

Phytochemical analysis and antimicrobial activity of *nyctanthes arbor-tristis* and *curcuma caesia* leaf extract against pathogens

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Abstract

Medicinal plants have bioactive compounds which are used for curing of various human diseases. Medicinal plants have antibacterial activities. The present study involves two different medicinal plants *Nyctanthes arbor-tristis* and *Curcuma caesia* leaves locally available in Bhilai region of Chattisgarh, India. The main objective of the research work was to check the presence or absence of the phytochemical constituents in all the selected medicinal plants. The results of the phytochemical analysis of these medicinal plants showed that the terpenoids, phlobatannins, reducing sugar, flavonoids and alkaloids were found to be present in afore mentioned medicinal plants. Comparative study of bioactive compounds present in both plant extracts revealed their probable pharmaceutical use in the treatment of diseases in human being.

Keywords: Phytochemicals, *nyctanthes arbor-tristis*, *curcuma caesia*

INTRODUCTION

Plant based medicines are the basis of many of the modern pharmaceutical industries present for our various ailments. Medicinal plant having one or more of its parts contain substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs (Sofoworo, 1982). Most of the plants used in traditional Indian system of medicine have been found active against a wide variety of microorganisms (Khan et al., 1994; Ahmad et al., 1998; Ahmad & Beg, 2001). Phytochemical compounds are present in plant parts are the natural bioactive compound found in plants, which work with fibers and nutrients to form an integrated part of immune system against stress conditions and various diseases. In the present study we not only evaluated the important bioactive compounds of both plants but also did its comparative study. The quantitative estimation of various trace elements concentration is necessary for effective dose concentration and also for understanding their pharmacological actions.

Phytochemical compounds are unit gift in plant elements area unit the natural bioactive compound found in

plants, that work with fibers and nutrients to create an integrated a part of system against stress conditions and numerous diseases. The present study deals with the phytochemical standardization and antimicrobial activity of chloroform extract of *Nyctanthes arbor-tristis* and *Curcuma caesia* leaves. The most aim of study is to screening the phytochemical compound and there antimicrobial susceptibility against pathogen of crude extract of *Nyctanthes arbor-tristis* leaves and roots. The extract therefore obtained when standardization could also be used as healthful agents.

Classification of *Nyctanthes arbor-tristis* figure 1:

Kingdom:	Plantae
Clade:	Angiosperms
Clade:	Eudicots
Clade:	Asterids
Order:	Lamiales
Family:	Oleaceae
Genus:	Nyctanthes
Species:	N. arbor-tristis

Classification of *Curcuma caesia* figure 2:

Kingdom:	Plantae
Clade:	Angiosperms
Clade:	Monocots
Clade:	Commelinids
Order:	Zingiberales
Family:	Zingiberaceae
Genus:	<i>Curcuma</i>
Species:	<i>C. caesia</i>

MATERIAL AND METHODS**Sample collection**

The plant parts of *Nyctanthes arbor-tristis* and *Curcuma caesia* leaves were collected from field of Durg and Bhilai, Chattisgarh and then the plant identified taxonomically and was preserved for extraction.

Extraction

The Extraction of *Nyctanthes arbor-tristis* and *Curcuma caesia* leaves from Hot and Cold Extraction procedure was done by Soxhletion and Maceration method using organic solvent (Chloroform).

Readiness of solvent extracts for monocot family caecia rhizomes

The rhizomes of monocot sort *caecia* were legitimately washed with running water, at that point once evacuation rhizomes were shed dried and pulverized to ask powder. Dried powder of monocot variety caecia rhizomes in regards to 50gm were hot separated with 5 hundred cc fuel abuse soxhelt instrumentation. The soxheltion at 30°C was improved the situation multi week to get separate. The concentrate was vanished in water shower at 70°C to get rough for phytochemical investigation. When the all out vanishing, the heap of the concentrates was recorded and after that marked. The extractions put away independently at 4°C in impermeable.

Maceration technique for *Nyctanthes arbor-tristis* and *Curcuma caesia* leaves

After Dried powder of *Nyctanthes arbor-tristis* and *Curcuma caesia* leaves about 50gm were of cold extracted with 200 ml Petroleum Ether using rotary shaker and were incubated for 1 week at 25°C at least 5 times vibration per day. The extract were filtered exploitation textile material and gaseous exploitation rotary distillation instrumentation, this extract dried in 50°C oven for 24 hours and finally kept at 4°C temperature applied the phytochemical Test, the upper than phytoconstituents were found in monocot *Curcuma caecia* rhizomes extract.

