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Research Article

Phytochemistry and Anti-Acetyl Cholinesterase Activity of *Salix nigra*

*Wasim A, Mushtaq A, Nadia M

Department of Bio-Technology, Faculty of Biological Sciences, University of Science & Technology Bannu, Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Phytochemical costing is very significant for confirmation of various naturally occurring bioactive secondary metabolites in plants. The methanolic crude extract of *Salix Nigra* was evaluated for phytochemical screening which showed the presence of alkaloids, saponins, terpenoids, coumarins and betacyanins. Methanolic extract of leaves of Salix Nigra failed to inhibit acetylcholinesterase in TLC bioautographic detection. The rest of plant extracts, including methanolic extract of stem, hexane extract of both leaves and stems, were detected to have acetylcholinesterase inhibitory properties.

Key words: *Salix Nigra*, phytochemistry, Anti-acetyl cholinesterase

INTRODUCTION

Medicinal plants are the natural botanical source of medicines being manufactured by indigenous pharmaceutical companies in Pakistan. These are also the basic source of modern pharmaceutical market, although today it has become an entire medical world of synthetics, with higher prices a common man cannot manage to pay for. It is therefore the most suitable time to consider the development and organization of our medicinal plants industry to become independent in the provision of common indigenous natural drugs that are being used to treat diseases in most of our rural areas.

In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents. The plants as medicines are capitalized in Ayurvedic, Greek, Islamic, Chinese, and Allopathic (Western) systems of medicines [1]. Plants bring into being over 100,000 molecules[2] as part of their metabolism. Dependability on plants as the source of medicines is prevalent in developing countries where traditional medicine plays a major role in health care [3].

The acetylcholinesterase (AChE) is a biologically important enzyme that hydrolyzes acetylcholine (ACh), a neurotransmitter well studied to play role in the pathology of Alzheimer's disease [4]. One of the most important approaches for treatment of this disease involves the augmentation of acetylcholine level in brain using AChE inhibitors. Several kinds of AChEIs, such as dopepezil, galantamine and rivastigmine are existing for the symptomatic management of patients with mild-to moderate AD [5]. However, these compounds have been reported to have the problems associated with the gastrointestinal disturbances and bioavailability [6]. Several studies have revealed anti-cholinesterase activity of the plant extracts and drugs. Few reports have claimed that, a few herbal extracts can act on the central nervous system, thereby enhancing the learning and memory. One of the richest resources for new anticholinesterase drugs are natural products [7]. Interestingly, intake of polyphenols through diets rich fruits, vegetables and beverages such as red wine was stated to reduce incidence of certain age related neurological disorders including macular degeneration and dementia [8, 9]. The current project was design to study the phytochemistry and anti acetylcholinesterase activity of crude methanolic extract of *Salix Nigra.*

MATERIALS AND METHODS

Collection and identification of Plant Material: The whole plant including stem, leaves and roots was collected from Ghoriwala, District Bannu, KPK Pakistan. It was than properly identified by Dr. Faizan, Assistant professor, department of Botany, University of science & technology Bannu, KPK, Pakistan.

Sample preparation and extraction: The air-dried, grinded leaves (300 g) of *Salix Nigra* were socked in methanol for 72 hrs. The solution was filtered with using Whatman filter paper No 1. The filterate was subjected to extraction. The extraction process was repeated three times, the extracts combined and the solvent removed to yield 40 g of the crude methanolic extract. The methanol soluble extract (12 g) was triturated with hexane and filtered to give a hexane extract (6 g) and a left over methanolic extract (12 g). The similar procedure was done for the extraction of stems of *Salix Nigra*. 8 gram of methanolic extract of the stems was triturated with hexane and filtered to give a hexane extract (2.5 g) and methanolic extract (5.5 g). Each of the respective extracts was placed in bottle at 4°C until looked-for further experiment.

Preliminary Phytochemical analysis: Qualitative phytochemical analysis of the crude powder of *Salix Nigra*

*Author for correspondence

Table 1: Phytochemical screening of *Salix Nigra* methanolic crude extract

Type of Test	Result
Alkaloids	+
Tannins	-
Saponins	+
Terpenoids	+
Coumarins	+
Anthocyanin	-
Betacyanins	+
Flavonoids	-
Cardiac Glycosides	-
Emodine	-

