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Determination of *In-Vitro* Anti Microbial Activity and Anti-Diabetic Activity Of *Ixora Chinensis*

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ABSTRACT

Since thousands of years natural products have played a very important role in health care and in prevention of disease. To evaluate the anti-microbial & antidiabetic activities of leaf extracts (chloroform and methanol) of *ixora chinensis*. A complete literature survey of *ixora chinensis* revealed that most of the activities reported on leaves and flowers were anti-oxidants, anti-cancer, anti-microbial activities. For preliminary phytochemical screening of the extracts, we performed tests for alkaloids, carbohydrates, flavanoids, tannins, glycosides, saponins, proteins and steroids. From this study, we can conclusively state that methanol, chloroform leaf extracts of *Ixora chinensis* has anti-hyperglycemic activity, anti-microbial activity. These results support the use of the plant in folk medicine to manage microbial infections, diabetics and other related diseases.

Keywords: *Ixora Chinensis,* Phytochemical Screening, *In-Vitro* Anti Microbial & Anti-Diabetic Activity.

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INTRODUCTION

Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. However, the last few years have seen a major increase in their use in the developed world. India is sitting on a gold mine of well-recorded and well-practiced knowledge of traditional herbal medicine. But, unlike China, India has not been able to capitalize on this herbal wealth by promoting its use in the developed world despite their renewed interest in herbal medicines. The ancient civilization of Chinese, Indians and North Africans provide written evidence for the use of natural sources for curing various diseases (phillipson, *et al.,* 2001)[5].The earliest document is a 4000 years old Sumerian clay tablet that records remedies for various illness (Kong *et al.,* 2003)[4]. However it was not until the 19th century that scientists isolated components from various medicinal plants.

Importance of natural products as drugs:

Since Vedic period natural products remain a prolific source for the discovery of new drug and drug leads. Recent data suggests that 80% of drug molecules are natural compounds inspired or natural products (Harvey, *et al.,* 2008). Many studies from 1981 to 2008 reveals that almost 50% of drugs approved since 19947 are based on natural products (Butler, *et al.,* 2008). Eventually there are found to be those traditional medicinal plants which are reported in classic texts like Ayurveda and Charaka samhitha. The bio diversity of India has remained untouched as far as discovery of new chemicals entities is concerned. The conventional rediscovery aims to identify a single active constituent from an active extract and to estimate it in the crude drug. The good examples of drug discovery like Digoxin, Quinine, Morphine etc. which replaced the extracts of their respective plants was responsible for creating an idea that a single active ingredient must responsible for the bioactivity (patwardhan, *et al.,* 2009).Since thousands of years natural products have played a very important role in health care and in prevention of disease. The ancient civilization of Chinese, Indians and North Africans provide written evidence for the use of natural sources for curing various diseases. (phillipson, *et al.,* 2001).The earliest document is a 4000 years old Sumerian clay tablet that records remedies for various illnes (Kong , *et al.,* 2003). However it was not until the 19th century that scientists isolated components from various medicinal plants. Friedrich isolated Morphine from papavera somniferum in 1806. Since then natural products have been extensively screened for their medicinal purposes. According to recent studies conducted by WHO-World Health Organization, about 80% of the world

population relies on traditional medicine ("Traditional Medicine Stratergy") launched (W.H.O news; *et al.,* 2002). About 121 drugs pescrided in USA come either directly or in directly from naturals sources (Benowitz, *et al.,* 1996). 47% of anticancer drugs in the market come from natural products or natural product mimics. Between years 1981 to 2006, about a 100 anticancer agents have been developed of which, 25 were natural product derivatives, 18 were natural products mimics. 11 were derived from natural products pharmacophore and, 9 were pure natural products. Thus natural sources make a very significant contribution to the drug discovery and health care system (Newman, 2003, 2007). Medicinal plants are increasingly in demand in both developing and developed countries (de Silva, 1997). In an effort to improve the efficiency on the practice and to possibly incorporate it into modern medicinal practices, researches are increasingly turning their attention to evaluating the therapeutic potential of medicinal plants (Mc Graw *et al.,* 1997). Keeping in view of the immense uses of natural products as medicine- the *Ixora chinensis* plant was chosen for the perfect study.

