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Phytochemical Screening and Antioxidant Activity Evaluation of Papaver Somniferum L Seed Extract from Eastern Sudan

Khadiga G. Abd Elaleem[1], Badr Eldin A.E. Saeed[2], Hajer Alsiyed Ahamed[3]

[1]Department of Biology, Faculty of Science and Art, Alkhafji Hafr Elbatin University, Saudi Arabia

[1, 2, 3] Departments of Biology and Biotechnology, AL Neelain University / Faculty of Science and Technology, Khartoum/ Sudan

Abstract: ***In this study, petroleum ether, methanol and chloroform seeds extract of Papaver somniferum L were examined for yield percentage, phytochemical constitutes and antioxidant activities using DPPH assay. The extraction with methanol resulted in the highest yield percentage followed by chloroform, while lowest yield percentage was represented by petroleum ether. The extraction yields were found to be (39.0, 18.9, and 11.2) for the methanol, chloroform, and petroleum ether extracts, respectively. Phytochemical screening for seeds extracts indicated the presence of various secondary metabolites like alkaloids, flavonoids, tannins, phenols, saponins, terpenoids and steroids. The antioxidant activity of methanol extracts of the seeds was found to have strong reducing power as (76.17% and 74.7%) at concentration 1ml, 0.5 ml respectively. These results suggested that the methanolic extracts contain compounds that are capable of donating hydrogen to a free radical and remove odd electron which is responsible for radicals' reactivity, effects may be due to the abundant presence of phenolic compound in methanol extract.***

Keywords: Antioxidant activity, Extraction, Methanol, Phytochemical screening

1. Introduction

Oxidative stress in human body contributes to the pathogenesis of many human diseases. The elimination of free radicals by the intake of antioxidant is important to reduce the oxidative stress and hence for the prevention of chronic diseases [1]. Antioxidants have already been found in plant materials and supplements. Due to their natural origin, the antioxidants obtained from plants are of greater benefit in comparison to synthetic ones [2], [3]. The use of natural antioxidants from plants does not induce side effects, while synthetic antioxidants were found to have side effect [4], [5]. Therefore, the investigations of biological activity and chemical composition in plants as a potential source of natural antioxidants are numerous. Plants synthesize compounds as secondary products with biological activity, like antioxidant, which are mainly phenolic compounds and can use to avoid oxidative damage.

Antioxidants are usually added to foods to prevent the radical chain reactions of oxidation. They act through inhibiting the initiation and generation step leading to the termination of reaction and delay the oxidation process. However, synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxy toluene are restricted because of their potential for toxic and carcinogenic effects. Therefore, there has been a significant interest to find natural antioxidants to replace the synthetic compounds in food applications and a growing tendency in consumer preferences for natural antioxidants, all of which has given more impetus to explore the natural sources of antioxidants [6].

Papaver somniferum L. belongs to the Papaveraceae family, and is commonly known as “Opium poppy.” The plant is found wild in various parts of Europe, northern Africa, and western Asia [7]. It is traditionally used as an herbal medicine against coughing, bronchitis, sore throat, minor sleep problems, and possesses a sedative effect [8], [9].

Previous investigations on this plant have revealed its nutritional composition [10], content of alkaloids, [11] and ethnobotanical studies [9], [12] and [13]. However, no detailed reports are available on the antioxidant activity of *Papaver somniferum* L. seed from Sudan, the objectives of this study are; (1) to determine yield percentage and phytochemical screening of *Papaver somniferum* seed extracts, (2) to investigate the antioxidant activity of three extract using the Cecil-Elect Spectrophotometer, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging method.

2. Plant Material

2.1 Collection of plant materials

Aerial parts of *Papaver somniferum* were collected, during the flowering period in April 2016, from Beqaa region, eastern Sudan, about 1300 m above Red sea level.

2.2 Identification of plant materials

The plant was identified by a Taxonomist at the department of Biology, Faculty of Science and Technology, Al-Neelain University, Khartoum, Sudan.

2.3 Plant material preparation

P. somniferum seeds were washed with water, and then dried in shade for a week. The dried seeds were ground using mortar and pestle and then sieved to obtain e powder. The powdered sample was stored in a sealed sample container for the following experiments.

2.4 Extraction of Plant Materials

The plant materials were extracted separately using Petroleum ether, Chloroform and Methanol, thirty gm of dry P.somniferum seeds. The extraction was carried out by cold extraction method using (250 ml) of each solvent. The process was carried out for three days; the obtained extracts were evaporated by rotatory evaporator under reduced pressure at 600C to get a dried solid product, and then stored in dried bottles.

