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IN VITRO EVALUVATION OF ANTI-MICROBIAL AND ANTI-OXIDANT ACTIVITIES OF *TINOSPORA CORDIFOLIA* FOR THE SKIN DISEASES (ECZEMA)

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ABSTRACT

Tinospora cordifolia (Guduchi) , also known as 'heavenly elixir' has been used as traditional medicine since time immemorial. Our present study particularly focussed on its use against skin diseases like eczema. Hot extractions of the powdered drug of whole plant were done with water, methanol, chloroform and petroleum ether. In case of water, cold extraction was done as well. All the extracts after drying were subjected to phytochemical screening, antioxidant activity and anti-microbial activity. Escherchia coli, Salmonella typhi, Bacillus subtillis, Staphyllococcus aureous and Pseudomonas aeruginosa were the strains used for anti-microbial activity. Total phenolic content and flavonoid content was also determined. Among all the extracts, only aq. Hot and aq. Cold extracts showed significant activity against Pseudomonas aeruginosa.

Keywords : Activity, Aqueous, Eczema, Extraction, Tinospora cordifolia

1.INTRODUCTION

Eczema (from Greek ēkzema, "to boil over") is a medical condition where patches of skin become red, inflamed, cracked, itchy, and rough. Blisters may sometimes occur.The word "eczema" is also specifically used to talk about atopic dermatitis, the most common type of eczema[1]. Some people outgrow this condition.It is more common in babies and young children, and often occurs on the faces of infants. It also often appears inside the elbows and behind the knees of children, teenagers, and adults.In rare cases, atopic dermatitis can first appear during puberty or adulthood. It equally affects males and females [2]. When someone refers to eczema, he usually means atopic dermatitis. It is the most common and chronic type of eczema. Other types of eczema include atopic dermatitis, contact dermatitis, dyshidrotic eczema, hand eczema, nummular eczema, and seborrheic dermatitis. Signs of eczema include severe itchiness, burning sensations, blistered skin, fluid drainage, white or yellow pus. A severe infection can also cause fever and chills, as well as other symptoms that mimic the flu. Treatment of infected eczema depends on whether it was caused by virus, bacteria, or fungus.

Viral infections may be treated with anti-viral medications or allowed to heal themselves. Antibiotics are used in infections caused by bacteria. Mild bacterial-infected eczema is first treated with a topical antibiotic. A steroid cream may also be used to reduce inflammation. Oral antibiotics are used in more severe cases of infected eczema. Fungal infections may also be treated with steroids as well as other topical antifungal drugs[3]. These days multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases and is a challenge. The development of antibiotic resistance is multi factorial, including the specific nature of the relationship of antibiotics with the bacteria, the usage of antimicrobial agent, host and environmental factors. This situation forced scientists to search for new antimicrobial agents from various sources as novel antimicrobial chemotherapeutic agents. Some plants are known as medicinal plants because they contain active substances which have a profound influence on various human diseases. Due to this reason, different countries used various plants as a source of many potent and powerful drugs from different plant parts including root, stem, flower, fruit, twigs and modified plant organs . Medicinal plants play a critical role in health care with about 80% of the world's populations relying on the use of traditional medicine, which is predominantly based on plant products. Bacterial resistance to drugs and therapeutic agents created a global hue and cry regarding the search for newer safer drugs. This hurdle can be resolved through the new and innovative antimicrobials from plants. *Tinospora cordifolia* is one of the most versatile and rejuvenative herbs belonging to the family Menispermaceae. It is also known as amrita or nectar of life, as it is very useful in strengthening the immune system of the body and keeping the body functions in harmony . In Ayurvedic preparations ,the stem of T. cordifolia is used as an ingredient for the treatment of general debility, dyspepsia, fevers and urinary diseases. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. For the last few years, a number of studies have been conducted in different countries to prove such efficiency. *Tinospora cordifolia* commonly named as "Guduchi" is a genetically diverse, large, deciduous climbing shrub having[4] greenish yellow typical flowers, found at higher altitudes. In racemes or racemose panicles, the female flowers are solitary and the male flowers are clustered. The season of flowering expands over summers and winters. A variety of active components like diterpenoid lactones, aliphatics, alkaloids, steroids, and glycosides have been isolated from the various parts of the plant body including root, stem, and whole plant. These days, this plant is of great interest to researchers across the globe because of its reported medicinal properties like anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-diabetic, anti-periodic, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory and anti-neoplastic activities. In this paper, we focus our attention to the anti eczemal activity of its various extracts[5]. We determined the in vitro antibacterial activity of its various extracts against human pathogens viz., Gram positive bacteria (*S. aureus*), S*treptococcus*, *Bacillus subtillis* (*B. subtillis*) and Gram negative bacteria (E. coli), (P. aeruginosa) and *Salmonella sp*.

