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PHYTOCHEMICAL ANALYSIS INDIGOFERA TINCTORIA AND TO STUDY ITS ROLE IN MANAGEMENT OF MANDALI VISHA (VIPER BITE) - A REVIEW ARTICLE

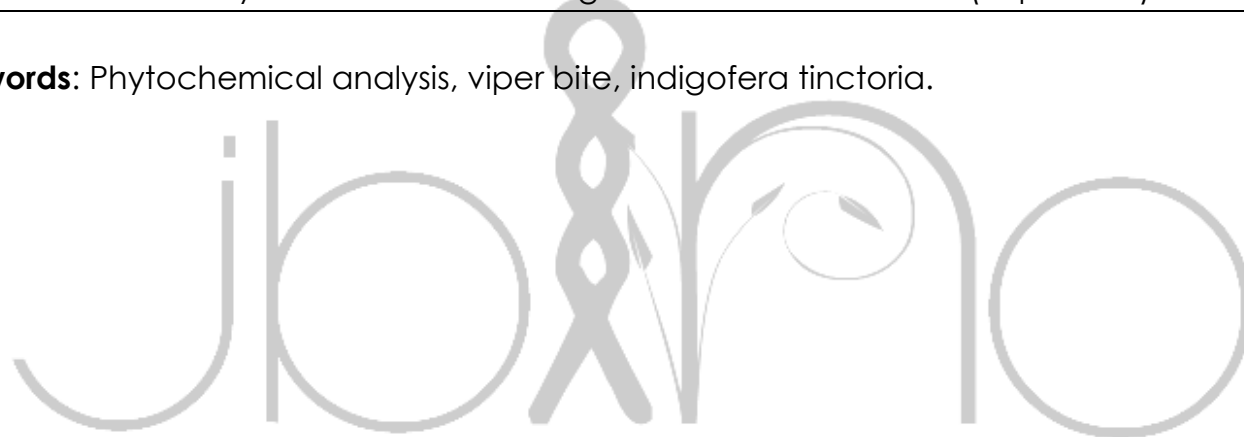
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ABSTRACT

The genera of indigofera (Family Fabaceae) are distributed throughout India and are medicinally useful. Indigofera tinctoria Linn (Fabaceae) has been extensively used in various folklore and traditional medicines. Studies on the plant reveal high LD50 thus low toxicity. In the present article we are studying the phytochemical analysis of Indigofera Tinctoria and to study the role in the management of Mandali Visha (Viper bite).

Keywords: Phytochemical analysis, viper bite, indigofera tinctoria.



INTRODUCTION

Preliminary Qualitative Phytochemical Screening/Analysis Preliminary qualitative phytochemical screening was done with method of Harbone (1998), Parekh and Chanda (2007). The pod of *I. tinctoria* extracts (i.e. soxhlet extract using ethanol, cold ethanolic, and aqueous extract) were all analyzed for the presence/ absence of saponins, flavonoids, alkaloids, tannins, and cardiac glycosides. Test for Saponins: The presence of saponins was determined by Frothing test. 2ml of the pod extract was vigorously shaken with 2ml distilled water and was allowed to stand for 10 minutes and classified for saponin content as follows: No froth indicate absence of saponins and stable froth more than 1.5 cm indicated the presence of saponins (Kapoor et al., 1969). Test for Flavonoids: 2ml of the pod extract was dissolved in dilute NaOH. A yellow solution that turns colourless on addition of con. HCl indicates the presence of flavonoids. Test for Alkaloids: 2ml of the pod extract was acidified with 1% HCl (i.e. 1ml of con. HCl in 99ml of distilled water) and was treated with few drops of Wagner reagents in a test tube. A reddish brown precipitates indicates the presence of alkaloids (Salehi-Surmaghi et al., 1992). Test for Tannins: 2ml of the extract was treated with about 10mls of distilled water and then few drops of 1% ferric chloride (FeCl_3) solution were added. The occurrence of blue-black, green or blue greenish precipitate indicates the presence of tannins (Segelman et al., 1969). Test for Cardiac glycosides: Keller-kiliani test was performed