was determined by using various methods. Tannins (200 mg plant material in 10 ml distilled water, filtered); a 2 ml filtrate + 2 ml FeCl3, blue-black precipitate indicated the presence of Tannins. Alkaloids (200 mg plant material in 10 ml methanol, filtered); a 2 ml filtrate + 1% HCl + steam, 1 ml filtrate + 6 drops of Mayor's reagents/Wagner's reagent/Dragendroff reagent, creamish precipitate/brownish-red precipitate/orange precipitate indicated the presence of respective alkaloids. Saponins (frothing test: 0.5 ml filtrate + 5 ml distilled water); frothing persistence indicated presence of saponins. Cardiac glycosides (Keller-Kiliani test: 2 ml filtrate + 1 ml glacial acetic acid + FeCl3 + conc. H_2SO4); green-blue color indicated the presence of cardiac glycosides. Terpenoids (Liebermann-Burchard reaction: 200 mg plant material in 10 ml chloroform, filtered); a 2 ml filtrate + 2 ml acetic anhydride + conc. H_2SO4. blue-green ring indicated the presence of terpenoids. Flavonoids (200 mg plant material in 10 ml ethanol, filtered); a 2 ml filtrate + conc. HCl + magnesium ribbon pink-tomato red color indicated the presence of flavonoids[10]. Coumarins **(**Exact 3 ml of 10% NaOH + 2ml of aqueous extract) Formation of yellow color indicates the presence of coumarin. Emodins **(**Exact 2ml of NH_4OH + 3ml of benzene was added to extract). Appearance of red color indicates the presence of emodins. Anthocyanin and Betacyanin (To 1g of plant extract, 1ml of 2N NaOH was added and heated for 5 minutes at 100^0C). Formation of bluish green color indicates the presence of anthocyanin and formation of yellow color indicates the presence of betacyanin.

Determination of acetylcholinesterase inhibition properties using TLC method: Anti-acetylcholinesterase activity was measured by using an adaption of the method described by Marston *et al*. (2002)[11]. Acetylcholinesterase (EC3.1.1.7) was dissolved in 150 ml of 0.05M Tris-hydrochloric acid buffer at pH 7.8. The stock solution was kept at 4°C.

5 mg of each hexane and methanolic extracts (both leaf and stems) was dissolved in 5 ml of chloroform and methanol, respectively (1000 ppm). Then the prepared samples were spotted on TLC plates. Migration of hexane fractions was conducted with hexane: ethyl acetate (4:1 v/v), while methanol fractions was eluted with varying dilutions using methanol and chloroform (6:4; 5:5; 4:6; 3:7; 2:8; v/v). The dried TLC plates were sprayed with AChE enzyme and incubated at 37°C for 20 minutes. For detection of the enzymes, solutions of 1-naphthyl acetate (250 mg) in ethanol (100 ml) and Fast Blue B salt (50 mg) in MiliQ water (20 ml) were prepared immediately before use. After incubation of TLC plates, 5 ml of naphthyl acetate solution and 20 ml of Fast Blue B salt solution were mixed and sprayed on the plates to give a purple coloration after 1-2 minutes. The inhibition of AChE was observed from the white spots on the purple colored dye background of the TLC plates.

RESULTS AND DISCUSSION

Antiacetylcholinesterase activity using TLC method: Phytochemical screening of *Salix Nigra* showed the presence of alkaloids, saponins, terpenoids, coumarins and betacyanins. Such phytochemical ingredients are ready to lend a hand in the preparation of natural drugs. The presence of secondary metabolites by phytochemical screening is shown in Table 1.

The AChE inhibition activity was done after the development of appropriate mobile phase solvent system for each extracts and plant parts. Both hexane extracts showed significant inhibition of AChE in mobile phase solvent system of hexane and ethyl acetate (hexane: ethyl acetate; 8:2; v/v).

The white spots were clearly seen on the purple colored dye background of the TLC plates. On the other hand, there was active white spot seen on the methanolic stems extract, while the methanolic leaves extract did not show any activity against AChE.

Various mobile phase solvent system (methanol: chloroform; 4:6; 5:5; 6:4; 7:3; 8:2; v/v) were used for the screening. This was due to the development of "tailing" effect (spot with comet-like tail) in the elution of the methanol fraction, and this tailing may affect the observations of white spot formations.

The overall results are shown in Table 2. AChE inhibitor is always the target of many Alzheimer & dementia drugs [12]. From the study, it was found that the hexane indicated higher anti-AChE activity than methanolic extract. Orhan *et al*. (2007)[13] observed that when polarity goes upward, the anticholinesterase effects of the plant extracts gradually decreased. This may be most likely due to anti-AChE activity of non-polar compounds found in high amounts within these extracts, which is in accordance with the possibility that the methanolic extracts, containing the polar compounds, exerted the less inhibitory activity. Houghton *et al*. (2006)[14] reviewed that terpenoids were acetylcholinesterase inhibitor, while there where a variety of studies identified the occurrence of different kind of terpenoids in *Salix Nigra* itself. This developed the understanding that the AChE inhibition properties of *Salix Nigra* extracts could be related to its terpenoids content.

CONCLUSIONS

The chemical analysis of *Salix Nigra* showed the presence of various secondary metabolites such as alkaloids, saponins, steroids, terpenoids, coumarins and betacyanins which are helpful in the synthesis of various drugs.

Table 2: AChE inhibition properties of *P. indica* extracts

Extract (1000 ppm)	Part tested	AChE inhibition
Hexane	Leaves	*
	Stem	*
Methanol	Leaves	Not Detected
	Stem	*
Hexane	Leaves	*

*Note: * indicated white spot*

With the exception of methanol leaves extract, all the extracts were able to inhibit AChE activity. Methanol stems extract, exhibited anti-AChE activity. From the findings of anti-AChE activity of methanol extracts of *Salix Nigra*, further studies need to be carried out to isolate and identify the bioactive components, especially from stems of *Salix Nigra.*

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