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Research Article

ANATOMICAL STUDY AND ANTI PYRETIC ACTIVITY OF *ZANTHOXYLUMLIMONELLA* FRUIT

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ABSTRACT

In holistic tradition, herbs aren't used to reduce fever unless there is also some positive benefit in treating the infection or inflammation that is causing the fever. Many alternative practitioners see fever as the body's natural response to a pathogen. Herbs such as willow, meadowsweet, black haw, cramp bark, birch, black cohosh and Indian pipe contain some derivatives of salicylic acid, and are often called "herbal aspirins". While aspirin and its chemical relatives are harsh on the stomach, the salicylin found in herbs is not nearly as strong as acetylsalicylic acid (aspirin) and the action of herbs for fever reduction is much different from synthetic aspirin. Like aspirin, herbs can act as inhibitors for inflammation-causing prostaglandins, but often they achieve this by addressing the imbalance that lead to an elevated incidence of prostaglandins. .In the present pharmacological evaluation the fuit extract (ethanolic) of Zanthoxylumlimonella plant was extensively investigated for its antipyretic activity against Brewer's yeast induced pyrexia model in rats. At the end of ourstudy, a strong conclusion can be drawn that,theeethanolic extract of Zanthoxylumlimonella .

Keywords: Zanthoxylumlimonella , Antipyretic , Salicylin , Aspirin.

INTRODUCTION

Contrary to what you may believe based upon what you may have been told-a fever is not sickness, but healing. A fever is the body's attempt to heal itself using heat for purposes of elimination via perspiration. A fever uses the body's skin and the function of perspiration to eliminate toxins from the body via the skin. Medically speaking, a fever is elevation of body temperature. The natural human body temperature is 98.6 degrees. A body temperature over 100.4 is considered pathological. Most fevers bring with them a natural fasting period, which is why the appetite usually disappears when a fever is on the scene. Metaphysically speaking, a fever denotes anger. Anger causes heat and heat is present during a fever; this is why the body temperature rises. **Fever** (also known as **pyrexia**[1,2,3]) is a common medical sign characterized by an elevation of body temperature above the normal range of 36.5–37.5 °C (98–100 °F) due to an increase in the temperature regulatory set-point.[4,5,6] This increase in set-point triggers increased muscle tone and shivering. As a person's temperature increases, there is, in general, a feeling of cold despite an increasing body temperature. Once the new temperature is reached, there is a feeling of warmth. A fever can be caused by many different conditions ranging from benign to potentially serious. There are arguments for and against the usefulness of fever, and the issue is controversial. With the exception of very high temperatures, treatment to reduce fever is often not necessary; however, antipyretic medications can be effective at lowering the temperature, which may improve the affected person's comfort. Fever differs from uncontrolled hyperthermia,[1,7,8,9] in that hyperthermia is an increase in body temperature over the body's thermoregulatory set-point, due to excessive heat production and/or insufficient thermoregulation.(10,11,12)

MEDICATIONS

Medications that lower fevers are called *antipyretics*. The antipyretic ibuprofen is effective in reducing fevers in children.[15] It is more effective than acetaminophen (paracetamol) in children. Ibuprofen and acetaminophen may be safely used together in children with fevers.[16,17)The efficacy of acetaminophen by itself in children with fevers has been questioned.[18] Ibuprofen is also superior to aspirin in children with fevers.[19] Additionally, aspirin is not recommended in children and young adults (those under the age of 16 or 19 depending on the country) due to the risk of Reye's syndrome.[20]

There are a number of medicinal herbs that have a long history of use as a natural treatment for fever, as well as traditional herbal combinations that are pleasant to the taste and provide a number of benefits when suffering from fever and chills.

Herbal treatment

In holistic tradition, herbs aren't used to reduce fever unless there is also some positive benefit in treating the infection or inflammation that is causing the fever. Many alternative practitioners see fever as the body's natural response to a pathogen. Fever has been shown to stimulate immune system production of antibodies and may also enhance the body's elimination of toxins. Fevers accomplish much for the body through stimulation of the circulation of both blood and lymph, bringing lymphocytes, immune globulins, and other infection-fighting agents to fight the cause of the disease. Fever also enhances the removal of lysed cells (cell death by breaking of the cellular membrane, often by viral or osmotic mechanisms), spent and infected cells, to be processed by the liver, spleen and lymph nodes. Other than those cases where a fever is too high, if a fever is suppressed, the individual loses the advantages from the body's natural defense system, and the illness may last longer. Therefore, herbalists try to use herbs that support the immune system and enhance other cleansing processes of the body. Diaphoretics help to lower body temperature by promoting perspiration; but they also help to detoxify the body. Circulatory stimulants increase blood flow to the skin and enhance the cleansing sweat. Alteratives may act through an anti-microbial action, through stimulation of the immune system, and help the body clear out the toxins.