Introduction of Plant:

Plants are considerably useful and economically essential. They contain active constituents that are used in treatment of many human diseases. Plants are rich sources of ecologically developed secondary metabolites, which are potential remedies for different ailments. *Ixora coccinea* (linn) (rubiaceae) is known as jungle of geranium or flame of the woods or vetchi in ayurveda. It is a common flowering shrub native to Asia. Its name derived from Indian deity, although there are some 400 species in the genus Ixora coccinea is a dense , multi- branched ever green shrub commonly 4-6ft (1-2-2m) in height, but capable of reaching up to 12ft (3.6m) height. It is traditionally used as anti-diabetic, hepatoprotective, chemo protective, antimicrobial, antioxidant, and anti inflammatory. Decoction of roots used for nausea, hiccups and anorexia. Powdered roots used for sores and chronic ulcers in indo-china, root decoction used to clarify the urine, poultice fresh leaves and stems for sprains, eczema.(Broza siliva fialova, Daniel Grcai *et al.*,2009)

MATERIALS AND METHOD

Plant Collection:

Leaves of plant *ixora cheninsis* were collected from JNTUH-H CAMPUS Hyderabad dist, in the month of December; The Plant material was identified and authenticated by Dr.(Mr), Badraiah and Ramchandra Reddy Head-Department of Botany, Osmania University Campus, and Hyderabad. Authentication vouchers No: 01711, S.No:175. The leaves were dried under shade and were ground to a coarse consistency in a mixer-grinder and kept in an airtight container.

Preparation of Plant Extract:

The powdered leaves of *ixora cheninsis* were successively extracted using solvents in order of increasing polarity, viz chloroform and methanol. After extraction, each time the marc was dried and later extracted with next solvent. The two extracts were dried by distilling the solvents in rotary vacuum evaporator.

PHYTOCHEMICAL SCREENING: (kokate, 2005)

The preliminary phyto-chemical screening on all the extract of *Ixora cheninsis* was carried out as per the procedure and test given below and results are discussed in table no-

The concentrated extracts were subjected to chemical tests as per the methods mentioned below for the identification of th.00e various constituents as per the standard procedures given by **Kokate (2005)** and **Khandelwal (1996).**

EXPERIMENTAL WORK:

***In-vitro* Anti diabetic activity:**

Protein tyrosine phosphate -1B inhibitory assay:

Protein tyrosine phosphatase inhibitory activity was determined according to the modified method of (Goldstein *et al*.2000). Rat liver homogenate was used as a source of protein tyrosine phosphatase 1B. The test compounds (5-50 µm, 5µL) were pre incubated with liver homogenate (3µl) in HEPES 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid) buffer (total volume 50µl) for 30 minutes. Drug assay was performed in a final volume of 200µl in a test mixture containing 10mm of p-nitro phenyl phosphate (Pnpp) in 50 mm HEPES buffer (pH 7.0) and 1mm DTT. After 10min of incubation at 37ºC, the reaction was stopped by addition of µl of 0.1 N NaOH and the absorbance was determined at 410nm. Sodium Orthovanadate was taken as standard for this enzyme assay (Kumar *et al.*, 2009).

***In-vitro* antimicrobial activity:**

Disc diffusion method:

The modified paper disc diffusion was employed to determine the antimicrobial activity of aqueous extract of the herbal preparations. Inoculum was spread over the agar plate using a sterile cotton swab in order to obtain uniform microbial growth. Then the prepared antimicrobial discs were kept over the lawn and pressed slightly along with control. Streptomycin 10µg/disc (Hi- Media) was used as positive control. The plates were incubated for 18 hr at 37ºC.The antimicrobial activity was evaluated and diameter of inhibition zones was measured. Experiment was carried out in triplicate and the average diameter of inhibition zones was measured. Experiment was carried out in triplicate and the average diameter of zone of inhibition was

recorded. The antibacterial activity was classified as highly active (>10mm), mild active (7-10mm) and slightly active (6-7mm) and less than 6mm was taken as inactive.

