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CASE REPORT

Antibacterial, Preliminary Phytochemical and Pharmacognostical Screening on the Leaves of *Vicoa indica* (L.)DC

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ABSTRACT

The aim of the present research was focused on the antibacterial, preliminary phytochemical and pharmacognostical properties of *Vicoa indica* (L.)DC. via in vitro approach. The aqueous and organic solvent (hexane, chloroform and methanol) extracts from the leaves of *Vicoa indica* (Asteraceae) were tested against *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Vibrio parahaemolyticus, Vibrio cholerae, Bacillus subtilis* and *Streptococcus pneumoniae* by agar cup plate method. The results showed promising antibacterial activity against the bacteria tested. Among these, hexane and aqueous extracts were found to have a more potent inhibitory effect comparing with the other extracts. Hexane and aqueous extracts showed several degrees of antibacterial properties against *E. coli, K. pneumoniae* and *S. typhi*. The chloroform extract expresses the maximum zone of inhibition against *K. pneumoniae, E. coli, S. pneumoniae, S. typhi* and *B. subtilis*; showing effectiveness of this extract for the treatment of infectious diseases. The methanol extracts exhibit excellent activity against *E. coli, K. pneumoniae* and *Vibrio parahaemolyticus*, which prove the potentiality of the plant extract for the treatment of various infections. This study includes preliminary phytochemical and pharmacognostic investigations on the taxon.

Keywords: *Vicoa indica, Phytochemical, Pharmacognostical, Antibacterial, Pathogens*

Plants have been used as folk remedies. For centuries, the ethno-botanical literature has described the usage of plant extracts, infusions and powders for diseases now known to be of viral origin. The ethnopharmacology provides an alternative approach for the discovery of antimicrobial agents, namely the study of medicinal plants with a history of traditional use as a potential source of substances with significant pharmacological and biological activities [1]. Herbal preparations are more frequently used to prevent and treat several diseases in world. In developing countries, the World Health Organization (WHO) estimates that about 80% of the population relies on plant based preparations used in their traditional medicinal system and as the basic needs for human primary health care [2]. In recent years, there is a need to study the plants having different values in their medicinal properties. Therefore, several medicinal plants have been evaluated for possible antimicrobial activity and potential cure from a variety of ailments especially of microbial origin [3, 4].

The traditional methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries too and moreover the use of herbal remedies has increased in the developed countries in the last decades. In this connection, plants continue to be a rich source of therapeutic agents. The remarkable contribution of plants to the drug industry was possible because of the large number of phytochemical and biological studies all over the world [5]. The Indian subcontinent is endowed with rich and diverse local health tradition, which is equally matched with rich and diverse plant genetic source. A detailed investigation and documentation of plants used in local health traditions and ethno-pharmacological evaluation to verify their efficacy and safety can lead to the development of invaluable herbal drugs or isolation of compounds of therapeutic value [6, 7].

Table 1. Preliminary Phytochemical screening of various extracts on the leaves of *Vicoa indica*

Constituents	Aqueous	Hexane	Chloroform	Methanol
Alkaloids	±	++	++	+++
Aminoacids	±	+	±	++
Anthroquinone glycosides	0	0	0	0
Coumarins	0	0	+	±
Flavones	0	0	0	±
Oils	0	0	0	0
Phenolic groups	±	++	++	++
Quinones	0	±	±	±
Saponins	0	0	0	±
Steroids	0	+	±	++
Sugars	++	0	0	+++
Tannins	0	0	0	±
Triterpenes	0	+	±	++

+ = Present, ++ = abundant, +++ = very abundant, ± = traces, 0 = absent

Vicoa indica DC. (= *Pentanema indicum* (L.) Y. Ling) is an herbal plant belonging to the family Compositae It is used by tribal population in India (especially northern states), acting as a contraceptive agent and female anti-fertility drug [8]. The ethnobotanical views show the infusion of whole plants were used in abortion [9], roots are remedy to cough and jaundice [10]. The major phytoconstituents, reported to contain germacranolide [11,12], vicoside A, vicodiol [13,14], Vicolides A, B, C and D, the sesquiterpene lactones [15], vicogenin, vicosigenin and vicoside B [16] oleanane triperpenoids [17], n-alkanes and their derivatives [18]. The previous biological investigation focuses the anti-inflammatory-analgesic properties [19], isolation of antiviral constituents [20] and pre-clinical toxicity studies [21]. Considering the above views, the present investigation deals with the antibacterial, preliminary phytochemical and pharmacognostical aspects of *Vicoa indica*, a common herb found in waste lands, agricultural fields and dry forest floors.

MATERIALS AND METHODS

Plant Materials and Extract Preparation

The aerial parts of *Vicoa indica* were collected from the plains of Tanjore district of Tamil Nadu, South India, during the month of January, 2005. The collected plants were shade-dried and coarsely powdered by using pulvarisor. These coarse powders were then subjected to successive extractions by various solvents of gradual increasing polarities such as hexane, chloroform and methanol by using Soxhlet apparatus. The collected extracts were then taken up for further investigations.