Herbs such as willow, meadowsweet, black haw, cramp bark, birch, black cohosh and Indian pipe contain some derivatives of salicylic acid, and are often called "herbal aspirins". While aspirin and its chemical relatives are harsh on the stomach, the salicylin found in herbs is not nearly as strong as acetylsalicylic acid (aspirin) and the action of herbs for fever reduction is much different from synthetic aspirin. Like aspirin, herbs can act as inhibitors for inflammation-causing prostaglandins, but often they achieve this by addressing the imbalance that lead to an elevated incidence of prostaglandins.

Anatomical study

Collection specimens[21,22,23]

The plant specimens for the proposed study were collected from Delhi, Mumbai and Kerala and authenticated by NISCAIR Delhi. Care was taken to select healthy plants and normal organs. Therequired samples of organs were cut and removed from the plant fixed I FAA(Farmalin-5ml+Acetic acid-5ml+70% ethyl alcohol-90ml). After

24hrs of fixing, the specimens were dehydrated with graded series

of tertiary Butyl alcohol as per the schedule given by Sass, 1940. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60 C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning[24,25,26,27]

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12micrometer. Dewaxing of the sections was by customary procedure(Johansen, 1940).The sections were stained with Toluidine blue as per the method published by O'Brien et al.(1964). Since Toluidine blue is apolychromatic stain. The staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls ,blue to the lignified cells, dark green to suberine, violet to mucilage, blue to the protein bodies etc. wherever necessary sections were also stained with safranin and Fast-green and IKI(for starch)

For studying the stomatalmorphology,venationpettern and trihome distribution pardermal section (section taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydrosideoe epidermal peeling of partial maceration employing Jaffry's maceration fluid(Sass, 1940) were prepared.Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerine medium after staining. Different cell component were studied and measured.

Photomicrographs[28,29,30,31]

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nicon lab photo 2 microscopic unit For normal observations brieght field was used.For the study of crystals, starch grains and lignified cells polarized light were employed. Since these structures have birefringent property under polarized light they appear bright under dark background. Magnification of the figures are indicated by the scale bars. Descriptive terms of the anatomical features are as given in the standard anatomy book(Easu ,1964)

HPTLC analysis

Sample were applied in duplicate on pre-coated silica gel 60GF254 aluminium sheets [(3x10) cm] with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software. Development of chromotogramAfter the application of spots, the chromatogram was developed in twin trough glass chamber [(20 x 10) cm saturated with respective solvents for specified time. Detection of spotsThe air-dried plates were viewed in ultra violet radiation of 366nm and 544nm after derivitisation in iodine chamber. The hromatograms were scanned and photo documentation were done. The Rf values and fingerprint data were recorded by WINCATS software.Peak development of different extracts

Two separate concentrations of 2.5µ L and 5µL of each extract were performed separately, and separate track was maintained for each concentration with separate peak development for each extract with two concentrations separately.Alcholic ,aqueus,chloroform and petroleum ether extract were collected and subjected to HPTLC.The test plates used were HPTLC pre-coated,silcica gel 60F 254(Merk),format 10/10cm,thickness 200micro meter,spotting volume 5 micro liter,separation technique ascending,separation chamber twin tough glass chmber 10/10cm(camag),mobile phace:wateralcohol,chloroform and petroleum ether,Relative humidity:55,temperature 30,Mode:absorbance/reflectance,wavelength:254,slide dimension0.3/4cm.

Antipyretic Activity

Antipyretic activity was studied according to (Raoetal).Pyrexia was induced in rats by injecting 20% w/v aqueous suspension of Breweriz yeast intramuscularly. After 18hr, the animals developed 0.5degree to 1degree rise in the rectal temperature. The animals were distributed in four groups of 6 each. Plant extract in the doses of 200mg/kg and 500mg/kg was administered to group 2nd& group 3rd orally. Group 4 was administered with acetyl salicylate (100mg/kg) through I.P. and the control group (group 1) was given 0.5ml normal saline i.p. Rectal temperature was noted at different time intervals. Percentage reduction in rectal temperature was calculated by considering the total fallin temperature to normal level as 100%.

