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Argemone species: Potential source of biofuel and high-value biological active compounds

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ABSTRACT

The *Argemone* genus includes weed species of great importance in traditional medicine due to biological activities attributed to secondary metabolites, mainly alkaloids, distributed in all tissues of this species. In addition, their seeds contain a large amount of oil (30 to 40%). For this reason, several authors have discussed the potential of this species as a non-edible source to produce multi-purpose raw materials and a low cost-crop for example in the production of biofuels such as biodiesel. *Argemone* species grows in poor soils with low water and nutrient requirements. This makes the *Argemone* species an attractive economical and environmentally friendly candidate for biofuels production. Furthermore, the *Argemone* species can also provide high-value by-products for the agrochemical and pharmaceutical industry. In this work, we compiled the ethnomedical information, biochemical features, and biofuel production efforts that have been published by testing different *Argemone* species, in order to compare the research efforts and analyze its biotechnological potential. After analyzing the literature, we conclude that the genus has great potential for high-value pharmaceutical products and energy production purposes, and also to control plant pests. We also consider that other species of the genus may have also potential applications in this field.

Keywords: Alkaloids, *Argemone*, *Argemone* oil, Bioenergy, Biofuels

1. Introduction

Weeds are invasive plants adapted to hostile conditions, they can grow in disturbed areas, with low nutritional and moisture requirements. Nevertheless, they have had great importance in traditional medicine due to their high content of secondary metabolites [1].

Several reports point out that a large part of the components of pharmaceutical products are derived from weeds [2]. Papaveraceae is an ethnopharmacologically important family considered as a weed, with 44 genera and over 760 species. Among them, *Argemone* genus is represented mainly by *Argemone mexicana* and *Argemone ochroleuca*, commonly known as the Mexican prickly poppy, flowering thistle, "cardo santo" or "chicalote". Both species are native to Mexico and have been used for the treatment of several diseases around the world, including skin diseases (leprosy, warts), tumors, microbial infections, malaria, etc. [3-5].

Argemone species have economic potential due to their oil content in seeds (30 to 40%) and therapeutic potential. The biological activities reported can be attributed to the high content of alkaloids throughout the plant, mainly benzylisoquinoline alkaloids [4, 6-9]. Alkaloids present in *Argemone* species include protopine, berberine, sanguinarine, among many others [3, 10, 11].

On the other hand, weeds have been identified as a source of non-edible crops utilized for the production of multi-purposes

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raw materials with potential to produce energy [12-14].

In the last decade, several studies reported the use of *Argemone* species as a candidate for low-cost crop to produce energy, due to its capacity to grow in poor soils, rough terrain, irregular rainfall, without specific environment variables. Furthermore, they can also provide high-value by-products [15, 16], making economically feasible the production of biofuels under the bio-refinery concept [8, 15, 17].

Patents in energy areas report the use of transesterified oil of *A. mexicana* as an additive (WO 2011/145101 A1, 2011/0283605 A1).

The first report on energy production using *Argemone* oil was in 2010 by the group of Singh and Singh at Devi Ahilya University (India). They detailed a process to generate and extract esters and biogas from *A. mexicana* seeds [8, 17]. Nowadays, several methods for biodiesel production have been published, including those using other species such as *A. ochroleuca* [18].

Many reviews about *A. mexicana* have been published focus on its chemical composition and pharmaceutical potential. So far it has not been found comparative studies with other species or the energy potential of the genus.

This article synthesizes and compares the peer reviewed published information related to the exploitation of the different species of *Argemone* genus, with an energy and ethnobotanical approach. The aim of compile the published information of all species of the genus was to compare and analyze the potential of new multi-purpose species and the perspectives of their use in the biofuel industry.

2. *Argemone* Genus

The first species of *Argemone* reported in the literature was *A. mexicana*, described as *Papaver spinosum* by Caspar Bauhin in 1595 and illustrated by Gerard in 1597. It was known by other names up to 1694 when was registered with the current name by Tournefort [19].

Argemone has been considered a genus with taxonomic inconsistencies from its initial descriptions. The first revision of the genus was attributed to Sir David Prain in 1895, whereas Ownbey [19] published a biography of the genus restricted to North America and Western India in 1958. The species *A. grandiflora, A. platyceras, A. polyanthemos* and some others have been cultivated occasionally according to Ownbey, besides pointing out that *A. sanguinea, A. polyanthemos and A. aenea* are the best species for gardens.

2.1. *Taxonomy* [20]

Kingdom: Plantae
Subkingdom: Traqueobionta
Superdivision: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Magnoliidae
Order: Papaverales
Genus: *Argemone*

The genus *Argemone is* originating from America, currently with presence in different countries outside its natural range of distribution. Karnawat [21] mentions that the genus comprises about 30 species. According to Ownbey [19, 22], there are 23 species in North America, highly distributed throughout Mexico and Central America, with the exception of very high areas, generally around 2400 meters above sea level. There are 4 species in South America, distributed in Paraguay, Chile, Argentina, Uruguay, Bolivia, and Panama.

In the region of Saudi Arabia, the first record of the genus was published in 1974 by Migahid, later Hussein reported in 1983 *A. mexicana* in desert areas; furthermore, *A. ochroleuca* have also been reported in the same country (as cited by Moussa [16]).

2.2. Distribution

Argemone species are plants adapted to semi-warm, semi-dry and temperate climates from sea level up to 2750 meters above sea level [23], they are tolerant to low temperatures and droughts [24]. They grow in abandoned agricultural lands, in agricultural areas, or on roadsides, associated with arid zones and low deciduous forest [20]. The presence of some species has also been reported in deciduous and evergreen tropical forests, xerophytic scrub, spiny forest, pine, mixed pine-oak and juniper [23].

A. mexicana and *A. ochroleuca* are native to America and can share habitats in tropical and subtropical areas where they are distributed around the world [16]. They are distributed mainly in the southern zone of North America [1]. However, these species are not restricted to any of 23 environmental variables such as frequency of rainfall, altitude, or soil type [16]. They are able to grow in regions with extreme conditions such as limited rainfall, high temperatures, and land with high salt concentrations, e.g. Saudi Arabia where sodium content varies from 0.15 to 2.25 meq/L [7, 16, 25].

A. mexicana and *A. ochroleuca* have been introduced in some regions such as Australia, India and West Africa [3].

2.3. Description

The taxonomic keys for the identification of the different species of *Argemone* can be found in *Flora del Bajío y de Regiones Adyacentes* [26], *Flora de Veracruz* [27] or *Monograph of the genus Argemone for North America and the West Indies* [19]. Classical references are widely used in current works. Distinctive features of the genus are mentioned in this work.