Identification tests for phytochemical constituents

The tests were performed to seek out the presence of active

chemical constituents like carbohydrates, proteins, starch, amino acids, steroids glycosides, flavonoids, alkaloids, tannins, saponins, phenols by the subsequent procedure. Phytochemical analysis was applied for all the extracts mistreatment normal ways.

Test for carbohydrate

Molisch's test: 3 ml of Molisch's reagent was added to the 3 ml of test solution, shaken for few minutes. Then 2 ml of concentrated sulphuric acid was added slowly from the sides of the test tube. The development of a purple ring at the junction of two liquids indicates the presence of carbohydrates.

Test for proteins

Biuret test: 3 ml of the test solution was treated with 4% sodium hydroxide (3- 5 drops) and 1% copper sulphate solution (3-5 drops). The appearance of blue colour indicates the presence of proteins.

Test for quinones

About 0.5 g of plant extract was taken and additional one



Figure 1: *Nyctanthes arbor-tristis*.



Figure 2: *Curcuma caesia*.

cc of extract and one cc of concentrate H_2SO_4 was additional formation of red color shows the presence of quinones. One drop of ethanolic Test resolution is placed on a filter-paper, followed by one drop of ethanolic phenylacetone nitrile resolution and one drop of 0.1 N hydroxide. A positive response is indicated by the looks of a blue or violet stain edged by a yellow ring.

Test for aminoacids

Ninhydrin test: Test solution (3 ml) and 3 drops of 5% lead acetate solution were boiled on water bath for 10 min. Change in the colour of solution to purple or blue indicates the presence of amino acids.

Test for Steroids

Salkowski test: Chloroform (2 ml) and 2 ml of concentrated sulphuric acid were added to 2 ml of test solution, shaken and allowed to stand. Change in the colour of lower chloroform layer to red and acid layer to greenish yellow fluorescence indicates the presence of steroids.

Test for glycosides

Keller-Kiliani test

Glacial acetic acid (3-5 drops), one drop of 5% $FeCl_3$ and conc. Sulphuric acid were added to the test tube containing 2 ml of T.S. Appearance of reddish-brown color at the junction of two layers and bluish green in the upper layer indicates the presence of glycosides.

Test for flavanoid

Shinoda test: To the powdered extract (10 mg), 5 ml of ethanol (95%), 3 drops of hydrochloric acid and 0.5 gm magnesium turnings were added. Change of colour of solution to pink indicates the presence of flavonoids.

Test for alkaloids

To the dry extract (20 mg) dilute hydrochloric acid (1-2 ml) was added, shaken well and filtered. With filtrate the following tests were performed.

Mayer's test: To the 3 ml of test solution 3 drops of Mayer's reagent (potassium mercuric iodide) was added. Appearance of reddish brown or cream precipitate indicates the presence of alkaloids.

Dragendorff's test: 3 ml of the test solution was mixed with Dragendorff's reagent (potassium bismuth iodide). Appearance of reddish brown precipitate indicates the presence of alkaloids.

Test for terpenoids

Salkowski test: Concentrated sulphuric acid (2 ml) was added to 2 ml of test solution. The solution was shaken and allowed to stand. The colour of lower layer changes to yellow indicating the presence of triterpenoids.

Test for Saponins

Foam test: Powdered extract (10-20 mg) was shaken vigorously with water (1 ml). Development of persistent foam which is stable at least for 15 minutes indicates the presence of saponin.

Test for phenols

Ferric chloride test: Test extract were treated with 4 drops of Alcoholic $FeCl_3$ solution. Formations of bluish black colour indicate the presence of Phenol.

Antimicrobial activity

The disc diffusion method (Kirby Bauer *et al.*, 1966) was used to test antibacterial activity of the extracts against pathogenic bacteria.

RESULTS AND DISCUSSION

Antifungal activity by disc diffusion assay

Antimicrobial activity by well diffusion assay:

Plants which shows that phytochemical constituent's i.e., terpenoids, flavonoids, alkaloids, reducing sugars and phenols etc. are either present or absent in these plants and the results were summarized in Table 1. In our studies it was investigated that alkaloids and flavonoids are present in *N. arbor-tristis* and *C. caesia* leaves extracts, whereas reducing sugars and terpenoids were found to be absent. In previous studies it was reported that flavonoids and terpenoids were present in chloroform extract of the *Nyctanthes arbor-tristis* and *Curcuma caesia* leaves extracts (Pietta, 2000) while alkaloids and phenols were found in it Figures 3-8 and Tables 1-5.

