

***In vitro* Evaluation of *Acacia nilotica* Pods for its Antioxidant, Acetylcholinesterase Inhibitory Activities and Phytochemical Screening**

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Abstract:

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by a progressive decline of memory and cognition. In the current study we have evaluate the antioxidant and anti-cholinesterase activities and also phytochemical screening of Acacia nilotica pods. We used the methods of (Shimida et al., 1992) and (Dinis et al., 1994) to

determine the antioxidant activity by DPPH and iron chelating assays respectively, the method of (Ellmam *et al.*, 1961) was followed to evaluate the Acetylcholinesterase inhibitory potential. IC_{50} values of DPPH assay was found to be $(4.0 \pm 0.03, 3.4 \pm 0.11, 3.6 \pm 0.05, 10 \pm 0.16)$ for chloroform, ethyl acetate, butanol, and aqua's fractions, and the acetylcholinesterase inhibitory potential was found $(0.35 \pm 0.04, 1.97 \pm 0.03, 0.74 \pm 0.09, 5.40 \pm 0.11)$ for the same samples. The results of phytochemical screening showed that all fractions of studied plant contain tannins, saponins, flavonoids, terpenes, steroids anthraquinones, proteins, amino acids and carbohydrates. The results we obtained could make *Acacia nilotica* pods an ideal initial source for antioxidant and anti-cholinesterase leads.

Key words: *In vitro*, *Acacia nilotica*, Antioxidant, Acetylcholinesterase, phytochemical screening

Introduction:

Alzheimer's disease (AD) is a neurodegenerative disease affecting the brain and it is the most common cause of dementia, leading to deterioration in vital cognitive processes such as memory, understanding, and speech. Symptoms can also include unpleasant behavioral changes, such as anxiety (Barnes *et al.*, 2006). The etiology of AD is complex as it involves many factors, all of which contribute and produce damage to the cortical nervous system. Currently there is no cure for AD.

To date the most promising target for the symptomatic treatment and slowing of AD progression is Acetylcholinesterase (AChE) inhibition, the enzyme which catalyses the breakdown of Acetylcholine (ACh), levels of this neurotransmitter can be elevated and function improved (Wenk, 2006).

Generation of highly Reactive Oxygen Species (ROS) is an integral feature of normal cellular function like

mitochondrial respiratory chain, phagocytosis, arachidonic acid metabolism. Their production however, multiplies several folds during pathological conditions. The release of oxygen free radicals has also been reported during the recovery phases from many pathological noxious stimuli to the cerebral tissues (Halliwell and Gutteridge, 1989). Generated free radicals can start chain reactions that damage cells and it may lead to cancer. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols (Sies, 1997).

The chemical diversity of the compounds found in nature makes plant and marine materials important potential sources of new drugs. Novel lead compounds and stereospecific structures for the synthesis of existing drugs. The most commonly used natural sources are plants and microorganisms, both in land and marine (Gareth Thomas, 2007).

Acacia nilotica Lam (Mimosaceae) is medium sized tree which has an inspiring range of medicinal uses (Ali *et al.*, 2012).

In the present study we assessed the antioxidant and Acetylcholinesterase inhibitory activities of ethanolic extract and fractions of *Acacia nilotica* pods, Sudanese origin, phytochemical study is also conducted.

Experimental:

Plant material:

Acacia nilotica pods were purchased from a herbarium shop in Khartoum, Sudan. Identified and authenticated in Herbarium of Medicinal and Aromatic Plants Research Institute, National Center for Research. Khartoum, Sudan.

