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Preliminary qualitative phytochemical analysis of *Acacia nilotica* fruits collected from Majdool Town, Southern of Libya

Saleha Mohammed[1], *Ali Hawad[2], Mahtab Alam[2], Abdulgader Ali[2]
[1]Chemistry Department, Faculty of Science, Sebha University, Sebha, LIBYA.
[2]Department of Biochemistry, Faculty of Medicine, Sebha University, Sebha, LIBYA.
*Corresponding author: ali.hwad@sebhau.edu.ly

Abstract
Background: Plants have many phytochemicals with various bioactivities, including antioxidant, anti-inflammatory and anticancer. Studies have shown that extracts from natural products, such as fruits, have positive effects against cancer, compared with chemotherapy or recent hormonal therapy. The *A.nilotica* can provide nutrients and therapeutic ingredients to preclude, mitigate or treat many diseases or conditions.
The Aim: This study aimed to analyze phytochemicals of the extracts of fruits of *A.nilotica* collected from majdool town. **Material and Methods:** These fruits were powdered and extracted with different solvents as methanol, chloroform, petroleum ether and water. Then, all the fruits extracts were analyzed quantatively looking for their phytochemicals constituents whether primary or secondary metabolites.
Results: These experiment's results showed that the *A.Nilotica* fruits extracts have several different phytochemicals among which phenols, Alkaloids, Flavonoids, Tannins, Terpinoids, Cardiac glycosides, saponins and resins, and secondary metabolites and other primary phytochemicals that may have bioactivities. The methanol and the aqueous medium are more efficient in extracting secondary phytochemicals than the other solvents. **Conclusion:** Overall, these results reveal that the *A.Nilotica* fruits may have medical therapeutic values due to the types of phytochemicals detected.
Keywords: Acacia nilatica, flavonoids, phytochemicals, seeds, pods, tannins.

دراسة أولية للتحليل النوعي للمواد الكيميائية النباتية لثمار القرض (Acacia nilatica) التي جمعت من منطقة مجدول جنوب ليبيا

صالحة ابوالقاسم محمد [1]و *علي فرج هوّاد[2] و مهتاب العالم[2] و عبدالقادر صالح علي[2]
[1]قسم الكيمياء– كلية العلوم– جامعة سبها، ليبيا
[2]قسم الكيمياء الحيوية– كلية الطب– جامعة سبها، ليبيا
*للمراسلة: ali.hwad@sebhau.edu.ly

ملخص تحتوي النباتات على العديد من المواد الكيميائية النباتية، ذات الأنشطه الحيوية، واتي تشمل مضادات الاكسدة، مضادات الالتهاب، مضادات السرطان. ولقد أظهرت الدراسات أن مستخلصات المنتجات الطبيعية لها تأثيرات ايجابيية ضد السرطان مقارنة مع العلاج الكيميائي، والعلاج الهرموني. نبات القرض (Acacia nilotica) يزود بمواد مغذية، كما توجد به مركبات علاجية تحد، أو تسكن أو تعالج العديد من الأمراض أو الحالات. تهدف هذه الدراسة الى تحليل المواد الكيميائية النباتية في مستخلصات ثمار نبات القرض. جمعت الثمار وتم طحنها، واستخلصت المواد الكيميائية النباتية منها بأستخدام مذيبات عضويه مختلفة(الميثانول ،الكلورفورم، البترولیوم أثير) والماء. جميع المستخلصات أخضعت للتحليل الكيفي للكشف عن المواد الكيميائية النباتية سواء منها المواد الأيضية الأولية والثانوية. دلت نتائج هذه الدراسة على أن مستخلصات ثمار نبات القرض تحتوي على عدة مواد كيميائية نباتية مختلفه منها الفينولات والمركبات شبه القلويه والفلافنويدات والتانينات و التربينويدات والجلايكوزيدات القلبية والصابونينات و الراتينجات ومواد أيضية ثانوية وآخرى كيميائية نباتية أولية ربما يكون لها أنشطه حيويه. ولقد أظهرت هذه الدراسة أن الميثانول والماء كانا الأكثر كفاءة من حيث استخلاص المواد الكيميائية النباتية من كل من الكلورفورم و البترولیوم أثير. نستخلص من هذه الدراسة أن ثمار نبات القرض ربما تمتلك خصائص ذات قيمة علاجية بسبب وجود المواد الكيميائية النباتية في هذه الثمار.
الكلمات المفتاحية: نبات القرض ، الفلافونويد ، المواد الكيميائية النباتية ، والبذور ، الطلائع ، التانينات.

