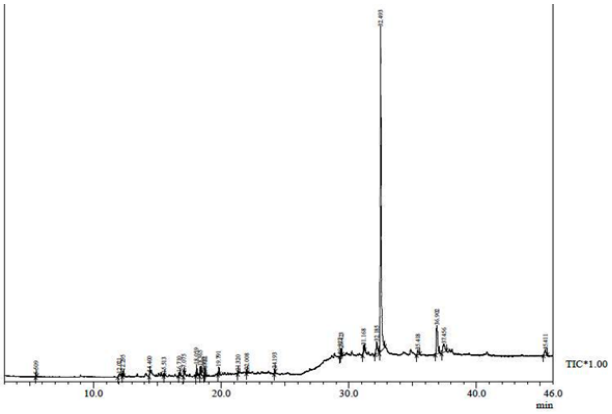

Figure 5: GC-MS Chromatogram of methanolic Stem extract of *Argemone mexicana*

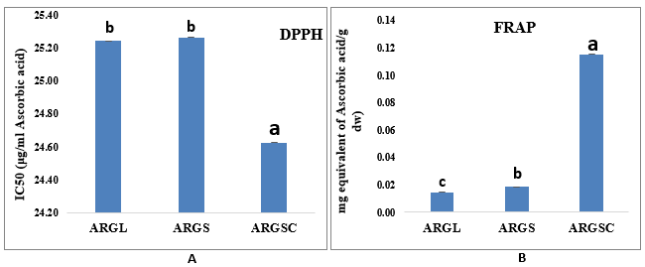

Figure 6: A. DPPH activity **B.** FRAP activity (significance level α = 0.05).

Conclusion

The present study demonstrated that *Argemone mexicana* contains adequate amounts of flavonoid and phenolic compounds, which can serve as natural sources of antioxidants, especially the callus. *A. mexicana* callus too has been found to be a rich source of bioactive and antioxidant compounds such as flavonoids, terpenoids, phenolics and tannins, which may be used for preparing novel functional products in pharmaceutical and food industries. The level of enzymes and antioxidant capacities were evaluated from *Argemone mexicana* plant (leaves and stem) and callus. GC-MS analysis of callus shows different valuable bioactive compounds such as 1, 3-propanediol, 2-(hydroxymethyl)-2-nitro-; Vitamin-E; Squalene, Stigmast-5-en-3-ol, (3.beta.)-; D:A-friedoolean-6-ene; Oxalic acid, monoamide; N-allyl-,dodecyl ester and 3A,4,5,5B,7A,12A,12 B,13,14,14A,14B-Dodecahydro.These bioactive compounds may be useful in the production of various valuable drug formulations. *Argemone mexicana* callus is found to have an unknown bioactive compound, which may be valuable after identification and clinical trial. Interestingly, leaves are supposed to have higher antioxidant potential, but in *Argemone mexicana* stem callus exhibited much higher antioxidant activity than reported by several researchers in highly accredited plant leaves. 3A,4,5,5B,7A,12A,12B,13, 14,14A,14B-dodecahydro has maximum area (38.20%) and unknown function, and one unknown compound found in callus, both compounds may be useful in new drug development.

References

Adnan M, Nazim M, Mostafa Kamal, Obyedul Kalam Azad, Arkajyoti, Shaikh BU, James W B, Mohammad OF, Cheol HP and Dong HC (2019) Investigation of the Biological Activities and Characterization of Bioactive Constituents of Ophiorrhiza rugosa var. prostrata (D. Don) &Monda Leaves through In Vivo, In Vitro, and In Silico Approaches. *Mol.* **24**:1367. doi: 10.3390/molecules24071367.

Ahn-Jarvis JH, Parihar A and Doseff AI (2019) Dietary flavonoids for immunoregulation and cancer: food design for targeting disease. *Antioxidants* (Basel) **8(7)**:202.

Alqahtani FY, Aleanizy FS, Mahmoud AZ, Farshori NN, Alfaraj R, Al-sheddi ES and Alsarra A (2019) Chemical composition and antimicrobial, antioxidant, and anti-inflammatory activities of *Lepidium sativum* seed oil. *Saudi J. Biol. Sci.* **26**:1089-1092. doi: https://doi.org/10.1016/j.sjbs.2018.05.007.

Amir H, Zakaria A and Nawaz K (2020) The Phytochemical Screening and Antioxidants Potential of *Schoenoplectus triqueter* L. Palla. Hind. *J. Chem*. 2020: Article ID 3865139, 8 pages https://doi.org/10.1155/2020/3865139

Arjun PJS, Murthy PN and Manik A (2010) Sharone Isolation and characterization of stigmast-5-en-3β-ol (β-sitosterol) from the leaves of *Hygrophila spinosa*. T. Anders. *Int. J. Phar. Sci. Res*. **1**(2):95-100.

Bizimenyera ES, Aderogba MA, Eloff JN and Swan GE 2007 Potential of neuroprotective antioxidant-based therapeutics from *peltophoruma africanum sond*. (fabaceae). *Afr. J. Trad. CAM*. **4(1):**99 – 106.

Bray HG and Thorpe WV (1954) Analysis of phenolic compounds of interest in metabolism methods. *Bio. Chem. Anal.* 1:27-52.