The different species of the genus except for *A. grandiflora* have thorny stems, leaves and, capsules [28]. *A. ochroleuca* has a whitish-green, with intense glaucous color, meanwhile, *A. mexicana* also presents a slightly glaucous tone with a more intense green [21, 29].

The leaves are alternate with serrated margins that ending in a spine. The bud forms are variable, they could be lobed, elliptical, spherical, or obovate. According to Ownbey (as cited by Peña [22]) the number of sepals varies from 2 to 6, being usually 3, with a corniculate appendage at the apex, the shape of the horns of the sepals is useful to distinguish them.

The flowers of *Argemone* species are actinomorphic, with 6 or rarely 9 petals, in *A. ochroleuca* are 6 elliptical to obovate or obcuneiform, whereas petals of *A. albiflora*, *A. munita* and *A. gracilenta* are obovate or suborbicular [21, 28].

The color of the flower ranges from yellow to white, including lavender color. Flowers are hermaphrodites. The anthers are linear of two dehiscent cells, the pistils are composed of a stigma, a short style, the stamens in the different species are numerous,

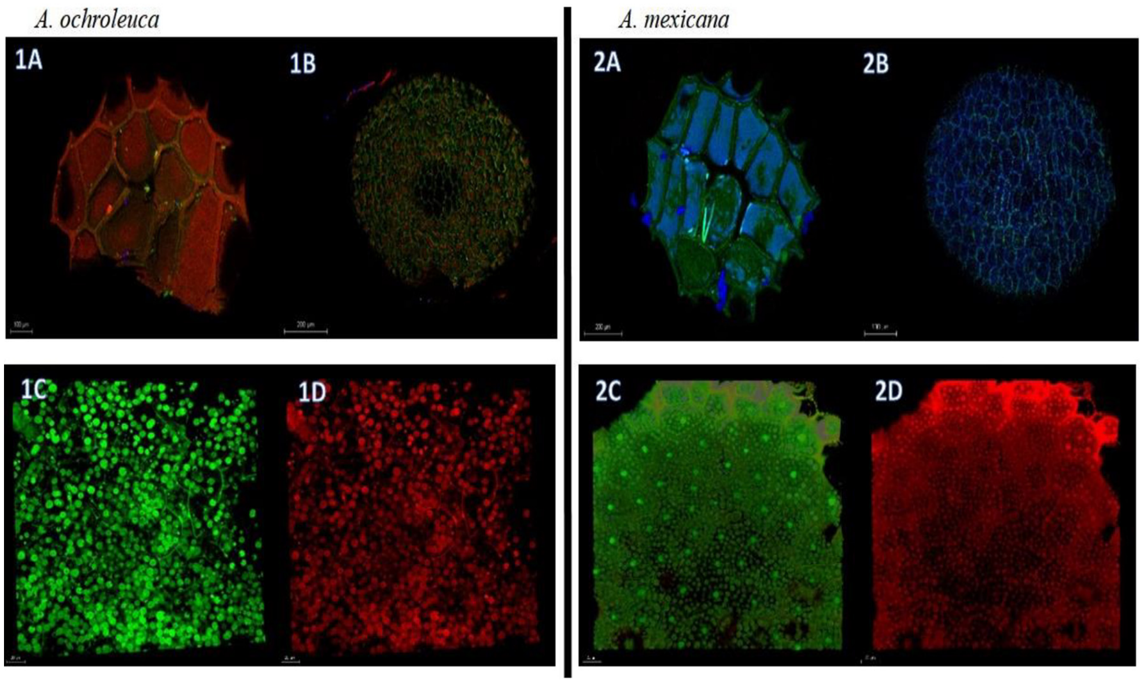

Fig. 1. Seeds of *A. mexicana* and *A. ochroleuca* in a fluorescence confocal microscope. Comparative using a laser with the same wavelength in both species *A. ochroleuca* (left) and *A mexicana* (right). With green, red and blue channels and merged images of the channels, showing autofluorescence in seeds due to the alkaloids composition. 1A and 2A seed testa surface (bars = 200 μm), 1B and 2B seed endosperm (bars = 200 μm), 1C, 1D, 2C and 2D (bars = 20 μm).

the number varies according to the species, Karnawat and Malik mention that *A. mexicana* has from 30 to 50 while *A. ochroleuca* from 20-75 [5, 22, 28, 30].

The different *Argemone* species produce a dry dehiscent fruit or capsules with 3-6 carpels and a great number of seeds. The capsules could show a narrowly elliptical-oblong, lanceolate, or ovate form [22, 28].

The seeds of *Argemone* are subspherical or slightly conical with a size between 1-2.5 mm. A slender peak often prominent is formed in the micropyle and the testa reticulate shows surface depressions [19, 28, 29]. Seeds cannot be visually differentiated, the measures of the depressions have not a taxonomic value because their color and size range is practically the same [21].

The roots of the *Argemone* species have a strong and slightly branched tap root. The primary root of *A. polyanthemos* can penetrate up to more than 60 cm deep, whereas other species develop lateral roots [5, 19].

Argemone seeds, present a composition of isoquinoline alkaloids, which exhibits properties of auto-fluorescence due to their molecular structure. The absorptions and emissions properties of sanguinarine are associated with the molecular structure acquired when dissolved in solution. The ionic form of sanguinarine as quaternary ammonium salt corresponds to a maximum emission of about 580 nm while the non-ionic form has a maximum peak at 450 nm [31].

In this work, we confirmed by examination using a fluorescence confocal microscope that the seeds from *A. mexicana* and *A. ochroleuca* show auto-fluorescence as shown in Fig. 1. This property can be applied as an important tool to evaluate the physiological state of the seed, as Yuan et al. [32] indicate.

The presence of autofluorescent molecules in different structures can be used also as a tool for morphological studies and to study the stress response to environmental stress, contributing to the assessment of the physiological state of the seeds or other tissues. This is possible since the chemical profile, e.g. alkaloid concentration changes with environmental factors such as exposure to light, soil fertility, soil moisture, among others [33].

The most widely studied species of the genus are *A. mexicana* and *A. ochroleuca* which are closely related because they present great morphological similarities which frequently lead to taxonomic confusions; but in fact, they differ in the shape of flowers, shape of bud, and petals color, showing bright yellow and pale yellow flowers respectively [21, 28, 29] as shown in Fig. 2.

