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Original article

Evaluation of antibacterial, antioxidant and nootropic activities of *Tiliacora racemosa* Colebr. leaves: *In vitro* and *in vivo* approach

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ABSTRACT

The antibacterial and antioxidant potential of *Tiliacora racemosa* leaf extracts in various solvents (methanolic, hexane, chloroform and ethyl acetate) was determined. Additionally, the presence of bisbenzylisoquinoline alkaloids in the plant prompted us to evaluate the nootropic activity of the methanolic extract in mice. Further, we seek to verify the nootropic effect by examining the anticholinesterase inhibition potential of the methanolic extract. The leaf extracts in various solvents were evaluated for their antibacterial and antioxidant activity by agar diffusion technique and α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging method, respectively. The *ex vivo* acetylcholine esterase inhibitory activity of the methanolic extract was carried out by Ellman's method in male Wistar rats. The nootropic capacity of the methanolic extract was examined in Swiss albino mice by utilizing the diazepam induced acute amnesic model. The chloroform/*n*-hexane and ethyl acetate fraction showed promising antioxidant and antibacterial (Gram positive and Gram negative bacteria) property, respectively. The methanolic extract was able to diminish the amnesic effect induced by diazepam (1 mg/kg i.p.) in mice. The extract also showed significant acetyl cholinesterase inhibition in rats. The findings prove that the memory enhancing capability is due to increased acetyl choline level at the nerve endings. The strong antioxidant nature and potential nootropic activity shown by the extract suggests its future usage in the treatment of neurodegenerative disorders such as dementia and Alzheimer.

1. Introduction

India is a vast repository of medicinal plants that are used in traditional medical treatments [1]. The rich source of Indian medicinal plants and its applications are well documented in indigenous systems as in Siddha, Ayurveda, Unani and Allopathy [2]. Plants have been used traditionally for centuries for the treatment of various disorders. They still play a vital role in disease prevention and treatment among majority of population in developing countries [3]. Plant-derived molecules are rich sources of diverse scaffolds that serve as the basis for rational drug design in medicinal chemistry [4]. It is a known fact that different plant parts exhibit wide-ranging therapeutic effect. Hence, any promising plant should be explored for their diverse medicinal properties [5].

Tiliacora racemosa Colebr. (Menispermaceae) is one such plant, which has triggered researcher's interest owing to its diverse biological activity [6–9]. It is locally known as Tiliacoru, Kelelata or Bhaglata. It grows wildly in Eastern India and is an evergreen climbing shrub. It is used by local tribal population (Santhals, Mundas, Lodhas, Bhumijs, Oraon and Kherias) of paschim medinipur, West Bengal, India for the treatment of topical skin infections, snake/insect bites and filariasis [7–10]. In Ayurvedic system of medicine it is known as krishnavetra which offers remedy to a number of diseases mainly the cancerous ones [11]. The roots, leaves and fruits of the climber are rich source of bisbenzylisoquinoline (BBI) alkaloids [12]. The BBI alkaloids isolated from the plants are tiliacorine, tiliacorinine, tiliamosine, tiliaresine, tiliarine, N-methyltiliamosine, tiliacosine and tiliasine [13,14]. These alkaloids exhibits nootropic, antimicrobial, antifungal, antineoplastic and hypotensive effects [15–17]. The roots of the

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plant have been reported to possess antimicrobial and antitumor activity. Additionally, the novel alkaloids isolated from the fruits of the shrub were found to be potent against few strains of bacteria [18]. However, the leaves of this plant or the novel alkaloids obtained from them were never explored for their biological activity potential. Furthermore, the BBI classes of alkaloids were reported to possess significant nootropics activity [19]. Plants containing these alkaloids were revealed to boost memory by enhancing neurogenesis in the hippocampus of brain [20]. Hence, they can be used to treat various cognitive disorders like Alzheimer's disease, dementia, etc. Additionally, several biologically important secondary metabolites such as anthraquinones, catechins, coumarins, flavonoids, phenols, quinones, saponins, steroids, sugar, glycosides, tannins and xanthoproteins have been detected from the leaves of a related species, *T.a cuminata* [21].

The aforestated research findings prompted us to evaluate the antimicrobial and antioxidant potential of leaf extracts in methanolic, chloroform, hexane and ethyl acetate solvents. Additionally, an acute toxicity study of the methanolic leaf extracts was carried out in mices. This is for the first time the nootropic property of *T. racemosa* was evaluated. The methanolic extracts were further examined for their nootropic potential by various *ex vivo* and *in vivo* pharmacological models. The results of the above studies are presented in this paper.

