Chapter-32
Significant Phytochemical Plant: *Indigofera tinctoria*

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Abstract: -

The significance of herbal drug trends lies in the global shift toward **natural, holistic, and cost-effective healthcare solutions**, driven by consumer demand for fewer side effects compared to conventional pharmaceuticals. Indigofera species are widely employed in Ancient traditional medicine all around the world, against many ailments. India has blessed with the *Indigofera* is the third-largest genus in the family of Fabaceae. Thus, based on these medicinal properties, various investigations have been undertaken in order to appraise the pharmacological activities and the chemical composition of these species. In this study we have done some preliminary phytochemical screening which provide us the knowledge of plants which will use as reservoir of chemical agents. Flowers of ***Indigofera tinctoria Linn.*** were collected. The water, pet ether, ethanolic Extracts of air-dried flower sample were examine for the presence of Tannins, Alkaloids, flavonoids, Saponins etc. The screening is carried out with reference to the standard methods found in literature.

Keywords: Phyto-chemicals, Indigofera tinctoria, extracts, Ancient traditional medicine plants.

Introduction

Herbal drugs have gained a reputation in recent years because of their safety, efficiency, and cost-effectiveness. In the present era most of the people depends on plant-based medicines as their first choice for encounter diseases and maintaining the health [1-3]. ***Indigofera tinctoria*** the plant will grow 2 to 3 feet tall and wide and does well in moist well-drained fertile soils with a pH 6 to 7. As with all plants in the bean family, it fixes nitrogen in the soil. It will tolerate full to partial sun conditions but does appreciate some afternoon shade in hot climates It had been cultivated and highly valued for centuries as the main source of indigo dye. The sp. ***Indigofera tinctoria*** is a medicinal plant shows activity like antibacterial [4], anti-inflammatory [5], antidiabetic [6], anti-HIV [7], immunomodulatory[8], antidepressant, and nootropic activity[9]. However, various reports are currently available in the literature regarding the antimicrobial and antioxidant activities of leaves and roots extracts of the plant but it is observed that research on the flower extracts are unfocused. In order to evaluate the medicinal information, the present study deals with phyto-chemical screening.

Material and Methods:

1.1 Collection of Sample:-

The flowers of ***Indigofera tinctoria*** were collected from the Caves of Aurangabad region. The collected plant material was botanically identified and confirmed by the Head of the Botany Department. The herbarium specimens were preserved. The flowers

were chopped into small pieces, dried and coarsely powdered. The coarse powder was then subjected to successive extraction with organic solvent such as petroleum ether, water and ethanol by Soxhlet method [10.] The extracts were then collected and distilled off on a water bath at atmospheric pressure and the last race of the solvents was removed and stored at 4°C. They were used for preliminary phytochemical screening [11].

Fig 01:-Flowers of *Indigofera tinctoria*

1.2 Preparation of Extracts:

The coarse powder of flower were subjected to successive hot continuous extraction with various solvent each time before extracting with next solvent the powdered material will be air dried (weight of crude extract 500 gm). Aqueous extract, Pet-ether, ethanolic extracts of the flower were obtained by mixing 5g of the plant material with 100ml of pure solvents. The solution was allowed to boil in a microwave oven for 2min and then filtered using Whatsmann filter paper NO.1 and the analysis was carried out immediately without storage.

Fig: Preparation of extract

1.3Traditional Uses :- The roots, stems and leaves are bitter, thermogenic, laxative, trichogenous, expectorant, anthelminthic, tonic, naturopathy, splenomegaly, echolalia, cardiopathy, chronic bronchitis, asthma, ulcers, skin diseases, diuretic and are useful for promoting growth of hair. The juice extract from the leaves is useful in the treatment of hydrophobia. An extract of the plant is good for epilepsy and neuropathy. The plant possesses anti-toxic property .The plant is stimulant, alternative and purgative. Indigo is antiseptic and astringent. The Juice of the leaves and indigo in powder are used mixed with honey in enlargement of liver and spleen, epilepsy and other nervous affections. In hydrophobia two ounces of fresh juice with an equal quantity of milk is given in the

morning for 3 days as a prophylactic; Juice is also given in asthma, whooping cough, palpitation of heart, in some lung diseases and kidney complaints as in dropsy. An Infusion of root is given as an root is given as an antidote in cases of poisoning by arsenic. Externally, leaves crushed are used as stimulant, Poultice or plaster in various skin affections, to hemorrhoids etc., and to cleanse an heal wounds and ulcers. Powdered indigo also is used for sprinkling on ulcers. It is applied mixed with castor oil to the navel of children to promote the action of the bowels and mixed with warm to the pubes and hypogastria as it stimulates bladder and therefore useful in cases of retention of urine[12]. Indigofera tinctoria is used in constipation, liver disease, heart palpitation and gout[13].

1.4 Mineral Assay: -

The minerals analyzed were Potassium using atomic absorption spectrophotometer, calcium and phosphorus by flame photometer. Ascorbic acid in flower was estimated by standard process [14.]

