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Anti-inflammatory screening on the flowers of *Trichodesma indicum* Linn.

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Abstract

Phytochemical screening and *invitro* anti-inflammatory activity of the alcoholic and aqueous extracts of the flowers of *Trichodesma indicum* were studied. The phytochemical screening revealed the presence of flavonoids, terpenoids and steroids. During the screening the prevention of hypotonicity induced HRBC membrane lysis was taken as a measure of the anti-inflammatory activity. The anti-inflammatory activity of the ethanolic extract of the flower was comparable to that of the standard drug indomethacin. The ethanolic extract of the flower has significant anti-inflammatory activity in comparison with the aqueous extract of the same plant.

Keywords: Anti-inflammatory, *invitro*, *Trichodesma indicum*, HRBC membrane stabilisation

Introduction

Inflammation is a complex reaction to injury that comprises of vascular responses and migration and activation of leucocytes. It basically start as the body's defense reaction, but may turn potentially harmful. Inflammation can be classified in to two types like acute and chronic. Acute inflammation is a transient process which occurs within seconds of injury, last for hours or days, represents early body reaction and is usually followed by repair, a process by which tissue is restored to its original state as far as possible. Chronic inflammation occurs when causative agent of acute inflammation persists for a long time. Fibrosis and tissue necrosis usually accompany chronic inflammation (Geetika Khanna Bhattacharya, 2011). Although infection is caused by a microorganism, inflammation is one of the responses of the organism to the pathogen. Without inflammation, wounds and infections would never heal. Similarly, progressive destruction of the tissue would compromise the survival of the organism. Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants (A.Mohini et al, 2012).

Trichodesma indicum (Boraginaceae) is an erect, spreading, branched and annual herb. The flowers are usually violet, light blue or purple in colour. Flowers occur singly in the axils of the leaves. Calyx is green, hairy, and 1 to 1.3 cm long, with pointed lobes. Corolla is pale blue, with the limb about 1.5 cm in diameter, and the lobes pointed (S.Nesamony, 2001). The plant is reported to be an antimicrobial (S.S. Saboo et al, 2013), antitussive (K Srikanth et al, 2002) and antidiarrheal agent (S.K Sharma and K.K Pillai, 2005). *Trichodesma* is also used in swellings of the joints and in treating snake bite (K.R.Kirtikar and B.D.Basu, 2005) The plant is also known as Indian Borage. The plant is distributed through out India and can be found near all most all river banks.

Materials and methods

Plant material

The flowers of *Trichodesma indicum* were collected from the Perumba river banks of Payyannur village of northern Kerala. The plant material was identified, authenticated and a voucher specimen was deposited in the department of Pharmacognosy of Academy of Pharmaceutical Sciences, Pariyaram, Kerala.

Preparation of extracts

The flowers were dried under shade and finally powdered. The powder was transferred to soxhlet extractor and subjected to extraction with ethanol and distilled water.

After extraction, the solvents were distilled off and the extracts were concentrated on water bath to a dry residue and kept in a desiccator.

Phytochemical evaluation

Preliminary phytochemical evaluation has been carried out as per the standard procedure. The following specific tests were performed for steroids, terpenoids and flavonoids (C.K. Kokate, 1999 and K.R. Khandelwal, 2000).

Test for Steroids

Salkowski reaction

To the 2ml of extract, add 2ml chloroform and 2ml Con. H₂SO₄ through the sides of the test tube. And Shake well.

Liebermann - Burchard reaction

Mix 2ml of the extract with chloroform. Add 1-2 ml acetic anhydride and 2 drops of Con. H₂SO₄ through the sides of the test tube.

Liebermann reaction

Mix 3ml of the extract with 3ml acetic anhydride. Heat. Add few drops of Con. H₂SO₄ through the sides of the test tube

Test for Volatile Oils

Hydro distill the drug material. Separate the volatile oil from distillate and perform the following tests like ;Check the Odor; Place a drop of oil in the filter paper and observe, and also check the solubility in 90% alcohol.

Test for flavonoids

Shinoda test

0.5g of magnesium turnings and few drops of Con. HCl from the sides of the test tube is added to the extract.

Evaluation of anti-inflammatory activity

The HRBC membrane stabilization has been used as a method to study the anti-inflammatory activity(R.Gandhidasan, 1991). Blood was collected from healthy volunteers. The collected blood was mixed with equal volume of sterilized Alsever solution The blood was centrifuged at 4000 rpm and packed cells were washed with isosaline and a 10 % v/v suspension was made with isosaline. The assay mixture contains the drug at various concentration, 1 ml phosphate buffer, 2 ml of hyposaline and 0.5 ml of HRBC suspension. Indomethacine was used as the reference drug. Instead of hyposaline 2 ml of

distilled water was used in the control. All the assay mixtures were incubated at 37°c for 30 min and centrifuged. The haemoglobin content in the supernatant solution was estimated by using spectrophotometer at 560 nm. The percentage hemolysis was calculated by assuming the hemolysis produced in the presence of distilled water as 100 %. The percentage of HRBC membrane stabilization or protection was calculated by using the following formula; Percentage Protection = 100 – (OD of sample /OD of Control) x 100, where OD is the optical density.