Percentage reduction=(B-C)×100/B-A

Where 'A' denotes normal temperature, 'B' denotes temperature 18hrs after yeast induction & 'C' denotes temperature at time intervals such as 1st h,2ndh &3rdh.

Statistical analysis: The data were statistically analyzed using one way ANOVA followed by Dumettz test for individual comparison of groups with control. 'P' values below 0.05 were considered as significant. Here all values of statistical analysis are expressed as Mean±SEM.

TRANSVERSE SECTION RESULTS

The fruit is a capsularcocci. It is dry deliscent fruit breaking vertically along the sutures.The fruit is 1 mm thick along the pericarp. The pericarp consist of parenchymateousmesocarp consists of parenchymaqteousmesocarp and sclerotic endocarp (figure 1.1).The mesocarp has outer boundary of epidermis which is broken and disintegrated at several places, but retained at certain places (fig 2.1)The epidermis of the mesocarp is 20 micro metre thick and has thick cuticle. The tissues of the mesocarp include thin walled. Compact parenchyma cells. These are wide circular lysigenous secretory cavities distributed all along the circumference of the pericarp (1.1, 2.1)There are also circular vascular strands situated in the mesocarp, the vascular strand consists of a central core of phloem and outer ring of xylum. The vascular strand is ensheathed by a thick cylinder of scelrenhyma cells (fi. 2.1, 2). The sclerotic endocarp is 500 micro meter thick. It consists of inner zone of radially elongated, thick walled cells with wide lumen (fig. 1.2). anarrow zone of squarish or polygonal sclerenchyma cells is located along the inner part of sclerotic region (fig. 1.2, 2.1). The elongated cells occupy 400 micro meter width and squarish cells are 100 micro meter wide.

POWDER MICROSCOPIC RESULTS

The powder preparation of the fruit exhibits the following elements when viewed under microscope.

1) Fragments of epicarp of the fruit are seen scattered in the powder. They appear in surface view (fig.3.1). The cells are polygonal, thin walled and compact, the walls are straight. The cells are 20 x 70 micro meter in size.

2) The inner part of the pericarp which is Sclerenchymatousare also seen in fragments. The cells are small, thick walled and angular in outline (fig. 3.2). They are 60 micro meter in diameter.

3) Isolated brachyscleroids are seen scattered with powder. They are rectangular in shape and have thick lignified secondary walls and prominent circular simple pits (Fig. 4.1, 2.3). The scleroids are 100 micrometer long and 25 micrometer thick. The cell lumen is wide and have no cell contents (fig. 5.1).

4) Fibrescleroid (fig. 5.2). These elements are occasionally seen in the powder, they are along, narrow and tapering at tip. They resemble the fibres but their cell walls have dense canal like pits, the cell lumen is wider than that of fibres.

Different types of scleroids (elongated and rectangular types and squarish types), where studies under the polarised light microscope. The scleroids appear bright against dark back ground, which exhibits lignified nature of their cell walls, (fig. 6.1, 2.3).

ANATOMY RESULTS

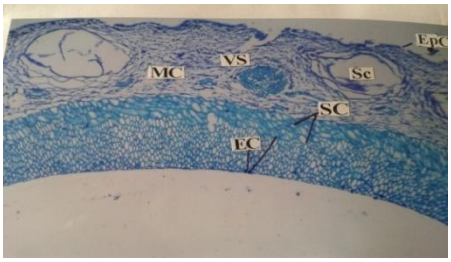


Figure 1.1 – TS of Pericarp of the fruit

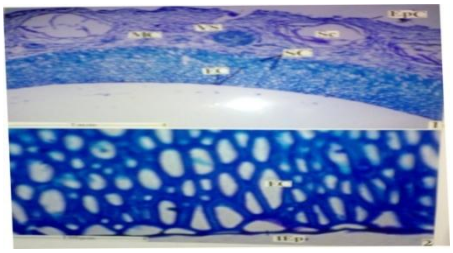
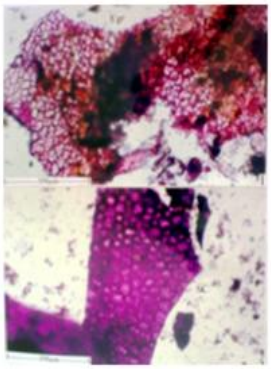