RESULTS AND DISCUSSION

Plant extract:

Table 1: Following are the yield of the extracts in various solvents

Solvent	% yield	Color	Physical status
Chloroform	6.9	Light green	Solid
Methanol	10.5	Brownish green	Semi solid

PHYTOCHEMICAL SCREENING:

Table 2: preliminary phytochemical screening of leaves extract

Phytoconstituent	Chloroform extract	Methanol extract
Alkaloids	+	+
Carbohydrates	-	-
Flavanoids	+	+
Tannins	+	+
Glycosides	+	+
Saponins	-	+
Proteins	-	-
Steroids	+	+

(+) present, (-) absent

For preliminary phytochemical screening of the extracts, we performed tests for alkaloids, carbohydrates, flavanoids, tannins, glycosides, saponins, proteins, and steroids. While in case of hexane extract, carbohydrates, flavanoids, tannins, glycosides, steroids were detected, in case of chloroform extract, alkaloids , carbohydrates, flavanoids, tannins and steroids were found, alkaloids, flavanoids, tannins, glycosides, saponins and steroids were detected.

***in-vitro* Anti-diabetic activity of Protein tyrosine phosphate -1B inhibitory assay:**

Table 3: Anti-diabetic activity of PTP1B Assay:

Abs without any drugs (no inhibition): 0296

Extract	Blanks	Abs	Actual volume	Max.abs without drug	% inhibition
Chlorofrom	0.463	0.649	0.186	0.296	37.16
Methanol	0.285	0.459	0.174	0.296	41.21
Standard-sodium orthovandate (Conc.10uM)	0.115	0.308	0.201	0.296	32.09

***In-vitro* Anti-microbial activity:**

Table 4: zone of inhibition of various extracts

Plant	Part used	Strains	Zone of inhibition (methanol)	Zone of inhibition (chloroform)
Ixora	Leaf	P.aurignosa	+++	--
Ixora	Leaf	E.coli	+++	--
Ixora	Leaf	S.aurius	++	++
Ixora	Leaf	M.leutisus	--	++

SUMMARY & CONCLUSION:

In first part of the present study, extraction of active constituents using solvents (water, chloroform, and methanol) and preliminary phytochemical screening of various leaf extracts was presented. For preliminary phytochemical screening of the extracts, we performed tests for alkaloids, carbohydrates, flavanoids, tannins, glycosides, saponins, proteins and steriods. While in case of chloroform extract, alkaloids, carbohydrates, flavanoids, tannins, and steroids were found. Alkaloids, flavanoids, tannins, glycosides, saponins, and steroids detected.

In the second part of the present study, pharmacological activities i.e., *in-vitro* anti-bacterial and anti-diabetic activities presented. Disc diffusion method was followed for the anti-microbial activity. Phytochemical screening reveals the presence of tannins, flavanoids, tritepenes, which may be responsible for anti-microbial activity.

Similar *in-vitro* anti-diabetic activity was done by PTP1B (protein tyrosin phosphate 1B) assay. Sodium orthovandate was used as a standard compound (concentration=10um). As the standard compound sodium orthovandate value was 32.09% of inhibition, comparatively methanol extract showed 41.21% of inhibition and chloroform extract showed 37.16% of protein tyrosine phosphate enzyme.

Literature survey revealed that *Ixora chinensis* is used in traditional medicine against different ailments like, gynaecological disorders, uteune fibroids, burns, diarrhoea. To our knowledge, the present study is the systematic positive report on the efficacy of methanol and chloroform extracts. Hence an attempt was made to study the in-vitro anti-diabetic and anti-microbial activities of the leaf extracts. The leaf extracts were effective against P.aurignosa and E.coli. We can conclude methanol and chloroform extracts were having significant anti-diabetic and anti-microbial activities. From this study, we can conclusively state that methanol, chloroform leaf extracts of *Ixora chinensis* has anti-hyperglycaemic activity, anti-microbial activity. These results support the use of the plant in folk medicine to manage microbial infections, diabetics and other related diseases.

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