A. ochroleuca Sweet was classified as a lower taxon of *A. mexicana* until 1903 (as cited by Ownbey [19]) when Rose [34] gave to *A. ochroleuca* a full specific rank. Some authors suggest that could have emerged as an autotetraploid of *A. mexicana,* because of its resemblance and having a degree of crosslinking [19, 21]. However, the molecular phylogenetic analysis revealed that these species have evolved independently [21].

3. Ethnobotanical Importance

Argemone species have been used in traditional medicine from ancient cultures. *A. mexicana* was described in the "De la Cruz Badiano" Codex, a compilation about the traditional use of the

Fig. 2. *A. ochroleuca* and *A. mexicana*. *A. ochroleuca* has a pale yellow color while *A. mexicana* has more intense colors.

medicinal plants by Aztecs [35]. According to the ethnobotanical interpretation of archaeobotanical and iconographic records, *A. ochroleuca* subsp. *stenopetala* was identified as a medicinal plant potentially used by Teotihuacan culture [36].

In Mexico, infusions of aerial parts of the plant are still used in the treatment of eye such as conjunctivitis, respiratory, dermatological, and oral infections, as well as for wounds. Some communities such as Tepotzotlán (State of Mexico), Ahuacatlán (Nayarit), among others also use these infusions because of their stimulant and hallucinogenic effects [37-39].

Other medicinal properties of *Argemone* species have been reported in different Latin American countries, such as analgesic use of *A. subfusiformis* in Argentina [19, 40], or in Bolivia, against cough and cold [41].

Moreover, *Argemone* species are also part of the traditional medicine of Saudi Arabia [42], and India (Tribe in Myeong area, Assam); where they have been used against diseases such as dropsy, jaundice, as well as eye and skin infections such as scabies and leprosy [3, 43].

3.1. Biological Activity

Several biological activities have been reported to *A. mexicana, A. ochroleuca, A. gracilenta, A. subfusiformis* and, *A. platyceras* such as antimicrobial, insecticidal, anti-inflammatory, cytotoxic, and anti-asthmatic [1, 3, 41, 44-47].

Since the biological activity could be modified by the extraction procedure, different raw tissues or extracts of *Argemone* species have been tested. Table 1 summarizes the plant part (leaves, stem, flowers, or whole plant), type of extract, and the biological model evaluated including rodents, insects (eggs, larvae, and adults), microorganisms (bacteria, fungi, and protozoal) as well as cancer cell lines.

As indicated in Table 1, crude extracts of different polarity (dichloromethane, ethyl acetate, acetone, ethanol, methanol, water, and hydroalcoholic mixture) of *Argemone* species have been tested on a variety of important pathogens such as the protozoan parasites *P. falciparum* and *T. cruzi,* which cause serious diseases such as malaria and Chagas; gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram-negative (*Escherichia coli* and *Vibrio cholerae*) bacteria, as well as yeast of *Candida* genus (*C. albicans, C. glabrata, C. krusei, and C. tropicalis*).

Insecticidal potential also has been evaluated in different species, for example against *Aedes aegypti*, responsible for the transmission of Dengue virus and yellow fever. In this species, Vidal et al. [57] report 100% mortality in larvae with 76.8 mg/L extract of *A. subfusiformis* after 12 h of exposure, with a lethal concentration LC50% of 6.24 mg/L after 48 h.

The ethanolic extracts of *A. ochroleuca* have an insecticidal effect on *Tribolium castaneum*. Bakhashwain and Alquiashi [54] report 78.9% mortality after 6 d of treatment (800 ppm). The ethanolic extract of this species also affects the feeding and larval

Table 1. Biological Activity Reported in *Argemone* Species

Species	Analyzed tissue	Potential Activity	Model	References
A. mexicana	Different parts, Rr	Antibacterial, wound healing, anti-inflammatory, antifeedant, cytotoxic, Nematicidal, anti-fertility, Antifungal Antifeedant	Rr	[1, 3, 4]
A. ochroleuca	Crude latex	Antifungal	*Candida albicans, C. glabrata C. Krusei, C. tropicalis and Drechslera halodes*	[11]
A. ochroleuca	Crude latex	Antibacterial	*Bacillus subtilis, Enterobacter aerogenes Micrococcus luteus, Escherichia Coli Staphylococcus aureus*	[48]
A. ochroleuca	Aerial parts (Methanolic Extract)	Antimicrobial Antibacterial Antifungal	*S. aureus, Vibrio cholerae, S. epidermis, B. subtilis, Cryptococcus neoformans and C. albicans*	[37]
A. ochroleuca	Branch, leaves and flowers (Dichloromethane extract)	Relaxant action mechanism	Guinea-pig trachealis muscle	[49]
A. ochroleuca	Latex	Insecticidal activity	Adults and eggs of *Culex sp.*	[50]
A. ochroleuca	Aerial parts (Methanolic extracts)	Antimicrobial	*Mycobacterium phlei, B.subtilis, Sarcina lutea, S. aureus*	[51]
A. ochroleuca	Aerial parts (Methanolic extracts)	Antiprotozoal	*Plasmodium falciparum GHA* and *Tripanosoma cruzi*	[52]
A. ochroleuca	Whole plant (Methanolic extracts)	Antifungal: Inhibition of spore germination	*Fusarium udum,* and *Helminthosporium*	[10]
A. ochroleuca	Stems, leaves and flowers (Ethanolic extracts, 15 and 30%)	Effect on larval growth and mortality	*Spodoptera frugiperda- 3rd instar larvae*	[53]
A. ochroleuca	Leaves (Ethanolic extracts, 800 ppm)	Repellency and insecticidal (in beetle)	*Tribolium castaneum*	[54]
A. ochroleuca	Aerial part (Ethanolic extracts)	Antimicrobial	*S. aureus* and *B. subtilis*	[47]
A. ochroleuca	Flowers (Decoction)	Antifungal: Inhibition of germination	*Aspergillus ochraceus*	[55]
A. gracilenta	Aerial part (Ethanolic extracts)	Antimicrobial	*S. aureus, B.subtilis, Enterococcus faecalis C. albicans*	[47]
A. gracilenta	Whole plant (Ethyl acetate fraction: alkaloids argemonine and berberine)	Anti-proliferative	Cancer cell lines (M12.C3F6 *murine B-cell lymphoma*, RAW 264.7 *macrophage, transformed by Abelson murine leukemia virus,* HeLa *Human cervix carcinoma*)	[46]