Introduction

Historically, Therapeutic plants have been recognized and used as the mean source for drug compounds, contributing greatly to human health and well being[1]. They have the capability to produce various chemical compounds which can be used to achieve important biological functions and to defend against attacks from predators such as insects, fungi, and herbivorous mammals. So far, at least 12,000 such compounds have been isolated; which are estimated to be less than 10%, such therapeutic plants exhibit various phytochemicals with different bioactivities; including antioxidant, anti-inflammatory and anticancer[2,3]. Recent studies have reported that

extracts from fruits, vegetables and medicinal herbs, have positive effects against cancer as compared with the chemotherapy or with the hormonal treatments[4,5]. The plant *Acacia nilotica* (*A.nilotica*) is a spiny tree; normally grow in dry climate places, widely distributed in tropical and sub-tropical countries. Recent studies have reported that *A. nilotica*'s powdered bark with little salt have been used for treating acute diarrhoea[6] and various other diseases such as colds, congestion, coughs, dysentery, fever, gallbladders, hemorrhages, leucorrhea, ophthalmia, sclerosis, smallpox and tuberculosis[7]. Furthermore, it has extensively been used for treating colds, bronchitis, and bleeding[8] because of its antibacterial bioactivity[9]. Approximately, 2000 of plants species are screened each year for anticancer property[10],. Among these species, *A.nilotica* can provide nutrients and therapeutic compounds[11]. Traditionally, the bark, leaves, pods, and flowers are used against cancer, cold, congestion, cough, diarrhea, dysentery, fever, hemorrhoid, opthalmia, sclerosis, smallpox, tuberculosis, leprosy, bleeding, leucoderma and menstrual problem[12]. Because of its availability, researchers have focused more on such plants, looking for effective and cheaper drugs. A recent review revealed that this plant extracts has different biological properties including; anti-bacterial, anti-malarial, antifungal, anti-diarrhea, anti-oxidant and anti-cancer[13]. This plant is widely distributed in Libya, especially in warm climate areas as the southern part, where it is traditionally used for treating different illnesses. However, during our intensive literature search), we did not come across any study concerning the phytochemical and bioactivity analysis of *A.nilotica*, at least for this plant growing in Majdool town, south of Libya. Most of the published works in Libya just focuses on its taxonomy. Therefore, we carried out this preliminary study to reveal the phytochemical constituents of the *Acacia nilotica* fruits.

Material and Methods: The *Acacia nilotica* fruits were collected from a tree growing in a local farm in majdool town, south of Libya. They were collected in December 2016. The climate of the region is marked by a long dry season for the most months of the year. Dry fruits were collected from the trees. The identification and the authentication was carried out in the Botany Department, Faculty of Science, Sebha University. They were, then washed thoroughly two times; with running tap water and, with sterile distilled water, to remove dust. The collected fruits were divided into three parts; the pods and the seeds, and whole fruits. Then, they were dried at room temperature**.** The samples were preserved in an air tight containers until used**[14].**

The dried parts and the whole fruits were grinded to fine powder and stored at 4°C until further use. forty grams of powder of each of the whole fruits and the parts were then extracted separately in 350 ml of each of mthanol, petroleum ether and chloroformby using Soxhlet method[15]. For the aqueous extract, samples (20 g) of the fine powder of whole fruits, pods and seeds of *A.nilotica* were macerated with 200 ml sterile distilled water in a flask for overnight. The macerate was first filtered through double-layered muslin cloth and then centrifuged at 4000 g for 30 minutes. Then supernatants were filtered using Whatmann No.1 filter paper this was achieved by maceration techniques according to the method described by Harbone **[16,17]**. All of the organic, aqueous extracts and phytochemical Qualitative analysis were achieved by using standard methods as described by many authors[11,18,19,20,21,22]. All the extracts subjected to phytochemical analysis. The detail procedures involved in the phytochemical screening are as described by Ushie and Adamu [23]**.**

Note: All the chemicals and reagents used in this study were of analytical grade and obtained from the chemical store of Sebha University.