Results

Phytochemical evaluation of aqueous and alcoholic extracts

The phytochemical evaluation revealed the presence of flavonoids, terpenoids, and steroids.

Plant Steroids

In *Salkowski reaction*, Chloroform layer appears red and acid layer shows green fluorescence indicate the presence of plant steroids. In *Liebermann - Burchard reaction*, initially a red colour appears which turns to blue and finally green indicating steroids in the extract. In *Liebermann reaction*, a clear blue colors appears through which the steroids can be confirmed.

Flavonoids

In *Shinoda test*, a Pink color observed which confirms the presence of flavonoids.

Volatile Oils (Terpenoids)

Volatile oil obtained from the flowers have characteristic odor and the filter paper was not permanently stained with the oil and more over the oil was completely soluble in 90% alcohol which confirms the presence of terpenoids in the alcoholic as well as aqueous extracts of the flower.

Table 1: Phyto chemical screening of alcoholic and aqueous extracts

Sl. No.	Test	Alcoholic extract	Aqueous extract
1	Phytosterols	+	+
2	Flavonoids	+	+

(+ present)

Table 2: Antiinflammatory activity of the flowers of *Trichodesma indicum*

SI No	Concen-tration mg/ml	Percentage Protection		
		Alcoholic Extract (Ethanolic extract)	Aqueous extract	Indomethacine (Standard)
1	Control	*****	*****	*****
2	100	79.8± 0.16	43.4 ± 0.29	90.19± 0.01
3	150	86.2 ± 0.01	59.6 ± 0.47	101.30 ± 0.27
4	200	93.7 ± 0.18	77.3 ± 0.39	135.65 ± 0.39
5	250	109.6 ± 0.22	98.9 ± 0.44	159.80 ± 0.22
6	300	145.3± 0.11	121.1 ± 0.50	199.77± 0.99

(Values are expressed as SEM of 3 readings)

Discussions

The anti-inflammatory activity of the alcoholic and aqueous extracts were concentration dependent, with the increasing concentration the activity was also increased. It was observed from the table. 2, that the ethanolic extract shows significant anti-inflammatory activity at the

concentration of 300 micro gm/ml which is comparable to the standard drug, indomethacine. The alcoholic extract of the flowers of *Trichodesma indicum* has significant anti-inflammatory activity in comparison to the aqueous extract of the same plant.

The HRBC membrane lysis is induced by the the

prevention of hypotonicity and which is taken as a measure of anti-inflammatory activity of the drug and HRBC method was selected for the *in vitro* evaluation of anti-inflammatory property because the erythrocyte membrane is an analogous to the lysosomal membrane. During inflammation process lysosomal enzymes will get released which can produce a series of diseases. The alcoholic as well as aqueous extracts of *Trichodesma indicum* were subjected to erythrocyte membrane stabilization induced haemolysis by hypotonic solution. The major mechanism of action of non steroidal drugs are by inhibiting the lysosomal enzymes or by means of stabilizing the lysosomal membrane (Rajendran Vadivu, 2008). The ethanolic extract of the flowers exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane.

Large number of herbal species has been used traditionally or as folk medicines against inflammatory disorders. Chemical classes of anti-inflammatory agents from natural sources have been reported to engage a vast range of compounds such as polyphenolics, flavonoids, terpenoids, alkaloids, anthraquinones, lignans, polysaccharides, saponins and peptides (Wan D et al, 2004, Chi Y et al, 2001). The phytochemical evaluation of our study shows that the flower extracts contain mainly flavonoids, steroids and terpenoids. Flavonoids are reported as major anti-inflammatory agent (Jang D et al, 2002). Some of them act as phospholipase inhibitors and some have been reported as TNF- α inhibitors in different inflammatory conditions. Biochemical investigations have also shown that flavonoids can inhibit both cyclooxygenase and lipoxygenase pathways of arachidonic metabolism depending upon their chemical structure (Jang D et al, 2002, Changa C et al, 2008). Terpenoids significantly inhibit the development of chronic joint swelling. Terpenoids may affect different mechanism relevant to inflammations arising in response to varied etiological factor (S.Kumar et al, 2013). In addition the flower also contain steroids as another constituent and plant steroids are also reported with anti-inflammatory activity (Snehal S et al, 2015).

Conclusion

From the above study it was concluded that the ethanolic extract of the flowers of *Trichodesma indicum* has significant membrane stabilization property and it was comparable to the standard drug indomethacine. The probable mechanism of anti-inflammatory action of the flower could be due to the presense of flavonoids, terpenoids and steroids. Further the compound isolation, purification and characterization for anti-inflammatory action has to be evaluated for the usage of *Trichodesma indicum* as a natural and potential anti-inflammatory agent.

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