2. MATERIALS AND METHODS

2.1 PLANT MATERIALS

The plant of *T. cordifolia* was collected and authenticated by "SRISTI, Gujarat University Campus, Ahmadabad". The whole plant was then shade dried and powdered.

2.2 PREPARATION OF PLANT EXTRACTS

The dried powdered plant of *Tinospora cordifolia* was subjected to successive extractions untill exhaustion with water (both hot & cold) , methanol (soxhlet) , chloroform (soxhlet) and petroleum ether (soxhlet). Every time, solvent was removed under vacuum to get the dry solid mass for the next extraction.

3 PRELIMINARY PHYTOCHEMICAL SCREENING

1µg of each dried extract was reconstituted in 1ml of its respective solvent of extraction and subjected to phytochemical testing for the presence of different types of compounds or chemical groups using standard methods[6].

Table 1: Phytochemical Screening of Tinospora cordifolia extracts

Phytoconstituents	***Tinospora cordifolia* powder extracts**				
	Aq.Cold extract	Aq.Hot extract	Methanolic extract	Chloroform extract	Pet.Ether extract
Carbohydrates	++	++	++	+	-
Saponins	++	++	-	+	-
Steroids	+	+	+	+	-
Flavonoids	++	+	+	+	-
Tannins	-	-	-	-	-
Alkaloids	+	+	+	+	-
Glycosides	++	+	+	+	-

Note: - represents absence, ++ means abundant; + denotes average

3.1. Determination of Total phenolic content

The total soluble phenolic content was determined by Folin-Ciocalteau reagent method (Malick & Singh, 1980) using gallic acid as a standard reference phenolic compound. 1 ml stock solution of each solvent extract was prepared (1g/ml), from which different aliquots were taken and put into test tubes. In each case, the volume was made up to 3 ml with distilled water. To it, the freshly prepared Folin-Ciocalteau reagent was added. After about 3 min, 2 ml of 20% sodium carbonate solution was added to each test tube and thoroughly mixed. The tubes were then placed in boiling water for one minute, cooled and the absorbance measured in a spectrophotometer at

650 nm against a blank reagent . The concentration of the total phenolic compounds in the extracts was obtained by extrapolating the absorbance of reaction mixture on standard gallic acid graph. Every time, the experiment was repeated thrice and concentration of total phenols was expressed as mg /g of dry extract.

3.2 Determination of total flavonoid content

The total flavonoid content was determined by colorimetric method (aluminium chloride) for all the extracts (Woisky & Salatino, 1998). 0.5ml of the stock solution of the extract(1g/ml), 1.5 ml methanol, 0.1ml potassium acetate (1M) was added to reaction test tubes and volume made upto 5 ml with distilled water. After incubating for 30 min at room temperature, the absorbance of the reaction mixture was measured at 415 nm. The total flavonoid content of all the extracts was calculated by extrapolating the absorbance of reaction mixture on the standard curve of rutin. Every time,the experiment was repeated three times and the total flavonoid content was expressed as equivalent to rutin in mg/ g of the extracts.

Table 2: Total phenolic and total flavonoid contents of *Tinospora cordiflora*

Tinospora cordifolia extract	Total phenolic content(mg/gm)	Flavonoid content (mg/gm)
Aq. Cold extract	1.05 ± 0.21	0.14 ± 0.03
Aq. Hot Extract	1.6 ± 0.24	0.15 ± 0.02
Methanolic extract	4.9 ± 0.29	0.49 ± 0.02
Chloroform extract	2.7 ± 0.29	0.31 ± 0.03

3.3. Evaluation of Antioxidant activities

Scavenging effect on 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH)

Radical scavenging ability of extracts was measured according to Mensor *et al.,* 2001 with little modifications. Briefly, one ml from 0.3mM methanolic solution of DPPH was added to 2.5 ml of samples containing different concentrations of herbal formulation extract[7]. Samples were kept at room temperature in dark conditions and after 30 min optical density (OD) was measured at 518 nm. Antiradical activity (AA) can be determined using following formula:

AA% = 100 – {[(Abs. Sample – Abs. Empty Sample) × 100] / Abs. control}

Where empty samples – 1 ml ethanol + 2.5 ml from various concentrations of all *T. cordifolia* extracts; control sample – 1 ml 0.3mM DPPH + 2.5 ml methanol. OD of samples, control and empty samples were measured in comparison with methanol.

