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## Evaluation of Anthelmintic Activity & Phytochemical Screening of the Peels of *Citrussinensis* & Rhizomes of *Curcuma longa*

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### ABSTRACT

Phytochemicals are secondary metabolites produced by all plants in which some have medicinal uses. The phytochemical analysis of peel & rhizome extracts in aqueous, ethanol, acetone, hexane, and chloroform extracts of indigenous medicinally important plants of *Citrussinensis* (peels) & *Curcuma longa* (dried rhizomes) were investigated. The phytochemical analysis revealed the presence of active constituents such as carbohydrates, flavonoids, alkaloids, terpenes, phytosterols, tannins, steroids, saponins, glycosides, phenols, and anthraquinones. This research supports the local use of the peel and rhizome extracts of orange and turmeric to show the potent nature of the sealants when using in combination to treat helminthiasis. These plants belong to family rutaceae & zingiberaceae respectively. The present study provides evidence that the solvent extract of *Citrussinensis* and curcumin along contains medicinally important bioactive compounds and this justifies the use of these plants in combination to treat helminthiasis & control mode growth in intestines.

### Key words:

Phyto chemical screening,  
Indigenous, *Citrussinensis*,  
*Curcuma longa*, peel extract, rhizome extract.

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## INTRODUCTION

The use of medicinal plants for the treatment of many diseases is associated to folk medicine from different parts of the world. Natural products from some plants, fungi, and other organisms, continue to be used in pharmaceutical preparations either as pure compounds or as extracts. An increasing interest in herbal remedies has been observed in several parts of the world and many of the herbal remedies have been incorporated into orthodox medicinal plant practice.

Diseases that have been managed traditionally using medicinal plant include malaria, epilepsy, infantile convulsion, diarrhea, dysentery, fungal and bacterial infections. Medicinal herbs considered to be a chemical factory as it contains multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene, lactones and oils.

Helminths are the parasitic worms which are large in size and so called macro parasites. The adult worms can be seen with naked eye. Many of them are transmitted via soil and infect the gastrointestinal tract, which makes the intestinal worms. Some parasitic worms including leech and monogeneans, are ectoparasites, thus, they are not classified as helminths, which are endoparasites. Any disease or infection caused due to helminth is known as helminthiasis, helminth infection. They often live in the gastrointestinal tract of their hosts, but they may also burrow in to their organs, where they induce physiological damage. Helminthiasis has been found to result in poor birth outcome, poor cognitive development, poor school and work performance, poor socio economic development, and poverty.

## MATERIALS & METHODS

### COLLECTION & PREPARATION

The fruit of *Citrussinensis* and rhizomes of *Curcuma longa* were purchased from the local market of Visakhapatnam, South India. The plant and the plant material were identified and authenticated by the department of botany. Citrus fruits were washed thoroughly by using tap water and were peeled off manually. All the peels were segregated into two halves where one half was dried at room temperature for 10 to 12 days. The dried peels were further made into small size and stored in air tight bag for the later extraction process. The rhizomes of *Curcuma longa* are washed thoroughly in water, cut into small pieces and air dried for 2 weeks at 35 to 40°C and stored in air tight containers for further studies.

### EXTRACTION

Extraction is the first step to separate the desired natural products from the raw materials. Solvent extraction is the most widely used method. The extraction of natural products progresses through the following stages: (1) the solvent penetrates into the solid matrix; (2) the solute dissolves in the solvents; (3) the solute is diffused out of the solid matrix; (4) the extracted solutes are collected. Soxhlet extraction is been used for the extraction process. Extractions use two immiscible phases to separate the substance from one phase into the other.

### PHYTOCHEMICAL ANALYSIS

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were

carried out in extracts as well as powder specimens using the standard procedures.

## PHYTO CHEMICAL SCREENING

### TEST FOR CARBOHYDRATES

#### MOLISCH'S TEST

To the test solution add few drops of alcoholic alphanaphthol then add few drops of concentrated sulphuric acid through the sides of test tube wall purple to violet colouring appears at the junction.

#### BARFOED'S TEST:

1ml of test solution is heated with 1ml of barfoed's reagent on water bath, if red cupric oxide is formed, mono saccharide is present. Disaccharide on prolonged heating (about 10 min) may also cause reduction, owing to partial hydrolysis to mono saccharides.

#### FEHLING'S TEST

Add 1 ml each of feehling's solution A & B to 1 ml of test solution and heat in a water bath, if red precipitate of cupric oxide is formed, it indicates the presence of carbohydrates.

### TEST FOR ALKALOIDS

#### MAYER'S TEST

Alkaloids give cream colour precipitate with mayer's reagent (potassium mercuric iodide solution)

#### WAGNER'S TEST

Alkaloids give red dish brown precipitate with wagner's reagent (iodine-potassium iodide solution)

#### 3) DRAGON DORFF'S TEST

Alkaloids give red dish brown precipitate with dragon dorff's reagent (potassium bisulphide solution)

### TEST FOR TANNINS

#### TEST WITH FERRIC CHLORIDE

Tannins give bluish black or brownish green colour with ferric chloride.

#### TEST WITH LEAD ACETATE

Tannins are precipitated by salts of lead.

### TEST FOR FLAVONOIDS

The extract (1 ml) was diluted in 1 ml of diluted sodium hydroxide, formation of yellow precipitate indicated the presence of flavonoids.