Results

Table.1. Shows description of the primary metabolites of carbohydrates, amino acids, and proteins in fruits of *A.nolatica* obtained from whole fruits, pods, and seeds of different extracts. On one hand, the methanol extract of the whole fruits, pods, and seeds showed traces of reducing sugars. On the other hand, the chloroform extract of the fruits and the other fruit parts did not show any traces of reducing sugars. Interestingly, the petroleum ether extracts, showed traces of reducing sugars in the whole fruits and seeds extracts, but did not show any traces of reducing sugars in pod's extract in all of the three aqueous solution extracts. The combined reducing sugars were absent, in most solvent extracts.

Table.1. Primary metabolites in chloroform, petroleum, and aqueous eth of whole fruit, pods, and seeds of *A.nilatica*. P = pods, W.S = Whole Fruits, S = Seeds. + =Trace , ++ = Moderate , - = Absent

Phytochemical Constituents	*Qualitative Tests*	*Solvents*											
		methanol			*Chloroform*			*Petroleum ether*			*Aqueous*		
		P	*WS*	*S*	*P*	*WS*	*S*	*P*	*WS*	*S*	*P*	*WS*	*S*
carbohydrate	*Bendict's*	+	+	+	–	–	–	–	+	+	–	–	–
	Combined reducing sugars	–	+	–	–	–	–	–	–	+	–	–	–
	Free reducing sugar (Fehling's test)	–	++	+	–	+	+	–	+	+	–	+	+
	Ketones	+	–	–	–	–	–	–	–	–	–	–	–
	Molisch's	+	+	+	+	+	+	+	+	+	+	+	+
Amino acids	*Ninhydrin test*	+	–	–	–	–	–	–	–	–	–	–	–
Proteins	*Biuret test*	–	–	–	–	–	–	–	–	–	–	–	–

Fixed oil and fats	*Spot test*	+	–	–	++	+	+	++	+	++	–	–	–

Table.2. Shows different phytochemical constituents of whole fruits, pods, and seeds of *A.nilatica* tree. It is obvious that there is trace amount of alkaloids in all of the three fruit parts (whole fruits, seeds and pods) detected in each extract of methanol, chloroform and aqueous solvents. Alkaloids were detected in all of the plant's extracted parts, as shown by the three tests; Mayer's, Hager, and Wagner tests. However, using the petroleum ether for alkaloids extraction, we detected trace amount in the three parts of the *A.nilatic* fruits only by the Hager test, but not by Mayer's and Wagner tests. In aqueous solution extracts all the three tests showed no alkaloids. H_2SO_4 test for detecting flavonoids, showed trace amount in pods and moderate amount in the whole fruits and seeds extracted petroleum ether and both of whole seeds and seeds of aqueous solution extract. The ketone sugars were present in traces amount only in the methanol extracts of pods, and absent in all of the other solvents extracts. The Molisch's test showed traces amount of sugar in all of the solvent extracts. Extraction in petroleum ether, showed no flavonoids in all of the three studied parts of *A.nilatica*. In addition, the aqueous solution extract showed flavonoids in all of the three studied parts of *A.nilatica*. The resins were detected in trace amount in pods extracted in methanol and in moderate amounts in both the whole fruits and the seeds extracted in aqueous solution, but, absent in the other parts extracted in methanol. The resins also were absent in all extracted parts either in chloroform or in the petroleum ether extracts of all the fruit parts. The tannins was detected in trace and moderate amount in methanol and chloroform extracts of all fruit parts and in aqueous solution extract as shown in Table.2. But, was absent in petroleum ether extracted parts and pods. Steroids were absent in the whole seeds and seeds extracted in methanol and in pods extracted in aqueous solution. We detected it in trace amount in all other fruit parts extracts as shown in Table.2. The phenols was

Table.2. Primary metabolites in extracts of methanol, chloroform, petroleum ether, and aqueous of whole fruits, pods, and seeds of *A.nilatica*.