Extraction and fractionation:

Extraction was carried out according to the method described by (Harbone., 1984). The shade-dried pods of *Acacia nilotica* were collected, powdered and extracted with 80% ethanol at room temperature for 3 days. Solvent was evaporated under reduced pressure using rotary evaporator apparatus to obtain a crude extract, which was dissolved in dist. H₂O and partitioned between Chloroform, Ethyl acetate, and n-Butanol. The yield a percentage was calculated as follows:

$$\text{Percentage} = \frac{\text{Weight of extract}}{\text{weight of the plant sample}} \times 100$$

Qualitative phytochemical evaluation:

Phytochemical screening was conducted to determine the presence of natural products in the extract obtained from the *Acacia nilotica* pods using standard methods of (Trease and Evans, 1989; Odebiyi and Sofowora, 1978).

Biological evaluation:

1. Antioxidant assay:

The antioxidant activity has been carried out using two assays.

DPPH free radical scavenging activity:

The DPPH radical scavenging was determined according to the method of (Shimada *et.al.* 1992), with some modification. The test samples were allowed to react with 2,2-di-(4-tretoctylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37°C in 96-wells plate. The concentration of DPPH was kept at (300µM). The test sample was dissolved in DMSO while DPPH was prepared in ethanol. After incubation decrease in absorbance was measured at 517nm using multiplat reader spectrophotometer. Percentage of radical

scavenging activity of the sample was determined in comparison with a DMSO treated control. All tests were conducted triplicate.

Iron chelating activity assay:

The iron chelating ability was determined according to the modified method of (Dinis *et.al.* 1994). in which the Fe⁺² was monitored by measuring the formation of ferrous ion-ferrozine complex. The experiment was carried out in 96 micrometer plat. The plant extract was mixed with FeSO₄. And the reaction was initiated by adding 5mM ferrozine. The mixture was shaken, left at 25°C for 10 min. and finally the absorbance was measured at 562 nm, using multi-plate spectrophotometer. EDTA was used as positive control, and DMSO as control. All tests were done in triplicate.

2. Inhibition assay:

Ellmam's *et.al.* (1961) method was used to measure AchE activity In 96-wells plate a total assay volume of 200 µL. This assay mixture contained 20 µL of enzyme, 140 µL to phosphate buffer (pH=8) containing 0.5 mM of 5,5'-Dithiobis (2-nitro benzoic acid) and 20µL acetylthiocholine iodide. 10µL of the extract (5mg of the extract dissolved in 1ml ethanol) was added. Absorbance in 412nm was measured. The percentage by which extract inhibited the enzyme activity was calculated as following formula.

$$\text{Inhibition\%} = 100 - \left(\frac{\text{rate of change in the absorbance of test}}{\text{rate of change in the absorbance of control}} \right) \times 100$$

IC₅₀ evaluations:

For the IC₅₀ experiment determination of the initial concentration of each fraction was dilute by half to make serial

dilution. Each fraction has the following concentration (50 µg/ml, 25 µg/ml, 12.5 µg/ml, and 6.25 µg/ml).

Statistical analysis:

All data were presented as means ± S.D. Statistical analysis for all the assays results were done using Microsoft Excel program. Student t test was used to determine significant difference between control and plant extracts at level of P < 0.05.

Results:

Ethanol extract of *Acacia nilotica* (pods) was able to scavenge the DPPH with 93%, all fractions appeared less activity shown in (table 1), it also acts as an Acetylcholinesterase inhibitor with 73%, chloroform fraction showed the highest activity with 91%. (Table 1).

Table 1: Antioxidant and anti-Acetylcholinesterase activities of *A. nilotica* pods.

Sample name	Yield %	Antioxidant	Acetylcholinesterase		
		% of Inhibition	IC ₅₀	% of inhibition	IC ₅₀
Chloroform	0.62	85±0.02	4.0±0.03	91.0± 0.02	0.35±0.04
Ethylacetate	58.58	86±0.01	3.4±0.11	88.8± 0.07	1.97±0.03
Butanol	32.00	85±0.01	3.6±0.05	89.1± 0.05	0.74±0.09
Water	8.80	81±0.12	10±0.16	50.4± 0.13	5.40±0.11

Phytochemical screening revealed that tannins, saponins, flavonoids, terpenes, steroids, anthraquinones, proteins, amino acids, carbohydrates are present, while alkaloids were absent. (Table 2)

Table 2: Phytochemical test of *A. nilotica* pods extract and fractions.