Antibacterial Activity Study

Bacterial strains Used

About seven human pathogenic bacterial strains were used. Both the gram-negative (*Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Vibrio parahaemolyticus, Vibrio cholerae*) and gram-positive bacteria (*Bacillus subtilis* and *Streptococcus pneumoniae*) were included. All the bacterial cultures were procured from Institute of Microbial Technology, IMTECH, Chandigarh, India.

Screening of Antibacterial Activity

Antibacterial activity was screened by cup-plate method [22]. Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with eight-hours-old broth culture of respective bacteria. Using the sterile cork borer, the well (3mm wide) was made into the each Petri-plate. Various concentrations of aqueous (cold, boiled and autoclaved), hexane, chloroform and methanol extracts (12.5, 25, 50, and 100 mg/ml) with respective solvents (as control drugs) were added into the wells by using sterile micropipettes [23] and simultaneously the standard antibiotics (as positive control) were tested against the pathogens. Then the plates were incubated at 37ºC for 24-48 hours. After the incubation period, the diameter of the inhibition zones of each well was measured

Table 2. Florescence analysis of aerial part of *Vicoa indica* under normal and UV-light

Chemicals/ Reagents	Normal light	UV light
Powder as such	Bluish green	Black
Benzene	Dark red	Brown
Chloroform	Reddish brown	Brown
Petroleum ether	Purple green	Green
Ethyl acetate	Reddish brown	Brown
Ethanol	Reddish brown	Brown
Water	Dark Brown	Brown
1N HCL	Bluish green	Brown
Aq. 1N NAOH	Purple green	Brown
1N NAOH in methanol	Dark brown	Purple brown
50% HNo_3	Reddish orange	Yellowish orange
50% H_2So_4	Dark brown	Brown

Table 3. Antibacterial activities of aqueous extracts of *Vicoa indica* against different microorganisms

Test Organisms	Aqueous Extract						Standard*
	Cold (%)		Boiled (%)		Autoclave (%)		
	50	100	50	100	50	100	
Escherichia coli	20	24	0	0	0	0	24(A)
Klebsiella pneumoniae	26	31	20	23	16	19	34(A)
Salmonella typhi	26	31	14	18	0	0	36(Cf)
Vibrio parahaemolyticus	27	30	20	24	15	17	24(T)
Vibrio cholerae	23	26	26	30	25	28	26(T)
Bacillus subtilis	25	27	20	25	13	17	30(S)
Streptococcus pneumoniae	0	0	0	0	0	0	33(Ce)

picillin (30 µg/ml); Cf - Ciproflaxacin (30 µg/ml); T - Tetracycline (30 µg/ml); S - Streptomycin (30 µg/ml); Ce - Cephalosporin (30 µg/ml); 0 = No activity

and the values were noted. Triplicates were maintained in each extract and the average values were calculated for the eventual antibacterial activity. The organic solvent extracts of the plant materials were evaporated, emulsified with tween 80 or Dimethyl sulfoxide (DMSO) solutions and stored in screw-caped bottles for testing the antibacterial screening.

Preliminary Phytochemical and Pharmacognostical Screening

The preliminary phytochemical studies were carried out by the methods described by Harborne [24] and Kokate et al. [25]. The plant extracts were screened for the presence of alkaloids, proteins, free amino acids, anthraquinones glycosides, flavonoids, tannins, phenolic compounds, carbohydrates, saponins, phytosterol and triterpenes.

The pharmacognostical investigations were conducted in terms of flourescence analysis [26], Physicochemical parameters such as total ash, water-soluble ash, acid insoluble ash and loss on drying were determined [27]. The successive extraction with organic solvents in the order of increasing polarity using a Soxhlet apparatus was carried out following the Indian Pharmacopoeia [28]. The percentage of solubility was calculated.

RESULTS

Preliminary Phytochemical Screening

The results of preliminary phytochemical screening of hexane, chloroform and methanolic leaf extracts of *Vicoa indica* are presented in Table1. The hexane and chloroform extract showed abundant occurrence of phenolic groups, triterpenes and trace amount was noticed in alkaloids, aminoacids, coumarins, quinines, steroids, but the complete absence was observed in anthroquinone glycosides, flavones, oils, saponins, sugars and tannins. Alkaloids, sugars and triterpenes are abundantly found in methanol extract followed by aminoacids, phenolic groups. While, the steroids, quinines, saponins, tannins are occurred in rare amount, but the rest of constituents were not found in the same extract.

