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PHYTOCHEMICAL INVESTIGATION AND IN VITRO ANTIOXIDANT ACTIVITY OF *ARGEMONE MAXICANA* LINN

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ABSTRACT

The present study was aimed to investigate the phytochemicals and antioxidant potential of crude extract of *Argemone maxicana linn* The Phytochemical screening showed the presence of alkaloids, flavonoids, tepinoids, carbohydrates, saponins and tannins. The antioxidant property of the extract was assessed by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method.

KEYWORDS:phytochemicals antioxidant *Argemone maxicana* linn, 2, 2-diphenyl-1-picrylhydrazyl (DPPH).

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INTRODUCTION

The plant *Argemone maxicana* linn belongs to family Papaveraceae, commonly known as prickly poppy or maxican pop, is an indigenous herb. It grows wild and is troublesome weed it is an erect prickly annual herb with milky latex and .grows to 0.6m by 0.45m. It is glabrous, branching herb with yellow juice and showy yellow flowers. Leaves glaucous, oblong oblanceolate, pinnately lobed, 1/2-3/4 to midrib, both surfaces sparsely covered with prickles along veins, margins somewhat sinuate-dentate, the teeth tipped with a prickle, sessile, upper ones usually somewhat clasping the stem The plant contains alkaloids as berberine, protopine, sarguinarine, optisine, chelerytherine etc. It is traditionally used as analgesic antispasmodic, antitussive, demulcent, emetic, expectorant, hallucinogenic, purgative, sedative, skin, warts.[1] The plant is used as a medicine in several countries. In India and Mexico the seeds are considered as an antidote of snake venom. The fresh yellow latex contains protein dissolving substance effective in the treatment of warts sores and skin infections. It is also used as curin dropsy and jaundice. Previous studies have proved the antibacterial activity of *Argemone maxicana* linn[2]. The seed oil contains myristic, palmitic, oleic, and linoleic acids[3] The whole plant is analgesic, antispasmodic, possibly hallucinogenic and sedative and contains alkaloids similar to those in the opium poppy (*P. somniferum*) and so can be used as a mild pain-killer[4] The fresh yellow, milky acid sap was known to contain protein dissolving substances which can be used in the treatment of warts, cold sores, cutaneous affections, skin diseases, itches. The root was known to be alterative and can be used in the treatment of chronic skin diseases. They are expectorant and can be used in the treatment of coughs and other chest complaints. The seeds were known to be demulcent, emetic expectorant and laxative.[5] The methanol extract, the partially purified fraction, and the pure compounds isolated from A. Mexicana significantly and in a concentration-dependent manner reported to reduced the morphine withdrawal. Since the pure compounds were identified as protopine and allocryptopine, the observed effects could be related to these compounds [6] Living cells may generate free radicals and other reactive oxygen species by-products as a results of physiological and biochemical processes. Free radicals can cause oxidative damage to lipids, proteins and DNA, eventually leading to many chronic diseases, such as cancer, diabetes, aging, and other degenerative diseases in humans.[7] Plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity [8-9]

MATERIALS AND METHODS

Plant material: The whole plant of *Argemone maxicana* linn was collected from the local surroundings at Bhopal city of M.P, during the month of November to December. The plant was acknowledged by a senior Botanist Dr. Jagrati Tripathi Head of the Department of Biotechnology, Unique college College Bhopal

Preparation of extract: The dried powdered of *Argemone maxicana* linn (2kg) was successively Soxhlet extracted using Ether, ethyle acetate and methanol (60-80o), for 72 h. The extracts were dried under reduced pressure using a rotator evaporator to get the crude. A dark green semi solid mass was obtained. It was stored below 4ºC until further used. When needed, the extract was suspended/dissolved in desired solvent and used.

Phytochemical screening

The Pet ether, ethyl acetate and methanolic extracts were subjected to a preliminary phytochemical screening to identify the various phytoconstituents present in them i.e. Alkaloids, Terpinoids, Glycosides, Steroids, Triterpenoids,

Flavonoids, Carbohydrates, Saponins and Tannins

Test for carbohydrates
Molish test
Treat the test solution with few drops of alcoholic alpha-napthol. Add 0.2ml of con. Sulfuric acid slowly through the sides of the test tube, a purple to violet color ring appears at the junction.

Test for alkaloids
Mayer's test: Crude extract was mixed with Mayer's reagent (Potassium mercuric iodide solution) Cream color ppt. was formed alkaloids
Hager's Test: To the 2-3 ml of filtrate Hager's reagent was added. Yellow precipitate was formed showing the presence of alkaloids

Test for Terpinoids
Salkowski Test*:* To 2 ml. extract, 2 ml of chloroform and 2 ml of conc. H_2SO_4 was added. The solution was shaken well. A reddish brown coloration of the interference indicated the presence of terpinoids.

Teat for flavonoids*:*
Shinoda test: Crude extract was mixed with few fragments of magnesium ribbons and conc. hydrochloric acid was added drop wise. Pink scarlet color appears after few minutes, indicated the presence of flavonoids

Zinc hydrochloride test
To the test solution add a mixture of Zinc dust and conc. Hydrochloric acid. It gives red color after a few minutes**.**

Test for triterpenes: To the extract chloroform and conc. H_2SO_4 was added. Appearance of red colour indicated the presence of triterpenes

Test for tannins*.*
$FeCl_3$ Solution Test: On addition of 5% FeCl3 solution to the crude extract, deep blue black color appeared, indicated the presence of tannins

Test for saponins: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously. Persistent froth indicated the presence of saponins

Teat for Amino acids
Ninhydrin test To the 3ml of crude sample 3 drops 5%ninhydrin was mixed and heated for 10min in boiling water bath. Purple or bluish color indicated presence of amino acids.