to assess the presence of cardiac glycosides. 2ml of the extract was treated 2ml of glacial acetic acid containing 1 drop of ferric chloride solution. To this solution a few drops of concentrated sulphuric acid (H_2SO_4) was added. A brown ring formation at the interphase indicates the presence of deoxy sugar characteristics of Cardiac glycosides (Ajaiyeobu, 2002). Test Organisms (Bacterial and Fungi) Pure culture of 24 hours *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhi*, and *Escherichia coli* of bacteria origin and an already cultivated culture of *Aspergillus terreus* were all collected from Anthony Van-Leeuwenhoek Research Centre Ihiagwa Owerri, Imo State in Nigeria. Screening for Antimicrobial Assay Antimicrobial activity was screened by agar well diffusion method (Perez et al., 1990). The plant extracts were tested for antimicrobial activity against bacterial pathogens such as *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Salmonella typhi*. And *Aspergillus terreus* as fungal pathogen. Screening for Bacteria Pathogen 12 different plate were prepared, containing the solidified MHA, then the test bacteria were inoculated into each of the plate by streak plate method, using sterile inoculating loop, with each test organisms inoculated into three different plate and labeled appropriately with marker, the plates were separated into three different sets such as A, B, and C. Each set, contain the test bacterium. They were evenly spread over the medium by streaking. Then two (2) wells of 6mm were made in each of the plate with a sterile cork borer. The

plant extracts were also labeled using code such as 1 and 2, where 1= Soxhlet Ethanolic extract and 2 = Aqueous extract of pod of *I. tinctoria*. In the first set of plate labeled 'A', the extracts were pipetted without any dilution and filled the hole, while in the second set label 'B', the extracts was diluted with sterile water in a ratio of 1:1 and then pipetted to fill the hole, conversely in the last three set, the extracts were diluted with sterile water in a ratio of 1:2, before pipetting into the hole. Finally the plates were incubated in an upright position for 24 hours at 37°C, after incubation the plates were observed for the formation of clear inhibition zone around the well indicating the susceptibility of the extracts. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well. Screening for Fungal Pathogen Two (2) conical flask with aluminum foil cover were sterilized alongside with already prepared medium of Potato Dextrose Agar (PDA) with autoclave. The medium were poured into the conical flask after autoclaving, and the undiluted ethanolic and aqueous extracts of *I. tinctoria* pod, were added to the medium and shaken thoroughly for proper mixing. The mixture was poured into two different sterile petri dish labeled according to the type of extracts used and allowed to solidify. The spore of *Aspergillus terreus*. Were inoculated into the medium using sterile inoculating loop and the plate was incubated in an upright position for more than 24 hours, after which the plate was observed for the degree of susceptibility.

Role in treatment of snake bite

Susruta acharya has mentioned the intake of 'sa sarpishkam niliniphalam' (intake along with ghee) to induce virechana (purgation) in the management of pakswasaya gata visha (visha located in lower Gastro Intestinal Tract) [8]. Raja marthanda has indicated the intake of neeli moolam (root of Neeli)grinded in tandula jalam (prepared by soaking pounded rice grains in water) for the management of mandali visha (viper bite).[1] A similar reference, regarding the intake of neeli moola kalka in warm water along with its application at the damsha sthana (bite site) of mandala(viper), is available in Prayoga sammucchayam, a keraleeya visha chikitsa grantha (Classic text books followed in Kerala for the treatment of poisonous conditions) [9]. External application and internal administration of the roots of Nimba (*Azadirachta indica*), Neeli and Karanja (*Pongamia pinnata*) is said to have the power for quick mitigation of visha [10]. They are considered as the Trinity in the treatment of Visha by visha vaidyas (Traditional Ayurvedic toxicologists of Kerala). Quite different from the other classics, Prayogasammucchayam explains 16 types of mandali and its treatment. Neeli has been mentioned for the treatment of kumbha mandali and rakta mandali (two varieties of viper snake) [11-12]. Internal and external use of Neeli moola along with Shireesha moola (root of *Albizia lebbek*) grinded in their patra swarasa (juice extracted from leaves)