Table 1. Continued

Species	Analyzed tissue	Potential Activity	Model	References
A. subfusiformis	Leaves (Acetone extracts)	Antibacterial	*E. colli, Salmonella pooni and S. pyrogens*	[45]
A. subfusiformis	Leaves (Ethanolic extracts 200 mg/Kg)	Protective effect in gastric mucosa	Gastric mucosa of *Mus musculus*	[56]
A. subfusiformis	Leaves (Ethanolic extracts 76,8 mg/L)	Insecticidal	*Aedes aegypti* fourth instar larvae and pupae	[57]
A. subfusiformis	Aerial parts (Ethanolic extracts 70%)	Antiprotozoal	*P. falciparum*	[41]
A. platyceras	Leaves and flowers (Methanol extracts)	Anti-asthmatic	Tracheae of Guinea-pig	[44]
A. platyceras	Seeds (Hexane, methanol, ethyl acetate and acetone extracts)	Insecticidal	*Aedes aegypti*	[58]

Rr: Review reference for more detail

development of *Spodoptera frugiperda* [53]. The mechanism of action has not been elucidated, but authors suggest that could be due to nervous system toxicity or enzymatic inhibition [57].

Martinez-Tomás et al. [59] reported that the aqueous extract of *A. mexicana* (5%) has an effect on the whitefly (*Bemisia tabaci*), reducing the population by 97.6%. *Bemisia tabaci* is a plague feared due to its high degree of resistance to numerous insecticides and its tendency to transmit viruses [60].

A more detailed review of *A. mexicana* and its biological activities which have been widely published can be found in Sharanappa and Vidyasagar [1], Brahmachari et al. [3], or Rubio-Piña and Vázquez-flota [4].

It is also worthy of note the muscle relaxing potential of leaves and flower extracts of *Argemone* species (*A. mexicana, A. ochroleuca,* and *A. platyceras*), which could be used to obtain new drugs to treat the symptoms of asthma [61].

In addition, the crude latex of *A. ochroleuca* shows antifungal, antibacterial, and insecticidal activity [48, 62, 63].

Different authors have investigated the inhibitory effect of *A. mexicana* and *A. gracilenta* extracts on the growth of various cancer cell lines. Studies about the sanguinarine alkaloid located in roots and mature seeds of *A. mexicana* and *A. ochroleuca* have demonstrated the effect against some human cancer cell lines such as squamous cell carcinoma, pancreatic carcinoma, colorectal cancer, leukemia, bone cancer, bladder cancer, lung, among others [64-72].

3.2. Alkaloids of Argemone

The wide variety of biological activities in *Argemone* species can be attributed to the high content of benzylisoquinoline alkaloids (ABI) such as protopine, berberine, sanguinarine among many others distributed in the different tissues of the plant [3, 35]. ABI are derived from S-norcoclaurine (1-benzylisoquinolines backbone), which is produced by the condensation of 4-hydroxyphenylpyruvate and dopamine [73].

Some ABI produced by *Argemone* species have been individually evaluated to determine different biological activities such as antibacterial, antiviral, cytotoxic activity, among others [35, 74, 75].

The mechanism of sanguinarine-induced apoptosis involves the cell death signaling pathway and the ability of the molecule to intercalate in DNA, inhibiting replication. Sanguinarine is a planar molecule of cationic nature, which easily penetrates the membranes, by binding to proteins with negative charge. Its reactivity with the SH groups of proteins results in the inhibition of cytosolic and membrane enzymes, such as Na + K + ATPase [3, 76, 77].

Despite cytotoxicity and DNA damage, sanguinarine has a differential effect on normal and cancer cell proliferation, inducing apoptosis in the latter [64]. Results of toxicology evaluation of a sanguinarine and chelerythrine mixture in pigs suggest daily oral safe doses of up to 5 mg per kg [78]. An assay on reproductive toxicology of sanguinarine reports maternal oral toxicity of 60 mg/kg in rats and 25 mg/kg in rabbits, without selective effect on fertility or fetal and neonatal development [75].

In addition, recent research through molecular docking studies, make efforts to search for molecules that may be inhibitors of the SARS-CoV-2 virus that causes the current COVID-19 pandemic, some works have evaluated various alkaloids present in *Argemone* species [79-81].

Pandeya et al. [80] proposed that *A. mexicana* could be a candidate against the infection, by the inhibition potential of their alkaloids on the RNA-dependent RNA polymerase of SARS-CoV-2. The authors point out the potential of protopine and allocryptopine, with a binding energy of -6.07 kcal/mol and -5.75 kcal/mol, respectively.

On the other hands Agrawal et al. [81] reported the molecular coupling between the COVID-19 Protease enzyme ($3cl^{pro}/M^{pro}$ PDB ID: 6LU7) with sanguinarine and berberine, the authors mention that although sanguinarine has good binding energy (-7.7720 kcal/mol), the formation of any hydrogen bond with the viral enzyme is not achieved.

4. *Argemone* Oil

The seed oil content in *Argemone* species is between 30 to 40% [4, 6-9]. However, these values have been reported strictly to *A. mexicana* and *A. ochroleuca,* and in some cases the species is not defined.

Mariod et al. [29] reported that the seeds of *A. mexicana* have about 35% oil and 24% protein, and also contain small amounts of starch and free sugars. The oil is composed mainly of linoleic acid (54% - 61%) and oleic acid (21% - 33%).

According to Ahmed et al. [42] *A. mexicana* oil is composed of 90% triglycerides, 2.3 - 2.8% diglycerides and 1.5 - 1.8% monoglycerides. Where 92% are neutral lipids, 5.5 - 5.8% glycolipids and 1.5% - 1.7% phospholipids.

The composition analysis of fatty acids from *Argemone* spp. has been focused mainly on *A. mexicana,* besides *A. grandiflora, A. ochroleuca,* and *A. platyceras.*

All reports are in agreement with the presence of linoleic and oleic acids as the main fatty acids, with some variations in the degree of unsaturation, very similar to the oil composition of other vegetable sources, such as sunflower oil [9, 82].

Table 2 shows the fatty acid composition of seed oil reported to different *Argemone* species, where palmitic acid (7 - 14.7%), stearic acid (3.8 - 6.75%), linoleic acid (36.6 - 61.4%), oleic acid (18.5 - 40%) and ricinoleic acid (9.8 - 10%) are mainly present.