DPPH radical-scavenging activity[10]
The method of Anna Floegel was used for the determination of scavenging activity of 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) free radical in the extract solution. A solution of 1mM DPPH in methanol was prepared as a control and 50µl of this solution was added to 2.95 ml of all the extract solutions prepared in different concentrations (100 to 200µg/ml). The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30min. The absorbance of the mixture was measured spectrophotometrically at 517nm. Lower absorbance of the reaction mixture indicates higher free radical-scavenging activity

Scavenging of hydrogen peroxide[11]
A solution of hydrogen peroxide (20mM) was prepared in phosphate buffer saline (PBS) (pH 7.4) various concentrations of the extract or standard in methanol(1ml) were added to (2 ml) of hydrogen peroxide solution in PBS. After 10 min, the absorbance was measured at 240nm

RESULTS

Table 1 represents the various phytochemicals present in different extracts. The petroleum ether extract contains terpinoids, saponins, carbohydrates, steroids and triterpinoids. The ethyl acetate extract contain alkaloids, flavonoids, carbohydrates. The methanol extract contains terpinoids and carbohydrates

Table 1
Preliminary phytochemical screening of crude extract of Argemone maxicana linn

S.No.	Tests	Observation for extracts		
		Pet. Ether	Ethyl acetate	Methanol
1	Test for carbohydrates			
	Fehling's Test	–	+.	+
2	Test for Alkaloid	–	–	+
	Wagner's test	–	–	+
3	Test for Flavonoids			
	Shinoda test	–	+	+
	Alkaline reagent test	–	+	–
4	Test for Terpenoids			
	Salkowski test	+	–	–
5	Test for Saponins	–	+	–
	Foam test		+	
	Test for proteins	–	+	+

+ present - absent

The ethyl extract of *Argemone maxicana* linn was subjected to dose dependent studies to calculate IC_{50} values. The ethyl acetate extract of *Argemone maxicana* Linn showed prominent IC_{50} value of 108.045μg/ml by DPPH method (table No. 2) and 1213 μg/ml by Hydrogen peroxide method (table No. 3). The standard ascorbic acid showed an IC_{50} value of 251.57 μg/ml μg/ml by DPPH method and IC_{50} value of 18.09 ug/ml μg/ml by the hydrogen peroxide method. Thus results showed that the ethyl acetate showed more potent antioxidant activity than standared ascorbic acid

TABLE 2
DPPH radical scavenging activity of ethyl acetate extract of Argemone maxicana

S.No	Concentration ((μg/ml))	% inhibition	% Inhibition of ascorbic acid
1	25	18.43	30.75746
2	50	24.53	37.49044
3	100	47.57	39.3267
4	150	69.47	43.07575
5	200	89.46	45.37108
6	IC_{50}l	108.045 ug/ml	251.57 μg/ml

TABLE 3
Hydrogen peroxide oxide radical scavenging activity of ethyl acetate extract of Argemone maxicana Linn

SNo	Concentration	% scavenging activity of extract	% Inhibition of ascorbic acid
1	100	7.36	51.94611
2	200	15.03	54.64072
3	400	24.41	57.48503
4	600	26.74	60.92814
5	800	35.27	67.96407
6	IC_{50}l	1213.58 ug/ml	18.09 ug/ml

DISCUSSION

Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention.[12-13] Phenols and steroids were also found responsible for anticancer activity.[14] Thus, this compound may serve as a potential source of bioactive compounds in the treatment of cancer. Flavonoids have been shown to exhibit their actions on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase.[15] This property may explain the mechanisms of antioxidative action of *Argemone maxicana* linn. They serve as health promoting compound as a results of its anion radicals. Also, the plant extract contains saponins, known to produce inhibitory effect on inflammation. [16] The capacity of ethyl acetate extract to scavenge was measured and the results were shown in (table 2&3). The antioxidants react with DPPH, a purple colored stable free radical, and convert it into a colorless α-α-diphenyl-α-picryl hydrazine. The amount of reduced DPPH could be quantified by measuring the decrease in absorbance at 517 nm.[17] Thus the extract showed potent DPPH radical scavenging activity (89.46%) at concentration of 200µg/ml when compared with standard ascorbic acid. The result of antioxidant activity indicates that the plant was potently active and the plant extract contains compounds that are capable of donating hydrogen to a free radical in order to remove an odd electron which is responsible for radical's reactivity.

CONCLUSION

The plant *Argemone maxicana* linn contains phytoconstituets like alkaloids, flavonoids, terpinoids, carbohydrates, saponins and tannins. On the basis of our results it is concluded that the ethanolic extract of *Argemone maxicana* linn has significant antioxidant activity. The antioxidants, which are formed in the human body due to exogenous and endogenous factors, are found to be responsible for many diseases. The phytochemical antioxidants have potent potential to neutralize free radicals or oxidants responsible for the cell damage. From the above antioxidant parameters assayed, *Argemone maxicana* linn was found to be better antioxidant in DPPH radical scavenging activity.

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