### TEST FOR STEROIDS

The extract (1 ml) was dissolved in 2 ml of chloroform in a test tube, and then 1 ml of concentrated sulphuric acid was added, formation of reddish brown colour at the inter-phase indicated the presence of steroids.

## TEST FOR PHENOLS

### TEST WITH FERRIC CHLORIDE

The extract (1 ml) was added with 1 ml of 10 % ferric chloride. The formation of a greenish brown precipitate indicated the presence of phenols.

### TEST FOR SAPONINS

#### FROTHING TEST

2 g of extract was mixed and boiled with 20 ml of water and then filtered. 5 ml of distilled water is added in 10 ml of this filtrate and was shaken vigorously for stable persistent froth. The formation of froth shows the presence of the saponins in extract.

### TEST FOR ANTHRAQUINONES

0.5 g of the extract was boiled with 10 ml of sulfuric acid and filtered while hot. 5 ml of chloroform was used to shake the filtrate. 1 ml of dilute ammonia was added in the chloroform layer. The resulting solution was observed for colour changes.

### TEST FOR TERPENOIDS

To 0.5 g each of the extract was added 2 ml of chloroform. To form a layer, concentrated sulfuric acid (3 ml) was carefully added. A reddish brown appearance of the interface indicates the presence of terpenoids.

### TEST FOR GLYCOSIDES

#### TEST FOR CARDIAC GLYCOSIDES

#### LEGAL'S TEST

Treat the test solution with pyridine and alkaline sodium nitroprusside solution, blood colour appears.

### TEST FOR SAPONIN GLYCOSIDES

#### FROTH FORMATION TEST

Place 2 ml solution of drug in water in a test tube, shake well, stable froth for this formed.

### TEST FOR FLAVANOID GLYCOSIDES

To the extract add Sodium hydroxide solution, yellow colour appears now add dilute sulphuric acid and the colour disappears and this indicates the presence of flavanoid glycosides.

### TEST FOR PHYTOSTEROLS

#### SALKOWSKI TEST

Dissolve cholesterol in 2 ml of chloroform in dry test tube. Add equal amount of concentrated sulphuric acid ( $H_2SO_4$ ). Shake gently, the upper layer turns red and the sulphuric acid layer shows a yellow colour with green fluorescence.

#### LIEBERMAN-BURCHARD TEST

Dissolve 1 or 2 crystals of cholesterol in dry chloroform in a dry test tube. Add several drops of acetic anhydride and then 2 drops of concentrated  $H_2SO_4$  and mix carefully which gives a deep green colour.

## RESULTS & DISCUSSION

The data revealed that the various sex tracts obtained from the peels of *Citrus sinensis* & *Curcuma longa* as a combination showed anthelmintic activity at 50 mg/ml, while the ethanolic extract showed significant results, which makes it a standard solvent. The concentrations of 10mg/ml, 20 mg/ml, 50mg/ml paralyzed at the same time but the time taken for death differed, out of these three concentrations, the plant drugs showed optimum anthelmintic activity at 50mg/ml concentration.

Potency of the extract was inversely proportional to time taken for paralysis and death of earth worms. The results were compared to standard drug ivermectin of various concentrations.

Therefore the activity shown by a combinational drug is more potent when compared to individuals.

**Tab 1: Anthelmintic activity of various extracts obtained from *Citrus sinensis* & *Curcuma longa* with different solvents:**

S.No	Plant extracts	Conc(μ/ml)	Time taken for paralysis (min)	Time taken for death (min)
1.	Vehicle (control saline)	-	-	-
2.	Chloroform extracts	10	42.16±0.61	75.51±0.41
		25	35.29±0.28	68.28±0.12
		50	25.48±0.35	34.14±0.50
3.	Ethanolic extracts	10	37.75±0.52	68.66±0.13
		20	27.25±0.21	50.18±0.73
		50	20.11±0.72	26.09±0.76
4.	Hexane extracts	10	30.14±0.16	51.61±0.52
		20	18.52±0.15	34.49±0.58
		50	9.41±0.13	30.12±0.62
5.	Acetone extracts	10	40.10±0.57	65.48±0.38
		20	32.51±0.27	52.20±0.82
		50	23.81±0.32	32.10±0.45
6.	Ivermectin	10	16.24±0.84	42.14±0.21
		20	14.19±0.21	24.13±0.20
		50	7.14±0.22	19.32±0.27
7.	Ethanolic extract of volatile orange oil	10	15.22±0.60	18.30±0.24
8.	Ethanolic extract of volatile curcuma oil	10	14.21±0.21	14.13±0.21

9.	Ethanolic extract of both <i>Citrus sinensis</i> & <i>Curcuma longa</i> in combination	10	16.22±0.52	18.28±0.56
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## CONCLUSION

Ethanolic extract of *Citrus sinensis* at the concentration of 10mg/ml showed the time of paralysis & death at 15 min and 18 min respectively. Ethanolic extract of *Curcuma longa* at concentration 10 mg/ml showed the time of paralysis & death at 16 min and 18 min respectively. While the combination of both *Citrus* & *Curcuma* at concentration 10mg/ml showed the time of paralysis & death at 14.2 min and 14.3 min respectively. Finally it can be concluded that the combination of both *Citrus sinensis* & *Curcuma longa* gives a potent extract which shows significant anthelmintic activity against earth worms. The current study leads to a conclusion that ethanolic extract of the plants possesses a unique property when compared with the prevalent drug. Further investigation is needed in order to isolate the phytochemical constituents responsible for anthelmintic activity.

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