2.5 Phytochemical screening

Phytochemical screening was performed to assess the qualitative chemical compositions of the three different extracts using different standard methods with some modification.

2.5.1 Detection of alkaloids

To detect alkaloids Evan method was used [14]. Solvent free extract (50 mg) was stirred with few ml of dilute hydrochloric acid then filtered. The filtrate was tested carefully with various alkaloidal reagents as follows different methods of: Mayer´s reagents [14], Wagner´s reagents [15] and Dragendorff´s reagents [16]. Mixture was filtered and the filtrate was divided in to three equal portions. First portion was treated with a few drops of Mayer's reagent, the second portion treated with equal amounts of Wagner's, and the third portion treated with Dragendorff´s reagent. The samples were then observed for the presence of turbidity or precipitation. For first test tube, a white creamy precipitate indicated the present of alkaloid; few drops of Areddish brown precipitate confirmed the present of alkaloid. Dragendorff´s reagent was added to the third test tube, yellow precipitate indicated present of alkaloid.

2.5.2 Detection of saponins

Method used was adopted by Kokate [17]: About 50 mg of the seed extract was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. Formation of two layers of foam indicated the presence of saponins.

2.5.3 Detection of phenolic compounds

Ferric chloride test: Method of Mace was adopted [18]. The extract of 50 mg was dissolved in 5 ml of distilled water; few drops of neutral 5% ferric chloride solution were added to the extract. A dark green color indicated the presence of phenolic compounds.

2.5.4 Detection of tannins

For detection of tannin, two tests were used following Kokate method [19].

- **Ferric chloride test**: a few drops of 1% neutral ferric chloride solution were to added extracts. Formation of blackish blue color indicates the presence of tannins.
- **Gelatin test:** 1% solution of gelatin containing 10% sodium chloride was added to the extracts, formation of white precipitate indicates the presence of tannins.

2.5.5 Detection of flavonoids: For detection of flavonoid, the method of Peach and Tracey is adopted [20].

Lead acetate test: To different extract, a few drops of aqueous basic lead acetate solution were added. Formation of yellow precipitate indicates presence of flavonoids.

2.5.6 Detection of steroids and terpenoid

Detection of steroids and terpenoid was carried out following Harborne method [21].

Salkowski´s test: about 100 mg of dried extract were dissolved in 2 ml of chloroform, Sulphuric acid was carefully added to form lower layer. A reddish brown color at the interface indicates the presence of steroidal ring. For terpenoid 2 ml of chloroform and 1 ml of concentrated H_2SO_4 were added to 1 mg of extract. The observation of reddish brown color indicates the presence of terpenoid.

2.6 DPPH free radical scavenging activity

The antioxidant activity of the seed extracts and the standard (Ascorbic acid) were assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl1-2-picrylhydrazyl (DPPH) free radical activity by modified method of Braca et al [22]. The dilute working solutions of the test extracts were prepared in methanol, where petroleum ether extract of seed was prepared in dimethyl sulphoxide (DMSO). Ascorbic acid was used as standard. A solution of 0.004% DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately.

These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using Cecil-Elect Spectrophotometer. 1.0 ml Methanol with 1.0 ml DPPH solution was used as blank. The ability to scavenge DPPH radical was calculated using the following formula:
% inhibition of DPPH = [(A B)/ A]
A= Absorbance of the blank solution
B= Absorbance of the test solution.

3. Result

3.1 Extract Yield

The extraction was carried out with three different solvents, including petroleum ether, chloroform, and methanol, to obtain extracts from plant seed, used in phytochemical screening and antioxidant assay. The percent yield of crude extracts following the removal of solvent using a rotary evaporator, represent that extraction with methanol resulted in the highest yield percentage followed by chloroform, while lowest yield percentage was represented by petroleum ether.
The extraction yields percentage were found to be (39.0, 18.9, and 11.2) for the methanol, chloroform, and petroleum ether extracts, respectively (Table 1)

Methanol extract showed the highest yield (0.39 %). The high polarity of methanolic extract may be responsible for the relatively high yield, the result agrees with [23]. These extraction yields percentage indicated that the solvents used

for extract preparation from *Papaver somniferum* L. seed showed different capacities to extract compounds and probably different compositions of the extracts.