There are few reports about the composition of *A. ochroleuca* seed oil. Fatima et al. [18] point out that the main methyl esters of *A. ochroleuca* are: eicosanoate acid (arachidic), palmitate, linoleate, oleate, 9- octadecenoate as the principal of the unsaturated, and methyl decosanoate (behenate) of the saturated. On the other hand, Fletcher et al. [87] reported in *A. ochroleuca* the composition of methyl esters, including some non-specific compounds (18% methyl linoleate and stearate) and two unknown components (47 and 26%, respectively) by their HPLC method.

The table includes unpublished data generated by the authors of this work about the fatty acids composition of *A. ochroleuca* seed oil. The obtained profile agrees with previously published works, where mainly linoleic and oleic acid are present.

Table 2. Fatty Acids Composition in *Argemone* Oil

Species	Fatty acids composition %												FFA%	References
	16:0 Palmitic Acid	16:1 Palmitoleic Acid	20:0 Arachidic Acid	18:0 Stearic Acid	18:1 Ricinoleic Acid	18:1 Oleic Acid	18:2 Linoleic Acid	18:3 γ-linolenic Acid	14:0 Myristic Acid	22:0 Behenic Acid	14:0 Methyl myristate	12:0 Laurate		
A. mexicana	7	-	-	-	10	23	58	-	-	-	-	-	1.83	[83]
A. mexicana	14.7	1.3	0.3	6.75	-	40	36.6	0.3	0.1	0.2	-	-	-	[9]
A. mexicana	14.5	-	1	3.8	-	18.5	61.4	-	0.8	-	-	-	-	c[14]
A. mexicana	7.95	5.87	-	5.95	9.8	21.79	48.02	0.58	-	-	-	-	-	[84]
*A. mexicana***	12.2	0.32	0.15	4.42	-	28.6	53.28	0.16	-	-	-	-	-	[85]
*A. mexicana***l	3.85	1.1	-	32.5	-	15	47.4	-	0.1	-	0.1	0.06	-	[9]
A. mexicana	5.4	-	-	2.8	-	14	76.7	1.3	-	-	-	-	-	[86]
*A. ochroleuca***	5	*	-	3	-	*	21, 18*	-	-	-	-	-	-	[87]
*A. ochroleuca****	12.3	0.11	-	2.11	-	20.01	64.74	0.14	-	-	-	-	-	This work***
A. grandiflora	10.9	-	-	3.2	-	15.7	70.2	-	-	-	-	-	-	[86]
A. platyceras	4.1	-	-	1.4	-	8.5	84.9	1.1	-	-	-	-	-	[86]

* In addition to two components unspecified (47 and 26%); **Methyl form; ***Unpublished results

5. Energy Production

The use of biofuels is of great interest, given that it originates from renewable sources. Numerous investigations have shown environmental benefits of the use of biodiesel, considering it as a clean fuel, which contributes to the reduction of the emission of toxic gases [88].

However, there are limiting factors for its application, such as high production costs and competition with food source, for this reason, the use of alternative raw materials is important because more than 95% of the raw materials used for the production of biodiesel come from edible vegetable oils, having an impact on food security [89, 90].

Different works about the use of *Argemone* species as a raw material with energy potential have been published. The first study to generate and extract esters and biogas from *A. mexicana* seeds was made in 2010 by Singh and Singh [17].

Since then, published papers (from 2010 to 2020) about obtaining biofuels from *A. mexicana* seed oil have been carried out mainly in India, in at least 8 different research centers, and two works carried out in Korea and Pakistan. Those studies reflect the interest in the genus *Argemone* due to its energetic potential, however, they are mostly exploratory works.

Moreover, the use of *A. ochroleuca* seed oil was reported for the first time in 2017 by Fatima et al. [17] from Quaid-i-Azam University in Pakistan.

The biodiesel obtained showed similar physicochemical properties to conventional fossil fuel [91], such as viscosity, density, calorific value, cetane index, flash point, fire point, and cloud point. Those characterizations are important to understand the quality and stability of methyl esters generated.

In Table 3, physical and chemical properties of the methyl esters from *Argemone* seed oil produced in different research works are summarized. The table compiles the works related to the use of *Argemone* seed oil from 2010 to 2019, whether raw, esterified or in mixtures with conventional diesel. Some authors report data of mixtures at 10, 20, 30, 40, and 100%, of which only the data of the lowest and highest mix have been taken.

It can be seen that the majority of synthesized biodiesel with *Argemone* seed oil has a density between 860 - 870 kg/m^3, which decreases when it is blended with petroleum diesel, as reported by Singh and Singh [8] densities of 810 and 790 in mixtures of 50 and 25%, respectively.

The viscosity of esters is slightly greater compared to conventional diesel according to ASTM standard (3.5 - 5 cSt 40°C [9]), however, some papers report viscosity values lower than 5 cSt, such as Agarwall et al. (3.94 cSt) [86], Parida et al. (5.07 cSt) [92] and Anjum et al. (5 cSt) [93].

Other authors mentioned that biodiesel blends are an option to reduce these values, such as a 10% mixture by Singh et al. (3.9 cSt) [83], 24% by Ilag et al. (2.8 cSt) [94] or 20% by Parida et al. (4.1 cSt) [95]. The flash and the cloud point of esters are also high, and the biodiesel blends reduce them [8].

In addition to the production of biodiesel, the use of waste for biogas production has been reported by Singh et al. [17], who obtained 52% methane through the anaerobic digestion of the seed waste generated in a reactor. The caloric content of seed waste was 4,621 kcal/kg.

The vast majority of the reports about the synthesis of biodiesel were performed in *A. mexicana*, except the study of Fatima et al. [18] where a single step transesterification of *A. ochroleuca* oil was made with a yield of 91%.

On the other hand, Table 4 shows the conditions for obtaining the biodiesel generated in *Argemone* spp., the type of alcohol, the catalyst, the reaction conditions (temperature and time) as well as the yield obtained by different authors.

The biodiesel production from *A. mexicana* oil has been mainly carried out by two-step esterification/transesterification reaction due to the high level of acidity reported by the authors [8, 17], employing an acid catalyst in the first stage and alkaline catalyst in the second stage, with a yield above 90% and using methanol as synthetic alcohol; whereas Agarwal et al. [86] reported a microwave assisted esterification process with a 91% yield.

Singh et al. [17] have evaluated the efficacy of ethanol and methanol as synthetic alcohol, obtaining a higher yield with methanol and NaOH as a catalyst. Moreover, the most commonly used catalyst is sodium hydroxide (NaOH), followed by potassium hydroxide (KOH), and manganese carbonate ($MnCO_3$). The best reaction yield (100%) was obtained by Singh and Singh [8, 17] with an alcohol-oil ratio of 5:1 using methanol, NaOH, and 60 minutes of reaction at 55 - 60°C. For different reports, the ratio 5:1 was the most used.