The IC_{50} value was calculated. The extracts with low IC_{50} value are potent scavenger than the extracts with high IC_{50} value. Chloroform extract was found to be the most effective scavenger with least IC_{50} value (0.64 ± 0.05 mg/ml) among all the extracts fallowed by methanolic, cold aqueous and hot aqueous extracts.

Table: 3 DPPH radical scavenging activity of extracts of *Tinospora cordifolia*

Experimental plant name	Plant part used	Solvent of Extraction	(IC_{50}) mg
Tinospora cordifolia	Whole plant	Cold Aq.	1.78 ± 0.04
		Hot Aq.	1.82 ± 0.06
		Methanol	0.76 ± 0.04
		Chloroform	0.64 ± 0.05

4. ANTIMICOBIAL ASSAY

4.1. Preparation of sample dilutions

The stock solution of extract was dissolved in 10% DMSO in water at a final concentration of 10 mg/mL (Konaté et al., 2012). The stock solution of extract and each fraction was sterilized by filtration through 0.22 µm sterilizing Millipore express filter. Negative controls were maintained by using discs impregnated with 10% DMSO in water and commercially available antibiotic diffusion discs ciprofloxacin (25 µg) were used as positive reference standards for all bacterial strains.

4.2.Well-diffusion assay

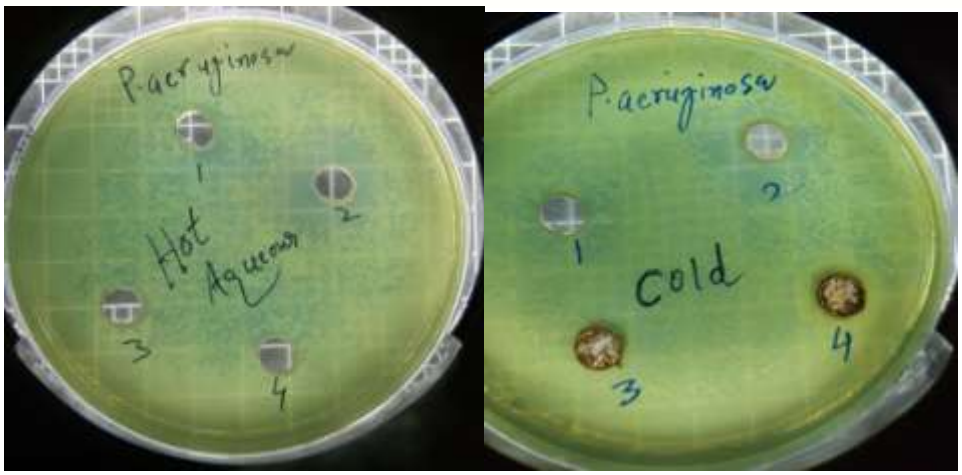
Mueller Molten agar petri plates were made by pouring 20 ml agar solution in each plate, followed by formation of wells. Thenafter inoculation of each plate was done with bacterial suspension(15ul). After complete drying under sterile conditions the wells were filled with test samples and ciprofloxacin was used as positive control and 10% DMSO as negative control. Finally all the plates were incubated for 24h at 37 C .The zone of inhibition was then evaluated . In Each extract the tests were performed in triplicate and the antimicrobial activity was expressed as mean of inhibition diameters.

5.Result

Table 4 : showing zones of inhibition by extracts of *Tinospora cordifoli*

	Zone of inhibition(mm)			
Concentration(µg/ml)	0.25	0.5	1.0	2.0
Aq. Hot	15	19		
Aq. Cold	14	19		
Chloroform				
Pet. Ether				

Fig. 8, Petri plates showing activity against *Pseudomonas aeruginosa*



6. CONCLUSION

Results of the present study reveal that chloroform and methanolic extracts are more potent towards DPPH scavenging activity and aqueous hot and aqueous cold extracts are more potent antimicrobial agents showing significant activity against *pseudomonas aeruginosa*. None of the extracts showed any activity against *Staphyllococcus aureus,* which is mostly involved in eczema. .Consequently, whole plant of *Tinospora cordifolia can* act as a potential source of antioxidants for pharmaceutical use or other purposes.

7. DISCUSSION

Tinospora cordifolia is a highly useful plant. Many effective compounds have been isolated from it, which range from anti-microbials , immune-modulators to anti neoplastics. In our present study, only aqueous hot and cold extracts showed sufficient activity against *Pseudomonas aeruginosa.* Till date, only activity of pet. ether extract against *Pseudomonas aeruginosa* has been reported[8]. In future, we would like to do antimicrobial activities against other pathogenic micro-organisms as well and also analyse the hot and cold aqueous extracts by various techniques like HPTLC, GC-MS, HPLC etc. so that we come to know the constituents responsible for the activity and exploit the same for the human well being.

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