Figure 2 – Inner sclerotic endocarp of the fruit.
(EC: Endocarp; E//pc.Epicarp, I Ep: Inner Epidermis of the endocarp ; MC.
Mesocarp; VS: Vascular strand)
Fig 2.1 : pericarp- a sector enlarged.
Fig 2.2 : vascular strand of the mesocarp - enlarged.(EP : Epidermis of the pericarps , EC : Endocarp , SC : secretory cavity ; Ph : phloem ; VS : Vascular strand', X : xylem)



Fegure:3 Powder Microscopy

1.surface view of the epicarp2. surface view of the endocarp(EP : Epicurus, SC :elerenehymatous endocarp

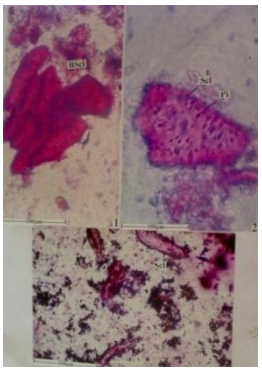
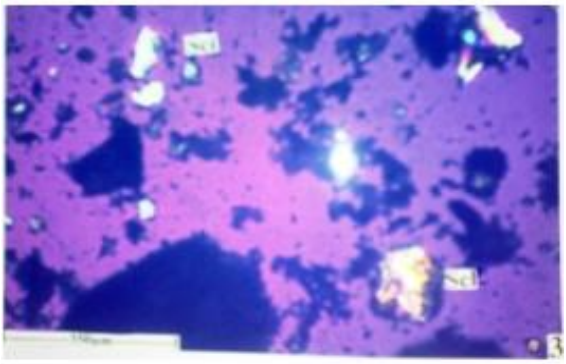


Figure: 4.1, 4.2, 4.3
Fiig 4.1 Elongated scleroid;

Fig 4.2 polyhedral brachyscleroid
Fig 4.3 Elongated sclereids



(Bscl : Braehysclereid; Pi : pit; Sol : scleroid)

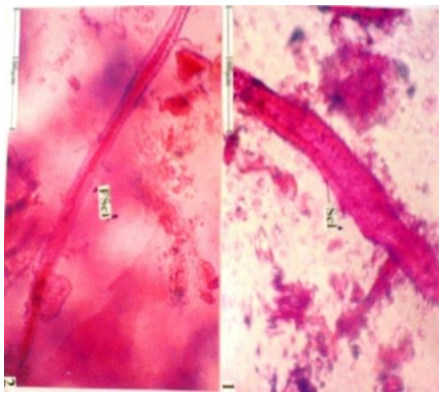


Fig 5.1 : one elongated scleroid - enlarged.
Fig 5.2 :fibrescleroid

(FScle : fibrescleroid; Scl : scleorid)

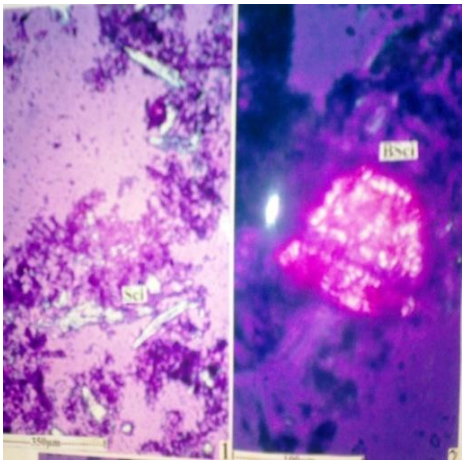
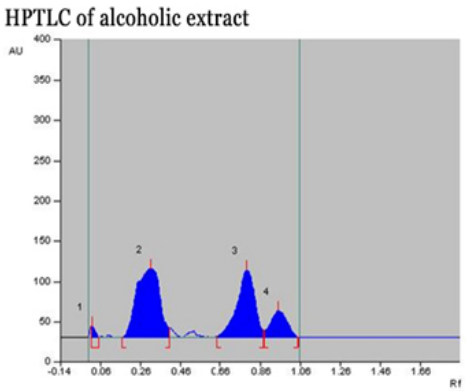
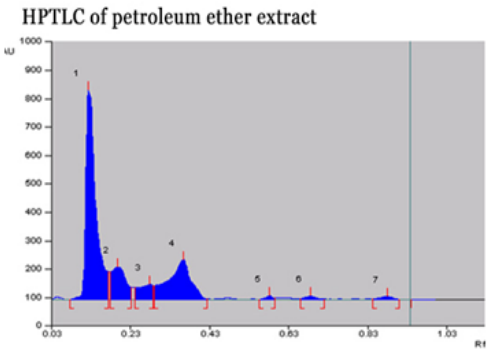