Phytochemical Constituent	Qualitative Tests	Solvents											
		methanol			chloroform			petroleum ether			Aqueous solution		
		P	WS	S	P	WS	S	P	WS	S	P	WS	S
Alkaloids	Mayer's test	+	+	+	+	+	+	–	–	–	+	+	+
	Hager test	+	+	+	+	+	+	+	+	+	+	+	+
	Wagner test	+	+	+	+	+	+	–	–	–	+	+	+
Flavonoids	H_2SO_4 test	++	++	+	+	+	-	–	–	–	++	++	+
Resins	Resins test	+	–	–	–	–	–	–	–	–	++	+	+
Tannins	$FeCl_3$ test	++	++	+	+	+	+	–	–	–	++	++	++
Steroids	Libermann test	+	–	–	+	+	+	+	+	+	–	+	+
Phenols	$FeCl_3$ test	++	++	+	+	+	+	–	–	–	++	++	+
Terpinoids	Salkowski test	–	+	+	+	+	+	+	+	+	–	++	++
Cardic glycosides	Killer kiliani test	–	+	–	–	–	–	–	–	–	–	++	+
Saponins	Foam test	+	++	–	–	–	–	–	–	–	+	++	-

P = Pods, W.S = Whole Fruits, S = Seeds.
+ = Trace, ++ = Moderate, - = Absent.

present in trace and moderate amount for methanol and Chloroform and aqueous solution extract. But, it was absent in petroleum ether extract. moderate amount in each of the whole fruits and in seeds extracted in methanol, and also in moderate amount in whole fruits extracted in aqueous solution, but only in trace amount in seeds, and absent in other extracted parts. seeds showed moderate amount in aqueous solution extracts. On other hand, the cardiac glycosides was detected in trace amount in pods extracted in methanol, and in moderate amount in each of whole fruits and seeds of aqueous solution extracts. But, it was absent in all other extracted parts in all of the solvents. The saponin was detected in moderate amount in each of the whole fruits and in pods extracted in methanol, and also in moderate amount in whole fruits extracted in aqueous solution, but only in trace amount in pods, and absent in other extracted parts.

Discussion: Plants produce diverse organic molecules، the great majority of which do not appear to participate directly in their growth and development. These compounds, traditionally referred to as secondary metabolites, often are differentially distributed among limited taxonomic groups within the plant kingdom[25]. Their function, for many of them remains unknown. However, their primary metabolites, in contrast, such as phytosterols، acyl lipids,

nucleotides, amino acids, and organic acids, are found in performing metabolic roles that are essential and usually evident[26]. In this primary study, our results present a number of primary and secondary metapolites consistent with several previous studies[27, 28,29]. Nowadays, medicinal plants are commonly used to extract a number of modern drugs. Such natural sources are used to cure various diseases worldwide. Plants have great diversity of bioactive compounds that make plants a prosperous source of different types of drugs[30]. The phytochemicals, detected in those plants as Tannins and polyphenols are previously reported to have anti-diabetic effects[31]. Furthermore, Saleem[32] reported that phenolic compounds of *A.nilotica* beans have strong anti-oxidant activity more than that of tocopherol. Plants anti-oxidants such as polyphenols and flavonoids are reported to have various biological properties as anticancer, anti-diabetic, anti-aging and prevents cardiovascular diseases[33]. Another recent study, demonstrated that presence of polyphenolic compounds like flavonoids have high level of anti-oxidant activity, which is based on their abilities to scavenge free radicals and active oxygen species[34]. In this work, we have used different solvents with different polarities, to extract different active compounds. The methanol and the aqueous solution extracts presented the highest activity compounds for all the anti-oxidant tested assays, and revealed that extracts obtained from polar solvents like methanol and aqueous solution have the maximum anti-oxidant activities in comparison with the others obtained from non-polar solvents, such observations were previously reported in classes of Lamiaceae[35]. Another study[36], speculate that evaluation of primary and secondary metabolites of plants would help to recognize variety of chemical compounds and would help also in their extraction, purification, and identification of the biological activity.

For instance, the detection of moderate amount of tannins and flavonoids in *A.nilotica* fruits including whole fruits, seeds, and pods would open the gate to take this research further a step ahead, since such compounds previously have been reported to have anti-oxidant, anti-cancer, and hepatoprotective activity[38]. Finally, we conclude that our overall results have revealed several phytochemicals constituents that are considered bioactive compounds, with therapeutic values. Furthermore, we suggest that more studies should be conducted to benefit from their biological activity for industrialized formulation either alone or in combination with other herbal extracts to prevent or treat various illnesses.

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