Family of compounds	Type of test	Inference				
		crude	CHCl ₃	n-BuOH	EtOAc	H ₂ O
Carbohydrates	Molisch's	+ ve	+ ve	+ ve	+ ve	+ ve
	Fehling's	+ ve	+ ve	+ ve	+ ve	+ ve
	Barfoed's	+ ve	+ ve	+ ve	+ ve	+ ve

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Tannins	Ferric Chloride	+ ve	+ ve	+ ve	+ ve	+ ve
Saponins	Frothing	+ ve	- ve	+ ve	+ ve	+ ve
Flavonoids	Alkaline	+ ve	+ ve	+ ve	+ ve	+ ve
	Lead acetate	+ ve	+ ve	+ ve	+ ve	+ ve
Terpenoid	Salkowski's	+ ve	+ ve	+ ve	+ ve	+ ve
Steroid	Salkowski's	+ ve	+ ve	+ ve	+ ve	+ ve
Alkaloid	Mayer's	- ve	- ve	- ve	- ve	- ve
	Wagner's	- ve	- ve	- ve	- ve	- ve
Proteins	Biuret	+ ve	+ ve	+ ve	+ ve	+ ve
Amino acid	Ninhydrin	+ ve	- ve	- ve	- ve	+ ve
Anthraquinones	Borntrager's	+ ve	- ve	+ ve	+ ve	+ ve

+ve = positive result -ve = negative result

Discussion:

When the ethanolic extract of *Acacia nilotica* were tested for their antioxidant potential, it exhibited a potent activity with (93%) using DPPH assay, may be due presents of flavonoids (Rauha *et al.*, 2000), all fractions had less activity possible to be due distribution of the active materials in all fractions. but when the same extract was evaluated for its antioxidant properties through alternative iron chelating assay it was found to be inactive, may be to the mechanism by which this extract extracted its antioxidant property.

In addition to previously mentioned antioxidant activity, ethanolic extract of *Acacia nilotica* was able to inhibit the activity of Acetylcholinesterase by (73.2%), the activity increased in chloroform, ethylacetate and butanol solvents and decreased in the residue (water) shown in (table 1), may according to polarity of the active component.

Table 2 indicate the presence of pharmacologically useful classes of compounds (saponnins, tannins, and flavonoids) tested for. These secondary metabolites have been shown to have therapeutic activities in plants and function in a synergistic or antagonistic fashion for the treatment of diseases (Trease and Evans, 1996).

Saponins have expectorant action which is very useful in the management of upper respiratory tract inflammation; saponins present in plants are cardiotoxic in nature and are reported to have anti-diabetic and anti-fungal properties (Finar, 1989; Trease and Evans, 1989; Kamel, 1991). Tannins are reported to possess physiological astringent and haemostatic properties, which hasten wound healing and ameliorate inflamed mucus membrane and also inhibit the growth of microorganisms by precipitating microbial proteins and making nutritional proteins unavailable for them; they form irreversible complexes with proline rich proteins, resulting in the inhibition of the cell protein synthesis. They have important roles such as stable and potent anti-oxidants (Trease and Evans, 1983; Tyler et al., 1988; Awosika, 1991; Ogunleye and Ibitoye, 2003). They act as binders and for treatment of diarrhea and dysentery (Dharmananda, 2003). Plant phenolic compounds especially flavonoids are currently of growing interest owing to their supposed properties in promoting health (anti-oxidants) (Rauha *et al.*, 2000).

Conclusion:

It can therefore be concluded that *A. nilotica*, in addition to possessing antioxidant and also exhibit anti-AChE properties and thus could potentially provide novel leads for polypharmacological, multi-target approaches to the treatment of AD.

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