Table 1: Yields of *Papaver somniferum* L. seed extract

Solvent	*Yield (%w/w)*	*Color*
Petroleum ether	11.2	Yellow
Chloroform	18.9	yellow
Methanol	39.0	Yellow

Antioxidant activity

Antioxidant capacities of plant extracts are largely dependent on the extract composition [24]. The measuring of antioxidant activity in food or biological systems is dependent on so many factors, including the properties of substrates, the conditions of oxidation, type of oxidizable substrate, and heterogeneity and heterophasic nature of the system [25]. There is a great multiplicity of methods used for antioxidant testing, but there are no standardized methods. Hence, in the present work, the antioxidant properties of *Papaver somniferum* L. seeds extracts were measured using the DPPH free radical scavenging activity assay phytochemical content was also screened in order to correlate with the antioxidant potential.

3.2 DPPH free radical scavenging activity

The methanolic, chloroform, and petroleum ether extract of P. somniferum seeds was used for evaluation the antioxidant activity of seed extract using DPPH assay. Inhibition percentage of DPPH free radical scavenging activity of P. somniferum seeds extract was presented in Table (3).

The highest scavenging activity was obtained from the methanolic extract of plant seeds (76.17%). These results suggested that the methanolic extracts contain compounds that are capable of donating hydrogen to a free radical and remove odd electron which is responsible for radicals' reactivity, effects were contributed to the abundant presence of phenolics compound , this result in agree with [26], [27], [28], and [29].

Whereas the chloroform and petroleum ether seeds extract have no antioxidant activity. Similar responses were observed for free radical scavenging capacity of *Papaver somniferum* [30].

Table 3: Antioxidant activity of P. *somniferum* seeds extract

Solvents	*Extract Concentration*	*Reading*	*DPPH (%)*
Chloroform	1.0 ml	0.803	-0.25%
Chloroform	0.5 ml	0.854	-0.27%
Methanol	1.0 ml	0.152	76.17%
Methanol	0.5 ml	0.161	74.7%
Petroleum ether	1.0 ml	1.307	-104.8%
Petroleum ether	0.5 ml	1.326	-107.8%

3.3 Phytochemical screening

The results of the phytochemical screening showed that the crude Petroleum ether and chloroform extract contains Alkaloids, saponins, tannin, flavonoids, and Steroids and Terpenoid; whereas the methanol extraction contains Alkaloids, saponins, tannin, flavonoids, Steroids and Terpenoid and Phenolic compound (Table 2).

Table 2: Qualitative Phytochemical Analysis of *Papaver somniferum* seeds extract.

Test	*Reagent*	*Petroleum ether*	*chloroform*	*Methanol*	*Observation*
Alkaloids	Mayer's	+ + +	+	+ + +	White creamy precipitate
	Wagner's	+ + +	+ ++	+ +	Reddish-brown precipitate
	Dragendroff's	+ + +	+ + +	+ + +	Yellow precipitate
Saponins	H_2O	+ + +	+ + +	+	Two layers of foam
Tannins	$FeCl_3$	+ + +	+ +	++	Black precipitate
	Gelatin test	+ + +	+ +	++	Yellow Precipitate
Flavonoids	Lead acetate	+ +	+	++ +	Yellow precipitate
Phenolic compound	$FeCl_3$	-	-	++ +	Dark green color
Steroids and Terpenoid	Salkowski's	++ +	+ + +	+	Reddish-brown color

Key:
(-): Negative test
(+): Weak positive test.
(+ +): Positive test.
(+ + +): strong positive test

4. Conclusion

In the present study, yield percentage, phytochemical screening and antioxidant activity of, methanol, chloroform, and petroleum ether extract of Papaver somniferum L. seed was carried out. The highest yield percentage obtained by methanol extract, followed by chloroform, while lowest yield percentage was represented by petroleum ether.

Phytochemical screening showed that the crude Petroleum ether and chloroform extract contains Alkaloids, saponins, tannin, flavonoids, and Steroids and Terpenoid; while the methanol extraction contains Alkaloids, saponins, tannin, flavonoids, Steroids and Terpenoid and Phenolic compound. Methanol extract showed the highest inhibition DPPH scavenging activity, petroleum ether and chloroform extract showed no antioxidant activities. General phytochemical screening was carried and antioxidant potential of Papaver

rhoeas L. Seed extracts was carried in vivo, further scientific work may be focused on their content of individual phenolic compounds and their in vitro antioxidant activity.

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