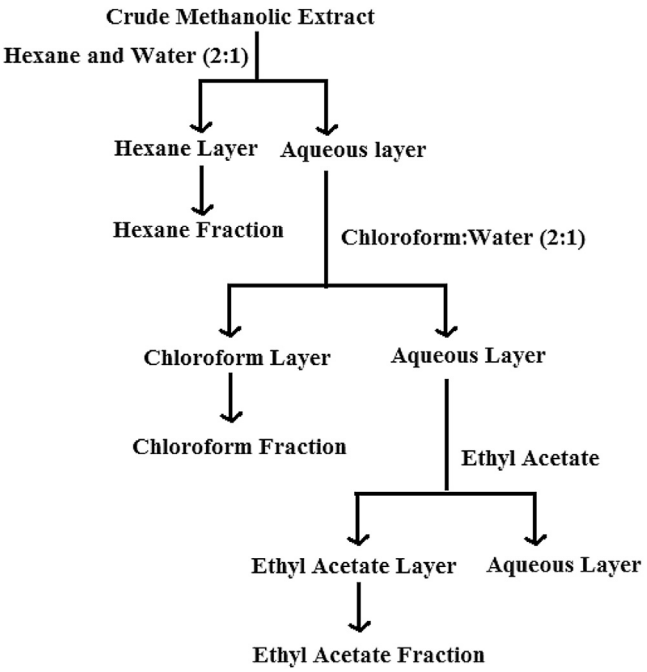

Fig. 1. General fractionation scheme for preparation of plant extracts.

2. Materials and methods

2.1. Plant materials

The fresh leaves of *Tiliacora racemosa* were collected during their vegetative stage in January to March 2015 from Jhargram, paschim Medinipur district of West Bengal, India. The plant was identified by Dr. K. Surekha, medical officer, Government Ayurvedic Hospital, Charminar, Hyderabad, India and a voucher specimen is preserved in our laboratories.

2.2. Preparation of extracts

Air-dried leaves (250 gm) were powdered and soxhleted with methanol (70–80 °C) for 6 h. The methanol was than evaporated under reduced pressure in a rotary vacuum evaporator. The methanolic extract was further suspended in 250 ml water and extracted with solvents of increasing polarity (*n*-hexane, chloroform and ethyl acetate). The solvent fractions were concentrated *in vacuo* and the extracts were preserved at 4 °C. The methanolic, *n*-hexane, chloroform and ethyl acetate extracts were obtained in 25%, 15%, 10% and 5% yield, respectively. The scheme for fractionation in different solvents is depicted in Fig. 1. All chemicals used were of analytical grade.

2.3. Phytochemical characterization of the extracts

A concentration of 20 mg/ml was prepared for each solvent fraction. The qualitative tests for flavonoids, alkaloids, tannins, saponins, steroids, terpenoids, glycosides, anthraquinones and phenols were performed according to a previously described procedure [22]

2.4. Antibacterial activity

2.4.1. Bacterial strains

The microorganisms were procured from Microbial Type Culture Collection (MTCC, IMTECH, Chandigarh, India). The plant extracts were evaluated for *in vitro* antibacterial against *Proteus mirabilis* 1429, *Pseudomonas aeruginosa* 424, *Salmonella enteric typhim* 98, *Bacillus subtilis* 121, *Staphylococcus aureus* 96, *Klebsiella pneumoniae* 109, *Bacillus cereus* 430, *Shigella flexneri* 1457 and *Escherichia coli* 443. The strains were maintained on slants of prescribed growth medium, and subcultured two days prior to the assays to prevent morphological and metabolic transformations.

2.4.2. Agar well diffusion method

The agar well diffusion method was used for evaluating the antibacterial potential of the extracts [23]. 0.1 ml of diluted inoculums of test organism was spread on Muller-Hinton Agar plates. Wells of 9 mm diameter were punched into the agar medium and filled with 50 μl of plant extract of 10 mg/ml concentration (500 μg/well, in DMSO solvent). The petri dishes were pre-incubated at 8 °C for 30 min to allow the complete diffusion of the samples and then incubated at 37 ± 1 °C for 24 h. For each bacterial strain pure solvents were used as controls. The antibacterial activity was determined by measuring the diameter of inhibited zone. The experiment was repeated thrice and the mean values were calculated. Chloramphenicol and streptomycin were used as reference standards. The antibacterial activity was calculated by measuring the inhibition zone diameter (mm) and was evaluated according to the following parameters: inhibition zone less than 9 mm, inactive; 9–12 mm, less active; 13–18 mm, active; 18 mm, very active.

2.5. DPPH free radical scavenging assay

The antioxidant potential of the extracts was evaluated by α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging method [24]. The different concentrations of the extracts were dissolved in methanol and added to 1 ml of DPPH (stock solution: 2.9 mg in 25 ml methanol). The solution mixture was further incubated at room temperature for 15 min. This was followed by measuring the absorbance of the solution at 517 nm in a uv visible spectrophotometer. A blank was carried out by taking methanol as the solvent. Quercetin (5–20 mg/ml) was used as the standard reference drug. The percent free radical scavenging capacities