Table 1: Mineral value of Indigofera tinctoria flower

Mineral	Value (gm/100gm)	Mineral	Value (gm/100gm)
Fe	0.36	Cr	0.03
Zn	0.15	Ca	8.78
Mn	0.04	K	2.10
Mg	4.21	Co	0.01
Cu	0.03	P	0.75

1.5 Phyto-Chemical Analysis:

The aqueous extracts, so obtained from the dried flowers powder of 10 plants were tested for the presence of phyto-chemicals. The water, pet ether, ethanolic Extracts of air-dried flower sample were examine for the presence of glycosides, Tannins, Alkaloids, flavonoids, Saponins, Resin. The screening is carried out with reference to the standard methods found in literature.

Fig: Lab work of Extraction

Test for Tannins:

To 5ml of the plant filtrate obtained a few drops of Ferric chloride were added the presence of a brownish green or blue black color indicated that the plant material possess Tannins.

Test for Phlobatannins:

To 10ml of the aq. Extract of the plant material was boiled with 1% HCl in a test tube the presence of Phlobatannins was confirmed by the deposition of red ppt in the test tube.

Test for Saponins:

To 10ml of the aq. extract of the plant material,3ml of distilled water is added and shaken well, so as to obtain froth. To the froth formed, a few drops of olive oil were added. The formation of emulsion indicates the presence of saponins.

Test for Flavonoids:

A few drops of 1% liquor ammonia were taken in test tubes, to which the aq. Extract was added. Yellow colorations of the solution confirmed the presence of flavonoides.

Test for Terpenoids:

Around 2ml of chloroform and 3ml of conc. Sulphuric acid were added consecutively to 5ml of the aq. Extract of the plant material. A reddish-brown interface in solution detonated the presence of Terpenoids.

Test for Steroids:

A couple of grams of plant powder were mixed with 10ml of chloroform, followed by boiling and filtration. To the above 2ml acetic anhydride and a few drops of concentrated sulphuric acid was added. Stable presence of blue-green ring in the solution confirms the presence of steroids.

1.6 Determination of antibacterial activity:- To determine the antibacterial activity of methanolic leaf and root extracts, cup-plate agar diffusion method was adopted. The bacterial cultures were grown in brain–heart infusion (BHI) broth and incubated at 37°C for 24 h. The cultures were later diluted with sterile medium, adjusted to 0.5 McFarland turbidity standard and inoculated on BHI agar plates by streaking the organisms over the surface of the medium using a sterile cotton swab and allowed to dry for about 10 min. Four wells of 6 mm in diameter and 4 mm in depth were cut using a sterile cork borer, maintaining a distance of 3 cm between them. The wells were filled with 100 µl of methanolic leaf and root extracts (100 mg/ml) dissolved in dimethyl sulfoxide (DMSO). Wells filled with 100 µl of nalidixic acid (500 µg/ml) and 100 µl of DMSO served as positive and negative controls, respectively. The plates were then kept at room temperature in an upright position for 2 h for diffusion of extracts and then incubated under the same growth conditions as mentioned above. Antibacterial activity was determined by measuring the inhibition zones formed around each well, averaged and the mean values were noted.

Result and Discussion :-The results of the phytochemical screening of the investigated water, ethanolic and pet ether I. tinctoria L. based on these medicinal properties, various investigations have been undertaken in order to appraise the pharmacological activities and the chemical composition of these species. The water, pet ether, ethanolic Extracts of air-dried flower sample were examine for the presence of Tannins, Alkaloids, flavonoids,

Saponins etc. Tannins are naturally occurring, water-soluble phenolic compounds, which precipitate proteins from aqueous media. The primary source of tannin, used as an active pharmaceutical agent. Topical applications of tannins serve as anti-inflammatory agent, treatment of wounds, burns, antiheamorrhagic and antiseptic potential. Saponins are bitter taste chemicals that possess foaming characteristic. Though saponins possess several beneficiary effects such as reducing the risk of cancer, serving as antioxidants, providing immunity, etc., certain saponins are toxic. In this study, Saponins were recorded in more. The modified or oxidized terpene is called as terpenoid. The antimicrobial activities of terpenes are reported. The presence of steroids as Phytoconstituents is reported next to flavanoids in this study. Steroids are one of the most widely used groups of drugs in present day anaesthetic practice. It revealed the medicinal potential value of petroleum ether and ethanol extracts against abdominal pain, diarrhea, fever, nausea, septicemia, urinary tract infections and vomiting, hospital-acquired wound infections, thus, it revealed the medicinal potential to develop broad spectrum antibiotics of therapeutic interest.

The antibacterial activity of ethanolic flower extracts of I. tinctoria L. was tested against five bacterial pathogens with nalidixic acid as a positive control and DMSO as a negative control . The ethanolic flower extracts of I. tinctoria L. showed promising antibacterial activity against a wide range of bacteria.Maximum in vitro inhibition was scored by E. faecalis, followed by S. aureus, S. paratyphi B, E. aerogenes, and K. pneumoniae, which presented inhibition zone diameter of 25.60±0.56 mm, 23.35±0.56 mm, 22.33±0.56 mm, 20.76±0.56 mm, and 19.33±0.56mm, respectively.

Conclusion

The results obtained from the present study suggest that I. tinctoria L. is a potential source of antibacterial .The I. tinctoria proved to bioactive molecules responsible for the antimicrobial activities against these tested microorganisms should be isolated identified and elucidated its structure to develop a new lead of therapeutic interest to cure various human ailments in India which provides knowledge on the availability of secondary metabolites from different plants. Studies of Indigofera tinctoria Linn has shows that it possesses low toxicity.

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