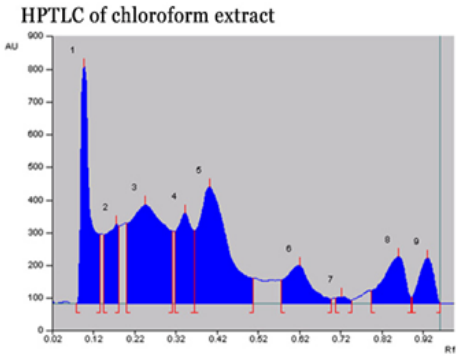
Figure: 6.1,6.2

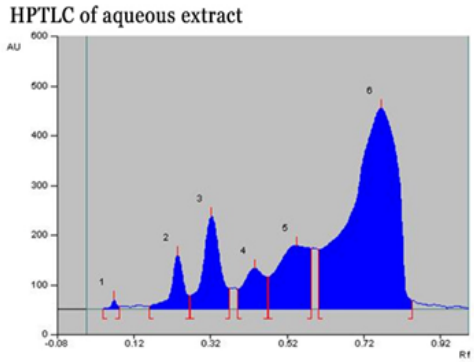
Figure: 6.3
Fig 6.1 - 3: scleroid as seen under the polarized light showing bright lignified wails
(Bscl - Bracyscleroid; Sc1: scleroid).

HPTLC RESULTS









Sl.no	Extract	Mobile phace	Detection	No. Of sports and Rf value
1	Alc.extract	100%chloroform	UV254	3(0.31,0.79 0.95)
2	Chloroformextract	Chloroform:benzene (2:8)	UV254	9(0.1,0.18,0.25,0.35,0.40,0.62,0.72,0.86,0.93)
3	Pet.ether extract	Pet.ether:diethyl ether (6:4)	UV254	7(0.12,0.19,0.28,0.36,0.58,0.68 0.88.)
4	Aques extract	Butanol:aceticacid:water 6:1:3)	UV254	5(0.24,0.33,0.44,0.55, 0.77)

ANTYPYRETIC ACTIVITY

Group	Treatment	Dose	Rectal Temperature(^{0}c)		Rectal Temperature(^{0}c)		
			Normal	18hrs after yeast induction	1 hr	2 hr	3 hr
Group 1	control		37.84±.04	38.91 ±.004[a]	38.91±.003[b]	39.02±.007[b]	39.04±.004[b]
Group2	Plant ext 1	200mg/kg	37.77±.01	38.90±.006[a]	38.49±.005[b] (19.24%)	37.82±.007[b] (50.70%)	37.61±.002[b] (46.94%)
Group3	Plant ext 2	500mg/kg	37.83±.01	38.83±.03[a]	38.78±.01[b] (25%)	38.31±.004[b] (26%)	37.90±.006[b] (46.5%)
Group4	standard	100mg/kg	37.74±.01	38.88±.003[a]	37.80±.01[c] (94.7%)	36.61±.004[c] (199.1%)	37.56±.01[c] (115.7%)

Mean±SEM 'p' value(V/s control by ONEWAY ANNOVA followed by Dunnett's test) is a<0.05 ,b<0.01 , c<0.001

CONCLUSION

The anatomical study and HPTLC indicates a large number of compounds in the plant *Zanthoxylumlimonella*.In the present pharmacological evaluation the fuit extract (ethanolic) of Zanthoxylumlimonella plant was extensively investigated for its antipyretic activity against Brewer's yeast induced pyrexia model in rats. At the end of ourstudy, a strong conclusion can be drawn that,themethanolic extract of Zanthoxylumlimonella . Antipyretic activity more or lessdepending on the dose levels. The methanolicextract of the plant at a dose level of 200mg/kgand5200mg /kg exhibited competent, potent andcomparable results promoting Zantoxylumlimonella.The mechanism of action of antipyretic study can be detected by intense molecular study.

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