6. Culture of *Argemone*

Argemone species are classified as perennial or annual [16, 26, 96]. The persistent primary root or the activation of axillary buds constitute and efficient perenization strategy in some plants [19].

According to Ownbey, in the year 1592 *A. mexicana* was introduced and cultivated in Europe for the first time, where it was popular in the gardens in the decade of 1830; whereas *A. ochroleuca* was introduced in 1790 and *A. grandiflora* in 1827 [30].

Argemone species are very versatile due to they can grow under different conditions, in poor soils, with different climatic conditions. For this reason, different plant growth stages can be found, even at the same time [16, 25]. It has been mentioned that these species bloom throughout summer until autumn. However, blooms of the plant can occur throughout the year. In western Mexico is more common to find developed plants in winter and at the beginning of spring [19, 30, 97].

Argemone species develop a deep primary root after germination, limited by the type of soil and moisture availability [98].

6.1. Requirements

The cultivation of *A. ochroleuca,* require well-drained and light soils, this species could be grown in acid, neutral and basic soils, but does not tolerate waterlogged soils. Moussa et al. [16] recorded the characteristics of the localities in Taif Governorate (Saudi Arabia) where grew naturally, they indicated slightly alkaline soils, with a lack of carbonates, high electrical conductivity (EC), and high content of Ca^{+2}, Cl^{-} and SO_4^{+2}.

A. ochroleuca were registered in sites with low phosphorus content, where it also grows well, in the case of *A. mexicana* has preference for sites with nitrogen deficiency [99, 100].

Table 3. Fuel Properties of *Argemone* Oil Biodiesel

Oil/ Methyl ester/ Blends Reference	*A. mexicana*																			*A. ochroleuca*
	100% ME [17]	Oil [17]	Oil [8]	100% ME [8]	75% ME [8]	50% ME [8]	25% ME [8]	10% ME [83]	100% ME [83]	100% ME [9]	100% ME [85]	RAO [14]	100% ME [15]	OIL 10% [6]	OIL 20% [6]	100% ME [93]	100% ME [92]	20% ME [95]	24% ME [94]	100% ME [18]
Density Kg/m^3 40°	870	910	910	870	830	810	790	824 (15°)	865 (15°)	860	875	904	888	809	816	870 15°	868 20°	843	-	860 (15°)
Viscosity cSt 40°	10.2 Ns	29.6 Ns	29.6	10.2	9.1	7.4	6.5	3.90	6.54	11.1	3.94	8.26 $x10^4$	5.18 $x10^4$	3.7	3.9	5	5.07	4.1	2.81	9
Calorific value (MJ/kg)	9.7 Kcal/kg	8.4 Kcal/kg	35.4	40.8	41.2	41.8	42.6	41.5	37.5	-	37.7	-	-	-	-	-	41.5	42.5	42	-
Flash point °C	170	235_a	235	170	80	75	68	69.7	193	171	156	-	-	34.2	41.3	-	170	81	76	72
Fire point °C	210	260	260	210	110	102	95	-	-	-	-	-	-	40.5	49.1	-	-	-	92	-
Cloud point °C	-	-	12	7	6	5	2	-	-	8	5	-2	-1	4	5	-	-	-	2	-7
Acid value mg KOH/g	Ns	-	76.2[a]	0.95	0.72	0.5	0.39	-	-	0.95	0.41	2.04	1.25	101.4	233.7	-	-	-	-	0.34
Saponification value mg KOH/ g	Ns	-	202.5[a]	166.4	149.6	71.1	8.8	-	-	167.5	-	-	-	134.6	168.2	-	-	-	-	-
Pour point °C	Ns	-12[a]	1	-1	-8	-14	-19	-	-	-14	+3	-	-	2	1	-	-	-	-1	-16
Cetane index	-	-	-	-	-	-	-	57.5	53	-	-	-	-	-	-	-	-	-	-	-
Peroxide value (meq/kg)	Ns	-	150[a]	130	110	60	30	-	-	131.4	-	-	-	-	-	-	-	-	-	-
Copper corrosion test	-	-	-	-	-	-	-	+	+	-	No.1	-	-	-	-	-	-	-	-	-
Carbon residue %	-	-	-	-	-	-	-	0.0019	0.010	-	-	-	-	0.2 gm	0.18 gm	-	-	-	-	-
Iodine value g/100g oil	-	-	-	-	-	-	-	-	-	-	-	190.3	134.6 NS	9.5 NS	16.5 NS	-	-	-	-	-
Smoke point mm	-	-	-	-	-	-	-	-	-	-	-	-	-	6	5	-	-	-	-	-
Lubricity μm	-	-	-	-	-	-	-	-	-	-	170	-	-	-	-	-	-	-	-	-
Oxidation stability (h)	-	-	-	-	-	-	-	-	-	-	6.03	-	-	-	-	-	-	-	-	-

a: Reported also in Rao 2012 NS: without units; ME: Methyl ester RAO: Refined *Argemone* oil TEO: Biodiesel transesterified of *Argemone* O10 : 10% oil *Argemone* +90% diesel, + = passed

Table 4. Processing Methods for Biodiesel in *Argemone* Oil

Species	Process	Alcohol	Catalyst	Oil/ Alcohol Radio	Reaction temperature	Yield %	References
A. mexicana	Two step	Methanol	NaOH	5:1	60 min 55-60°C	1,000 mL ester/L	[8]
A. mexicana	Two step	Methanol	NaOH	5:1	60 min 60°C	1,000 mL ester/L	[17]
A. mexicana	Two step	Ethanol	KOH	5:1	60 min 60°C	700 mL ester/L	[17]
A. mexicana	Microwave-assisted trans-esterification	Methanol 25%	KOH 1%	4:1	20 min at 280 W 420 rpm	91.6	[85]
A. mexicana	Two step	Methanol	$MnCO_3$	5:1	45 min < 75°C	90	[9]
A. mexicana	Two step	Methanol	NaOH	5:1	180 min 60°C 200 rpm 240 min, 50°C 250 rpm,	91	[15]
A. mexicana	Two-step	Methanol	Ns	6:1	120 min 60°C	94	[93]
A. mexicana	Two-step	Methanol	NaOH	5:1	180 min 60°C 200 rpm 240 min, 50°C 250 rpm	NS	[95]
A. ochroleuca	One Step	Methanol	NaOH	7:1	120 min 65°C 600 rpm	91	[18]

6.2. Reproduction

Argemone species reproduce sexually through cross-pollination, and their seeds are dispersed by surface water, agricultural machinery, or animals. Most of the seeds in species such as *A. ochroleuca* and *A. mexicana* do not germinate immediately after they are released, even until after several years, due to the seeds present morphological dormancy, most seeds germinate until the embryos are developed [98, 101].

