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INVESTIGATION OF HERBAL FORMULATION FOR SKIN ALLERGY

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Abstract:

Skin allergy is when skin becomes irritated because immune system reacted to some antigen. This is called allergic reaction. Temporary outbreak of red, bumpy, scaly or itchy patches of skin, possibly with blisters or welts. Most common skin complaints are Dermatitis, eczema, acne, urticarial, psoriasis, skin allergy etc. Allergic contact dermatitis occurs when your skin comes in direct contact with an allergen. The present study thus compile traditional medicines use in aiding to treat allergic skin disease. The herbs used for this study were *Tinospora cordifolia, Acorus calamus, Coscinium fenestratum, Smilax china , Terminalia chebula* . Methanolic extracts of these herbs were used to perform assays. The results of $Fe3^{+}$ Reducing assay showed a maximum percentage inhibition at 100 µg/ml (IC_{50} = 36.89 %). The anti-inflammatory activity of the combination mixture showed highest percentage inhibition at 80 µl (IC_{50} = 38.04%). The antibacterial activity showed resistance against *Stapylococcus aureus, Pseudomonas aeruginosa, Micrococcus luteus*. In GCMS compounds like Cayophyllene and Methyl eicosa-5,8,11,14,17-pentaenoate were found with anti-inflammatory property. A qualitative phytochemical screening revealed the presence of alkaloids, phenols, flavonoids, terpernoids, glycosides, tannins and saponins.

Key words: skin allergy, *Tinospora cordifolia, Acorus calamus, Coscinium fenestratum, Smilax china, Terminalia chebula*

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I. INTRODUCTION

The skin is one of the largest immunologic organs and is affected by both external and internal factors. many skin disorders are immune mediated.these immunologic mechanisms might have implications for potential targets of future therapeutic interventions (luz fonacier et al.,2009). herbal formulations have been used since ancient times as medicines for the treatment of range of diseases. herbal products are measured to be the symbols of safety in comparison to the synthetic products.(sarojini k et al.,). the use of plants are viewed to be creation of traditional medicine and most commonly ayurvedic ,unani are commonly used(teschke,2014).

Coscinium fenestratum has anti-microbial, anti-diabetic properties for curing diseases and root of this plant considered for wound healing, treating ulcers and also used for skin problems (ravisankar rai v et al.,2013). Tinospora cordifolia commonly known as guduchi. It is reported for medicinal properties like anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, antimalarial, hepatoprotective, immunomodulatory and anti-neoplastic activities. sweet flag is a herbaceous perennial belonging to family acoraceae. the most important health benefits of s.china are energy tonic, impotency and chronic arthritis(qi chung,2019) ,it is also reported to be used in treatment of skin diseases. terminalia chebula belonging family combretaceae possesses anti-bacterial and antifungal activities.All the above mentioned herbs which are able to treat skin allergy and have anti-inflammatory properties were used to brew a herbal tea formulation.

II. MATERIALS AND METHODS

Collection and preparation of plant material

The whole plant parts of Tinospora cordifolia, Acorus calamus, Coscinium fenestratum, Smilax china, Terminalia chebula were collected The leaves were separated from the whole plants and collected. The leaves were washed and dried at room temperature and were crushed coarsely in a mortar and pestle and used for methanolic extraction by adding methanol and left for hrs.

Infusion preparation

A boiling water extraction procedure was used to simulate household brewing conditions (Lusia Barek.et.al 2015). Foreign materials were carefully removed from all plant materials. In order to dry all leaves, they were rinsed in clean water, cut into small pieces, and spread out on paper for 24 hours. An infusion tea bag was filled with 0.25 grams of each herb. The tea bag was added with brown sugar 5 grams. Teabag was steeped in 200ml of water (100°C) for 3 minutes to 5 minutes.

QUALITATIVE ANALYSIS

Phytochemical analysis

Preliminary qualitative screening for phytochemicals, of all these plants was carried out with the following methods,

Test for Alkaloids

In the test tube, a reddish-brown precipitate is formed after adding a few drops of Wagner's reagent to few ml of the plant extract. (Wagner.H, „Pharmazeutische Biologic")

Test for Terpenoids

2 ml of extract was treated with 2 ml of acetic anhydride before adding a few drops of concentrated sulfuric acid. Blue and green rings appeared, indicating the presence of terpenoids. (SavithrammaN.et.al 2011)

Test for Flavonoids (Alkaline reagent test)

A few drops of 1N sodium hydroxide solution were added to 2 ml of extract, causing intense yellow colour to appear. When dilute hydrochloric acid was added, the yellow colour became colourless, indicating flavonoids are present. (ShaP.et.al 2014)

Test for Phenolic Compounds (Ferric chloride test)

5% aqueous ferric chloride was used to treat a few drops of the extract. A deep blue to black colour indicates that phenolic compounds are present in the extract (ShaP.et.al 2014)