Charles et al (2012) Ethnobotanical and current ethnopharmacological aspects of *Argemone mexicana* linn: an overview. *Int. J. Pharm. Sci. Res*. **3(7)**:2143-2148.

Chen C, Wang L and Wang R (2018) Phenolic contents, cellular antioxidant activity and antiproliferative capacity of different varieties of oats. *Food Chem.* **239**: 260–267.

Cowan MM (1099) Plant Product as Antimicrobial Agents. *Clin. Micro.* **12**:564-582. doi:10.1128/CMR.12.4.564.

Downey PJ, Levine LH, Musgrave ME, McKeon-Bennett and Moane S (2013) Effect of hypergravity and phytohormones on isoflavonoid accumulation in soybean (*Glycine max*. L.) callus. *Micro. Sci. Tech*. **25(1)**: 9–15.

Edge R, McGarvey DJ and Truscott TJ (1997) The carotenoids as anti-oxidants-A review. *J. Photochem. Photobiol*. 41(3):189-200.

Fardiyah Q, Suprapto, Kurniawan FA, Ersam T, Slamet A and Suyanta (2020) Preliminary Phytochemical Screening and Fluorescence Characterization of Several Medicinal Plants Extract from East Java Indonesia. *Mat. Sci. Eng.* **833**: DOI:10.1088/1757-899X/833/1/012008. Corpus ID: 225559405

Farina M Preeti B and Neelam B (2014) Phytochemical evaluation, antimicrobial activity and determination of Bioactive Components from Leaves of *Aegle marmelos. Hind. Pub. Corp. Bio. Med. Res. Int.* 2014:497606.

Hema C, Mukesh M Tansukh B and Kanika S (2020) Plant tissue culture as a perpetual source for production of industrially important bioactive compounds. *Biotech. Rep*. **26**:e00450.

Hertog, Feskens and Kromhout (1993) Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet* 342 (8878):1007-11.

Kailas DD, Satish RP, Pratik PD, Madhavi NP, Dilip DH, Nilam JJ, Vinit ND and Ashwini L (2021) More Abstract Biogenic synthesis of gold nanoparticles using Argemone mexicana L. and their cytotoxic and genotoxic effects on human colon cancer cell line (HCT-15). *J. Gen. Eng. and Biotech.* 19(1):9.

Machado KD, Islam MT and Ali ES (2018) A systematic review on the neuroprotective perspectives of beta-caryophyllene. *Phyto. Res*. 32(12):2376-2388.

Masuoka N, Matsuda M and Kubo I (2012) Characterization of the antioxidant activity of flavonoids. *Food Chem.***131(2):**541–545.

Olamide E, Adebiyi FO, Olayemi Tan Ning-Hua, Zeng Guang-Zhi (2017) In vitro antioxidant activity, total phenolic and flavonoid contents of ethanol extract of stem and leaf of *Grewia carpinifolia. Beni-Suef Univ. Jour. Basic and Appl. Sci*. **6(1)**:10-142314-8535.

Ontiveros M, Rinaldi D, Marder M, Espelt MV, Mangialavori I, Vigil M, Rossi J and Ferreira-Gomes M (2019) Natural flavonoids inhibit the plasma membrane Ca2+-ATPase. *Biochem. Pharm.* **(166)**:1-11. DOI: 10.1016/j.bcp.2019.05.004

Pathak T, Goel A and Tripathi SC(2021) Medicinal property and ethnopharmacological activities of *Argemone mexicana*: An overview. *Annals R.S.C.B.***25 (3)**:1615-1641.

Pellegrini RR Proteggente A, Pannala A, Yang M and Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *J. Free Radical Bio. Med.* **26(9-10)**:1231-1237.

Polovnikova MG and Voskresenskaya OL (2008) Activities of antioxidant system components and polyphenol oxidase in ontogeny of lawn grasses under mega polis conditions. *Russ. J. Plant. Physl.* **55**: 699-705.

Shettima AY, Karumi Y, Sodipo OA, Usman H and Tijjani MA (2013) Gas chromatography–mass spectrometry (GC-MS) analysis of bioactive components of ethyl acetate root extract of *Guiera senegalensis J.F. Gmel. J. App. Phar. Sci.* 3(03):146-150. DOI: 10.7324/JAPS.2013.30328

Tauheeda T, Muhammad AA, Aziz UR, Tayyaba S, Muhammad A and Khalid M k (2012) Phytochemical screening, free radical scavenging, antioxidant activity and phenolic content of *Dodonaea viscose* Jacq. *J. Serb. Chem. Soc.* **77 (4)**:423–435.

Wafa AM, Sabrina AA, Shihab A, Aiman A, ahlah MNN and Khaldon (2016) The antioxidant activity and phytochemical screening of Yemeni *Argemone mexicana* Linn. *World J. Pharm. Med. Res*. **2(4)**:22-26.

Zhang Q, Yang W, Liu J, Liu H, Lv Z, Zhang C, Chen D and Jiao Z (2020) Identification of six flavonoids as novel cellular antioxidants and their structure-activity relationship. *Oxid. Med. Cell Longev*. **2020**:4150897.

Zhishen J, Mengcheng T and Jianming W (1999) The determination of flavonoid content in mulberry and their scavenging effects on superoxide radicals. *Food Chem*. 64(4):555-559.