helps in fast relief from lootha visha (spider bite poison) [13]. Dhara (An external therapy in which the prepared medicine is continuously poured on the affected part) and pana (intake) of Sariba (Hemidesmus indicus) and Neeli moola kwadhitha jala (water boiled with the above mentioned two drugs) is indicated in case of loota visha. [13] Intake of ghrita (ghee) processed in Neeli patra swarasa along with Tulasi swarasa (juice of Ocimum sanctum), Nirgundi swarasa (juice of Vitex negundo) and certain other drugs is also indicated in loota visha. The same yoga is advised to be processed in coconut oil for external application which is available in the market under the name Neelidaladi keram [14]. Application of Neeli dala (leaves of Neeli), made into kalka (paste) in milk, over the abdomen has been explained by acharyas as one of the earliest method of diagnosis of the site of Garavisha (A condition of latent poisoning). The site where the paste does not dry is considered to be the site of visha. References regarding the usage of Neeli moola (Root of Neeli) for treating Manushya visha (human poison) and lizard poisoning are available in Sahasrayoga. Kodasseri margam, a unique keralaleeya visha chikitsa grantha has indicated the intake of Neeli moola in the swarasa (juice) of Dronapushpi (Leucas aspera) for the management cobra bite venom along with other drugs. References regarding Thala (treatment in which Application of medicinal paste on the vertex of head is done) and lehya yogas (lickable formulations) containing Neeli for the treatment of visha atura (A person

afflicted with poison) is also available in the text [20]. Traditional visha vaidyas of Kerala use Neeli as an extra addition to classical yogas to yields better results. Similarly, Neeli patra swarasa (leaf juice of Neeli is used for grinding certain Agada yogas in which bhavana dravya (Medium for grinding the drugs in a formulation) is not specified by acharyas (eminent scholars), in order to enhance its potency. In the context of pratyoushdha prayoga (antidote), pana (intake) and vilepana (external application) of Neeli is considered to be effective as a samanya pratyoushdha dravya

Though various references regarding the properties of the drug is available in the classics, most acharyas opines that the plant possess tikta rasa (bitter taste), laghu ruksha guna (light to digest and dry in nature), katu vipaka (becomes pungent after digestion) usna virya (hot in potency) and is kapha vata shamana (alleviates kapha and vata). Visha is said to be attributed with avyaktarasa (undefined taste) dosa pradhanya as vatapittothara (predominant with vata and pitta dosha), laghu ruksha guna and usna virya. Though it is said 'samanyam vridhi karanam' (exposure to situation or materials with similar properties results in the increase of the particular substance or condition), it can be inferred that the drug Neeli acts as vishahara due to its prabhava (unexpected unique pharmacodynamics of a drug), as Charakacharya rightly quotes. 'vishagnamuktam yath prabhavastatra karanam. (visha hara property of drugs is due to their prabhava)

. Though specific doses are not mentioned in most conditions, The Ayurvedic pharmacopoeia of India concludes the dose of Neeli patra Kashaya (decoction of leaves of Neeli) as 50 – 100 gm and that of root as 48 gm of drug for decoction. This dose may be chosen for ailments other than visha, as visha, quite different from other diseases, is considered to be atyaya (emergency) condition which demands intervention in muhurmuha: oushadakaala (frequent administration of medicine) . and in doses which depend on the condition of the victim and the potency of the visha inflicted, as Acharya Vagbhatta quotes ' sthana vega balaabala, alochya nipunam bhudhya karmanantharam achareth...' (Visha condition should be treated according to the logic of the physician after carefully looking into various aspects such as the location & severity of the poison in the body, the strength of the patient etc) . Hence the dose of Neeli to be administered in visha may vary depending on the condition of the patient and logic of the vaidya (physician). Ayurvedic classics reveal that Neeli is widely used in the management of numerous conditions of visha. Apart from the brihat trayis and the nighantus, the drug is frequently mentioned in keraleeya visha chikitsa granthas like Prayogasammucchayam, Kriyakaumudi, Kodasherymargam etc which throws light on its easy accessibility and its efficacy even in the management of high potent poisons like mandali visha. More research work is to be taken up in order assess the properties of each plant part to evaluate its action against any particular visha

condition. The plant, with its vishahara potency and its availability in abundance, can be utilized by practitioners to advice as a household remedy as well as for clinical practice in order to treat vishayukta conditions.

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