The germination of *A. subfusiformis* seeds was observed 10 years after sowing. Several thermal shock treatments have been implemented to break the morphological dormancy in *Argemone* seeds, including high and low temperatures to break the latency of hypocotyl and epicotyl before germinating [101].

Karlsson et al. [101] evaluate different germination conditions by modifying temperature and light. Maximum germination (100%) was achieved only in treatments with gibberellic acid and with the elimination of part of the seed coat (scarification). The authors explain that the latency may be due to an inhibiting substance present in the seed.

Moussa et al. [16] reported that the germination of *A. ochroleuca* seeds occurs mainly in December. On the other hand, Serrano-Gamboa [102] indicates a germination rate of 21% after two weeks; whereas Xool-Tamayo [103, 104] obtained a higher seed germination percentage (60%) after 3 d with gibberellic acid treatment.

7. The Environmental Importance of *Argemone* spp

In some countries, *Argemone* species are considered invasive species that can affect the biodiversity by their allelopathic activity. Also, some species such as *A. mexicana*, due to their toxicity, are seen as very dangerous for livestock and humans for the risk of poisoning from accidental consumption [25, 98].

Despite this, not all species have the same toxicity, it has even been mentioned that some animals have fed on *Argemone* plants without any impact on their health. In Madagascar, the native lemur species were observed feeding on *Argemone* stems in a period of insufficient resources [5]. In Mexico, *A. ochroleuca* was mentioned in 3 sites in the region of the Valley of Mexico as a forage plant [105].

In addition, the multiple biological activities of *Argemone* species allow the use of its compounds for the biological control of other weeds or numerous pests, resulting in a positive environmental benefit. Abd-elgawad et al. [106] described the phytotoxic potential of the essential oil of *A. ochroleuca*, therefore, its use as a bioherbicide could be an environmentally friendly option that counteracts the negative environmental and human health impacts caused by the use and accumulation of synthetic agrochemicals.

On the other hand, the biofuels implementations that can be obtained from *Argemone* oil provide environmental benefits as discussed in previous sections. This can be further improved by the chemical profile of these species and their high-value by-products, besides the growing conditions of these plants, which have low water requirements and can take advantage of nutrient-poor soils, without competing with food crops generating the possibility to make barren lands productive and even to grow sustainable crops [8, 15, 18].

Table 5. Patents List of *Argemone* Genus

N. Patent and Publication info	Title	Abstract	Inventor	IPC
WO/2011/145101 11.24.2011	Detoxified and transesterified *Argemone* oil as bio-additive	*Argemone* oil detoxified and transesterified as bio-additive to petroleum diesel and process for preparation thereof	Das Premananda [IN]	A23D C11B C11C
WO2009147685 10.12.2009	A biofuel additive for diesel engines	Biofuel additive for diesel engines and a process for the preparation from the weed *A. mexicana*	Dey S and Sen R [IN]	C10L 1/02
US20070218108A1 20.09.2007	Lipolysis promoter and food-and-drink and feed	Food, drink and feed containing one or more plants that include *A. mexicana* and others	Suetake Yoko, Yoshida Keishiro And Susumo Shimura (JP)	A61K36
WO2003057133A3 17.07.2003	Herbal composition for treating various disorders including psoriasis, a process for preparation thereof and method for treatment of such disorders	Herbal composition (and preparation process) for psoriasis, containing extracts of the leaves and/or stem of *A. mexicana* plant, with pharmacological and immunological activities	Arora Kumar, Gupta Kumar, Narendar, Srivastava and Dinesh [IN]	A61K35
WO2006025068A1 09.03.2006	A purified arabinogalactan-protein (AGP) composition	Describes a purified Arabinogalactan-Protein (AGP) and derivative composition isolated from leaves or stems of *A. mexicana*	Arora Kumar, Srivastava Vandita and Walunj Sameer [IN]	C07K14 A61K
CN105943691 21.09.2016 [CN]	Traditional Chinese medicine preparation for treating keloid and preparation method thereof	Preparation of traditional Chinese medicinal mixture with several herbs including *A. mexicana* for the treatment of keloids	Zhao Feng Zhang Jianhua [CN]	A61K33
CN105250748 20.01.2016 [CN]	Traditional Chinese medicine for treating cervical cancer and preparation method	Preparation of traditional Chinese medicinal mixture with several herbs including *A. mexicana* for the treatment of cervical cancer	Tan Zhen Jiang Yongjun [CN]	A61K36
1178/CHE/201 28.08.2015 [IN]	The herbal formulation for the treatment of hair fall	The formulation for the treatment of hair loss that includes various herbs including *A. mexicana*	R Meena [IN]	A61K36
CN104984271 A 21.10.2015	Medicine composite for treating child adhesive intestinal obstruction and preparation method	Medicine compound and preparation method with *A. mexicana* for treating child adhesive intestinal obstruction	Du Guoqiang Yan Rui [CN]	A61K35A61P1
CN104352774 A 18.02.2015	A pharmaceutical composition for treating infectious pancreatic necrosis of rainbow trout and preparation method of pharmaceutical composition	Preparation of a pharmaceutical composition for treating infectious pancreatic necrosis of rainbow trout.	Chen Hengping, Huang He and Li Xin [CN]	A61K36A61P
WO 2013/043031 A1 28.03.2013	Pesticide made of isoquinoline alkaloids, flavonoids and vegetable and/or essential oils	Organic pesticide formulated with extracts from *A. mexicana* and *Cirsium ehrenbergii* for biological control (control of white insects, low toxicity for mammals and low persistence in the environment)	Hernandez Romero Yanet , Rodriguez Narvaez Cristina, Saavedra Aguilar Mario [MX]	A01N65 A01N2 A01N43