Preliminary Pharmacognosy Studies

The fluorescence analysis of the powdered drug from the leaves of *Vicoa indica* in various solvents and chemical reagents was performed under normal and

Table 4. Antibacterial Activity of organic solvent extracts of *Vicoa indica*

Organisms Tested	Diameter of Zone of Inhibition (in mm)												Standard Antibiotic*
	Extract Concentration												
	Hexane (mg/ml)				Chloroform (mg/ml)				Methanol (mg/ml)				
	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	
Escherichia coli	22	24	0	0	0	0	0	0	20	18	16	14	24(A)
Klebsiella pneumoniae	26	31	20	22	0	0	0	0	20	18	14	13	34(A)
Salmonella typhi	24	30	15	19	0	0	0	0	0	0	0	0	36(Cf)
Vibrio parahaemolyticus	30	30	18	21	0	0	0	0	18	16	15	13	24(T)
Vibrio cholerae	24	25	29	29	21	21	20	18	0	0	0	0	26(T)
Bacillus subtilis	25	27	20	27	18	16	15	15	0	0	0	0	30(S)
Streptococcus pneumoniae	0	0	0	0	0	0	0	0	0	0	0	0	33(Ce)

* A – Ampicillin (30 µg/ml); Cf – Ciproflaxacin (30 µg/ml); T – Tetracycline (30 µg/ml); S- Streptomycin (30 µg/ml); Ce – Cephalosporin (30 µg/ml)
0 = No activity

Ultra Violet (UV) light (Table 2). The physico-chemical characters such as total ash value recorded was 3.2%, acid Insoluble ash value recorded was 2.0%, Water insoluble ash was 4.4% and loss on drying was 3.7%. The successive extraction value of powdered drug in various solvents recorded was 3.9% in hexane, 4.2% in chloroform and 5.6% in methanol.

Antibacterial Activity

The results of antibacterial activities of various extracts (aqueous, hexane, chloroform and methanol) from the leaves of *Vicoa indica* showed concentration-dependent activity against tested microorganisms (Tables 3&4). The different concentrations of hexane extracts (12.5, 25, 50,100 mg/ml) were tested against gram positive and gram negative bacterial strains. The higher concentration of the same extract had inhibitory effects towards the bacterial strains namely *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhi*, but the other extracts did not show any activity. The chloroform extract was found to have better inhibitory effect against gram negative strains namely *K. pneumoniae*, *E,coli, S. typhi* followed by gram positive strains *S. pneumoniae* and *Bacillus subtilis.* This effect was concentration-dependent. All the other organisms were found to be resistant. The different concentrations of methanolic extracts of *V. indica* had significant activity against *S. aureus*, *E. coli*, *K. pneumoniae* and *Vibrio parahaemolyticus*. The remaining organisms were not susceptible to the plant extracts.

DISCUSSION

All plant parts synthesize some chemicals in themselves which metabolize their physiological activities. These phytochemicals are used to cure the disease in herbal and homeopathic medicine. Nowadays, most of the people like to use the traditional methods to cure general diseases [29]. The present research was focused on the leaves of *Vicoa indica* extracts (using different extracts), for the presence of some phytochemical substances and pharmacognostic studies such as fluorescence analysis, ash value, extractive value, loss by drying etc. Several researchers contributed similar type of investigations in the different plant species namely *Grewia tilifolia*, *Tridax procumbens*, *Senna uniflora* and *Dysoxylum* species [30-33]. On the other hand, Aggarwal et al. [34] reported various chemical constituents isolated from *Achyranthes* species (*Achyranthes aspera*, *A. bidentata* and *A. fauriei*) including steroids, long chain compounds, saponins, organic acids and their esters, carbohydrates, alkaloids, triterpenes, polyphenols, anthraquinones, flavonoids and amino acids. Notable physiological activities from the various parts of the plant and its isolates are reported to contain several activities including antimicrobial activity [5-7].

The antibacterial activities of *Vicoa indica* extracts were tested against eight bacterial strains. The results showed promising antibacterial activity against the bacteria tested. Among these, hexane and aqueous extracts were found to have a more potent inhibitory effect than the other extracts. Similarly, Umadevi et al. [35] investigated the antibacterial and antifungal activity of chloroform, acetone, methanol and aqueous extracts of *Andrographis echiodes* at different concentrations against seven strains of bacteria. Likewise, Radha et al. [36] studied the antimicrobial activity of different extracts (chloroform, ethyl acetate, methanol and water) of *Heliotropium marifolium* by standard dilution test using Muller Hinton Agar (MH) medium. The findings showed potential antimicrobial properties against the organisms tested. The n-hexane and (MeoH) methanol (80%) extracts from *Mitracarpus scaber* leaves (which) to be removed exhibited a pronounced antibacterial and antifungal activity, based on their concentrations [37]. The results from the aqueous extract, contributed excellent activity against *Vibrio* species compared to standard antibiotics (Table 3).

In conclusion, the pharmacognostic investigations on physicochemical characteristics and fluorescence analysis shows that authentic botanical of this crude drug prevents adulteration, substitution and has a crucial role in standardization of crude drugs. The preliminary phytochemical screening of the leaves of *Vicoa indica* indicates the presence of secondary metabolities, having an essential role in medicine. Overall, the present study indicates the antibacterial properties of *Vicoa indica* and provides some idea about phytochemical and pharmacognostical investigation on *Vicoa indica*. This study paves the way for further attention/research to identify the active compounds responsible for the plant biological activity.

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