Test for Saponins (Foam test)

An extract of 2 ml and 6 ml of distilled water was added to a test tube. The mixture was shaken vigorously until foam developed, which indicates saponins are present. (SavithrammaN.et.al 2011)

Quantitative analysis

Determination of total phenols

Folin-Ciocalteau reagent method was used to determine the total phenolic compounds (SpanosG.A.et.al 1990) with slight modifications. One hundred µL of Composition of the Herbal extract (Table1.1) (1mg/mL) was mixed with 900 µL of distilled water and 1 mL of Folin Ciocalteau reagent (1:10 diluted with distilled water). After 5 min, 1 mL of ethanol solution of Na2CO3 (20%) was added. The mixture was then allowed to stand for 30 min incubation in dark at room temperature. The absorbance was measured by UV-VIS spectrophotometer at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent (µg/mg of extract), which is a common reference compound.

Determination of total flavonoids

The total flavonoid content of Composition of the Herbal extract (Table1.1) was determined using aluminium chloride colorimetric method with slight modification as described by LiuX.et.al 2007 One mL of extract (1mg/mL) was mixed with 0.5 mL of 5% sodium nitrite solution and incubated for 5 min at room temperature. Then, 0.5 mL 10% aluminium chloride solution was added and incubated for further 5 min at room temperature followed by 1 mL of 1 M NaOH solution was added. The total volume was made up to 5 mL with distilled water. Absorbance was measured at 510 nm using spectrophotometer. The result was expressed as (µg/mg of extract) quercetin equivalent.

DPPH radical scavenging activity

The antioxidant activity of Composition of the Herbal extract (Table1.1) was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhydrazyl (DPPH) free radical (BloisMS.et.al 1958.). One mL of 0.1 mM DPPH solution in methanol was mixed with 1 ml of various concentrations (100-600 µg/mL) of plant extracts. The mixture was then allowed to stand for 30 min incubation in dark. Distilled water was used as the reference standard. One mL methanol and 1 mL DPPH solution was used as the control. The decrease in absorbance was measured using UV-Vis Spectrophotometer at 517 nm. The percentage of inhibition was calculated using the following formula:

$$\%\ of\ DPPH^{\cdot}\ radical\ inhibition = ((Control - Sample)/Control) \times 100$$

Superoxide radical scavenging activity

Superoxide radical scavenging activity was carried out by the method of (Ravishankara.MN.et.al 2002). The reaction mixture contains different concentrations of Composition of the Herbal extract (Table1.1), 50 mM of phosphate buffer (pH 7.8), 1.5 mM of riboflavin, 12 mM of EDTA and 50 mM of NBT, added in that sequence. The reaction was started by illuminating the reaction mixture for 15 min. Immediately after illumination, the absorbance was measured at 590 nm and the IC50 was calculated. Ascorbic acid was used as positive control.

$$superoxide\ radical\ inhibition = ((Control - Sample)/Control) \times$$

ANTI-INFLAMMATORY ASSAY:

This test helps to analyse the second stage of wound healing process. Take various concentrations of sample (20-120 µL). Add 2 ml anti- inflammatory buffer and then add 200 µL. Incubate at 54°C for 30 minutes.

% Inhibition of haemolysis = [(Abs (control) – Abs (Sample)/Abs (Control)] x 100

ANTIBACTERIAL ACTIVITY:

Microbial strains:

The microorganisms of Gram positive strains such as *Micrococcus luteus* (ATCC 4698) and *Staphylococcus aureus*(ATCC 25923) as well as Gram negative strains such as *Pseudomonas aeruginosa*(ATCC 15442) were used for the evaluation of antibacterial activity. Antibacterial activity of extract was carried out using agar well diffusion method (Kubo, I., *et.al*., 2002). The solidified nutrient agar in the petri plates was inoculated by dispensing the inoculum using sterilized cotton swabs which is previously immersed in the inoculum containing test tube and spread evenly onto the solidified agar medium. Five wells were created in each plate with the help of a sterile well-borer of 8 mm diameter. The root tuber extract was then poured into each well containing 250, 375 and 500 µg/mL concentrations. All the plates with extract loaded wells were incubated at 37ºC for 24 h and the antibacterial activity was assessed by measuring the diameter of the inhibition zone formed around the well. Tetracycline (25 µg) was used as positive control.

FORMULATION OF FOOD PRODUCT

The tea was formulated by mildly crushing the above mentioned herbs after shade drying and was stuffed into tea bag.

III. RESULTS

A. PHYTOCHEMICAL ANALYSIS

Yellow colour precipetate appears indicates present of alkaloids. Dark brown colour indicates the presence of phenol. Blood red colour indicates the presence of glycosides. A reddish-brown ring coloration of the interface indicates the presence of terpenoids. A yellow colour formation within short period is positive for flavonoids. Observed for brownish green coloration for tannins present. No formation 2cm layer of foam indicated the absence of saponins. No Brown precipitate formation indicates for negative for steroids.