Table 5. Continued

N. Patent and Publication info	Title	Abstract	Inventor	IPC
JP3177642 B2 JPH0570360 A 23.03.1993	Androgenic hormone-resistant agent	An androgenic hormone-resistant agent contains the extract of herbs including *A. mexicana* for inhibition of sebum secretion, the treatment of acne, dandruff, itch and alopecia	Araki Keiko Hakamata Yusuke Hirayama Yutaka [JP]	A61K
IN161158B 10.10.1987 [IN]	An improved process for the isolation of sanguinarine and dihydrosanguinarine from the seeds of *A. mexicana*	Process for isolation of alkaloids sanguinarine and dihydrosanguinarine	Mahato Shashi Bhusan Sahu Niranjan Prasad [IN]	C07G5
WO2007089132A1 9.08.2007	An improved method for the manufacture of soap for hair treatment and active ingredients for shampoos, lotions, gels and creams and resulting soap	Treatment and active ingredients including *A. ochroleuca* extract for shampoos, lotions, gels and creams and soap	Francisco Alanis Cabrera and Mario Flores Vega [MX]	A61K
20060189512 24.08.2006	Compositions containing botanical extracts rich in phlorizin and methods for using such compositions in blood glucose modification and to affect aging	With phlorizin extract and a plant-based substance having hypoglycemic properties (such as *A. mexicana, A. ochroleuca, A. platyceras*) with influence on glucose and insulin	Ehrenkranz Joel R. L	A61K
WO/2012/145609 26.10.2012	Transoral methods and compositions for wrinkle reduction and cosmetic lip and facial augmentation	Cosmetic and therapeutic products for reducing wrinkle appearance and augmenting certain compartments of the facial integument of a mammal, especially a human	Bojanowski, Krzysztof Zhao Hui	A61K
2369/DEL/2004 19.02.2010 [IN]	Novel weed and industrial waste based biofertilizer	Biofertilizer comprised of invasive plant extracts (dry powders) including *A. mexicana*	Vinod Kumar, Kochhar Sunita and Kochhar Pushpangadan [IN]	C05F7
20161103114 03.03.2017 [IN]	Production of ayurvedic preparations from hers for treatment of liver cancer	Preparation made by 24 herbs extracts including *A. mexicana,* used for the treatment of liver cancer	Mr. Arun Kumar [IN]	A61K
245/KOL/2013 27.02.2015 [IN]	Herbal compositions for prevention or control of plant pests	Herbal compositions with pesticidal activity for prevention or control of plant pests which including *A. mexicana*	Prasad Vijay [IN]	A61K
WO 2000/076526 A1 21.12.2000	Method for the prevention and treatment of chronic venous insufficiency	Method for the prevention and treatment of varicose veins (hemorrhoids) by application of isoquinoline alkaloid. An improved method for isolating and purifying alkaloids (isoquinoline) from plants was included	Jia Qi, Qiu Zhihua Mahiou and Belaid	A61K
WO 2014/040632 A1 20.03.2014	Composition for the prevention and treatment of acute and recurrent urinary tract infections	Composition for the prevention and treatment of acute and recurrent urinary tract infections, a synergistic association of Arbutin and Berberine from plants such as *A. mexicana*	Sferra Daniela, Russo Valentina,Vergalito Franca	A61K

CN: China; IN: India; JP: Japan; MX: Mexico; IPC: International Patent Classification

8. Registered Intellectual Property Rights

The search of the state of intellectual property related to *Argemone* genus was performed using search engines of the World Intellectual Property Organization (WIPO-PATENTSCOPE), LENS, ESPACENET, and Google Patents.

For the patent search, the word "ARGEMONE" as a title, abstract or claims were used. For LENS, 123 results were obtained with these criteria, patents included in the jurisdiction of WIPO were selected, and according to LENS were 25 patents. The search was complemented with other search engines, selecting other patents of the local jurisdiction. The results are summarized in Table 5.

Most of the patents reported are included in the A61K classification, which refers to preparations for medical, dental, or toilet purposes. These patents protect mainly herbal formulations with different plants including some species of *Argemone (A. mexicana, A. ochroleuca,* and *A. platyceras*).

Most of these herbal preparations have pharmaceutical applications, such as hypoglycemic or lipolytic effect. As well as for the treatment of dermal diseases, cancer, and other cosmetics treatments such as hair loss.

The main countries with patents related to *Argemone* species are India, China, and the United States. *A. mexicana* is the main species described in these patents, *A. ochroleuca,* and *A. platyceras* are mentioned only in 2 patents (20060189512 and WO2007089132 A1).

Furthermore, only two patents were found in the energy sector, which refers to bioadditives prepared from *A. mexicana* oil to mix with diesel.

9. Conclusions

The genus *Argemone* includes several weed species of great importance due to the presence of benzylisoquinoline alkaloids produced in different tissues of the plant, which have important biological and physiological properties widely reported. Many of these alkaloids inhibit the growth of different microorganisms, which is promising for new drug development, such as antibiotics.

Due to the effect of extracts of different *Argemone* species against important pathogens or diseases such as malaria, or the cytotoxic activity in different cancer cells lines, they are a candidate for the isolation of new compounds that have not yet been elucidated and that could contribute to an advance in these health problems. Furthermore, the economic potential is evident for the manufacture of natural herbicides and pesticides that can provide protection against a variety of pests affecting commercial crops.

On the other hand, due to the high content of oil in the seeds, several authors have considered its potential use in the energy sector. Thus the integral potential use of the bioactive compounds and the oil of the seeds of the *Argemone* species could generate a strategy that contributes to improve the quality of life of small farm communities through the sustainable production and marketing of unconventional high valuable raw material for the chemical, pharmaceutical, and energy sectors. Furthermore, the low nutritional requirements or demand for water in the cultivation of these species make the *Argemone* crop an option with competitive advantages for the production of biofuels such as biodiesel.

The previous works reported, as well as the different patents that have been generated indicate the potential of the *Argemone* species, at the same time, it is clear that there is still a long way to go in improving the production of energy products, as well as in the investigation of new biological activities of species that have not yet been studied.

The potential of *Argemone* species also requires exploring more about their crop and management; in order to improve the yields generated in oil and secondary metabolites. Opening the possibilities for breeding programs for these species for various applications: ornamental, pharmaceutical, energy, or biological control purposes.

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Author Contributions

M.D.A.A. (Ph.D. student) developed the original idea and wrote the manuscript. DA.J. (Ph.D.) fully revised the manuscript. L.M.J. (Ph.D.) revised plant's phytochemical section. M.JC. (Ph.D.) revised oil and biodiesel section. G.M.A (Ph.D.) revised botanical and agronomy section. C.N.J. (Ph.D.) revised manuscript and did technical support in the area of microscopy.

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