Table 1:phytochemical test:

S.NO	Phytochemical test	Inference
1.	Alkaloids	Present
2.	Terepenoids	Present
3.	Phenolic compounds	Present
4.	Flavanoids	Present
5.	Tannins	Present
6.	Glycosides	Present

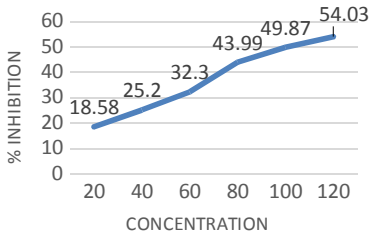
B. QUANTITATIVE ANALYSIS

The total flavonoid and phenol content was 52.04µg/mg and 642.3 µg/mg

Phytochemicals	Amount (µg/mg)
Flavonoids	52.4±0.31
Phenols	642.3±0.45

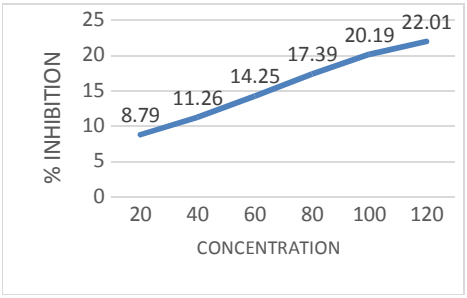
C. DPPH ASSAY

A DPPH-based radical (1,1-diphenyl-2-picrylhydrazyl radical) was used to assess the ability of extracts to scavenge free radicals (Kedare SB.et.al 2011). The maximum DPPH˙ radical scavenging activity was 49.87±0.50% at 100 µg/mL concentration. The IC50 was found to be 100.26 µg/mL concentration and was compared with standard (Ascorbic acid, IC50 = 26.41 µg/mL concentration).



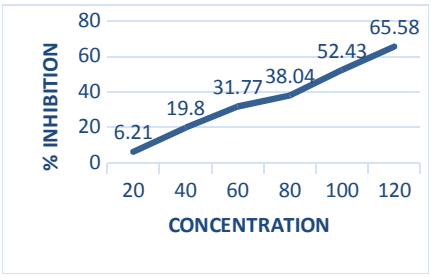
D. SUPEROXIDE ASSAY

Antioxidants are able to inhibit the blue NBT formation and the decrease of absorbance with antioxidants indicates the consumption of superoxide anion in the reaction mixture (Gülçin.İ.et.al 2002). At the concentration of 120 µg/mL, the extract was found to have the maximum superoxide radical scavenging activity of 22.01±0.73% and the IC_{50} was 272.60 µg/mL concentration. It was compared with the standard of ascorbic acid (IC_{50} = 18.12 µg/mL concentration).



E. ANTI-INFLAMMATORY ASSAY

Human red blood cell membrane stabilization (HRBC method) has been used as a method in estimating the anti-inflammatory property (Ejebe.et.al 2010). The maximum hemolysis inhibition was 38.04±1.91 at 80 µg/mL concentration and the IC_{50} was 105.15 µg/mL concentration. It was compared with the standard of Asprin (IC_{50} = 137.14 µg/mL concentration).



F. ANTIBACTERIA ASSAY

s. no	Organism	Zone of inhibition(mm)			
		STD	250µl	375µl	500µl
1.	*Staphylococcus aureus*(ATCC 25923)	29	18	20	21
2.	*Micrococcus luteus*(ATCC 4698)	27	20	22	23
3.	*Pseudomonas aeruginosa*(ATCC 15442)	22	21	23	24

G. EVALUATION OF FOOD PRODUCT UNDER VARIOUS PARAMETERS

S.NO	PARAMETERS:	OBSERVATION
	ORGANOLEPTIC:	
1	State	liquid
2	Odour	Characteristic (leafy smell)
3	Touch	watery
4	Appearance	Characteristic as green tea
5	Flow property	Free flowing
6	Colour	brown
7	Flavour	vegetal
8	After mouthfeel	Cooling, Herbaceous
	PHYSICO CHEMICAL:	
9	pH	6.9-7.2
10	Temperature	100□
11	Preservatives	Nil
12	Shelf life	7 days (room temperature)

The organoleptic parameters are by involving sense of organs .The naked eye test is just an observation of the formulated tea by eyes.The test results matched the characteristic of a tea. There are no preservatives added and

temperature is boiling point of water as tea formulated here is by infusion, so water is brought to boiling temperature and tea bag is steeped inside and left for infusion.

IV. CONCLUSION

The above herbs were used for preparation of herbal infusion (herbal tea). The present study spotlights the use of traditional herbs in modern on the go food formulation. The estimation tests proved the presence of flavonoids, phenols, phytochemicals, antibacterial and anti-inflammatory properties.

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