Many biochemical constituents of plants have been shown to possess excellent biological activities (Gupta *et al.*, 1993; Cowan, 1999; Iwu *et al.*, 1999; Ogunleye & Ibitoye, 2003; Tshikalange *et al.*, 2005). Both selected medicinal plants for screening were found to possess tannins. Tannins have amazing stringent properties. They are known to hasten the healing of wounds and inflamed mucous membranes. Flavonoids are also present in both selected medicinal plants as a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity (Rio *et al.*, 1997; Salah *et al.*, 1995). It also helps in managing diabetes induced oxidative stress. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer. Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, anti hyperglycemic and anti-inflammatory and immune modulatory properties (Rabi *et al.*, 2009; Wagner *et al.*, 2003). In addition, terpenoids can be used as protective substances in storing agriculture products as they are known to have insecticidal properties as well (Sultana *et al.*, 2008). In these present study the phyto-constituents

Table 1. Comparative Table of Phytoconstituents of *Nyctanthes arbor-tristis* and *Curcuma caesia* leaves extracts.

S.No	Phytochemicals	<i>Nyctanthes arbor-tristis</i>	<i>Curcuma caesia</i>
1	Alkaloids	+ve	+ve
2	Terpinoid	+ve	+ve
3	Phenols and Tannins	+ve	+ve
4	Saponins	+ve	-ve
5	Flavonoids	-ve	-ve
6	Quinines	-ve	-ve
7	Proteins	-ve	-ve
8	Steroids	-ve	-ve
9	Cardiac Glycosides	-ve	-ve
10	Carbohydrates	-ve	+ve
11	Amino Acid	-ve	-ve

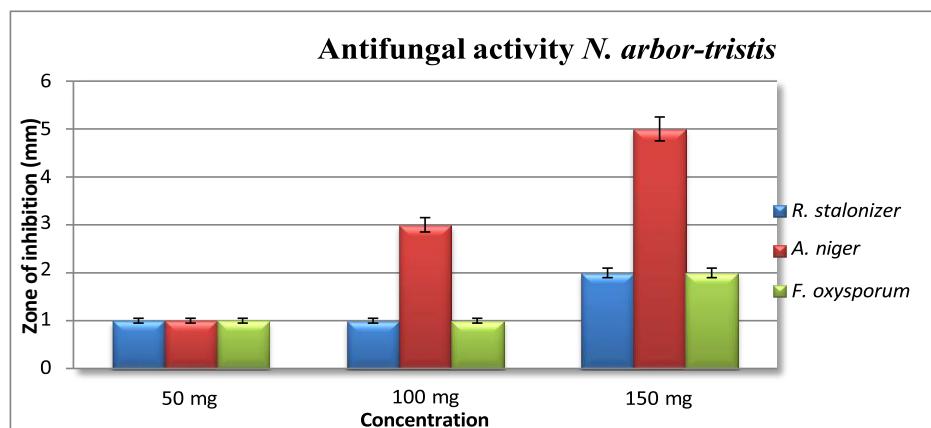
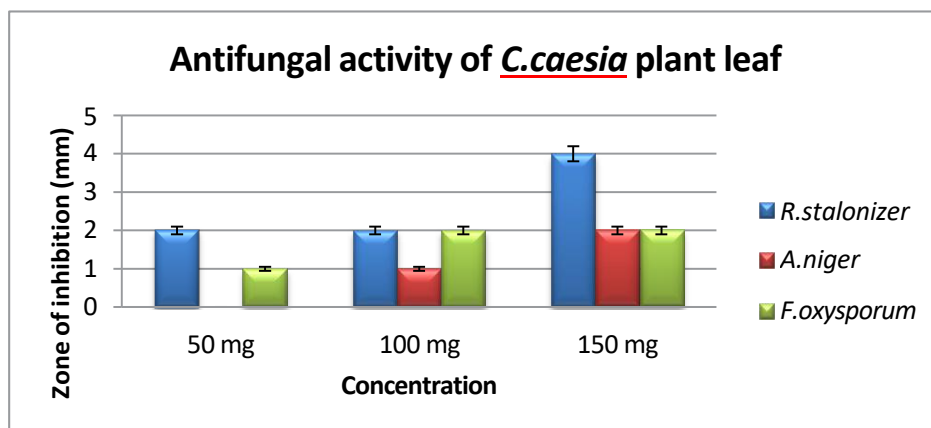
**Figure 3:** Antifungal activity of *N. arbor-tristis* and *C. caesia* leaves of different solvent system.**Figure 4:** Antifungal Activity of Chloroform Extract of *N. arbor-tristis* Plant leaf.**Figure 5:** Antifungal Activity of Chloroform Extract of *C. caesia* Plant leaf.



Figure 6: Antibacterial activity of *N. arbor-tristis* and *C. caesia* of different solvent system.

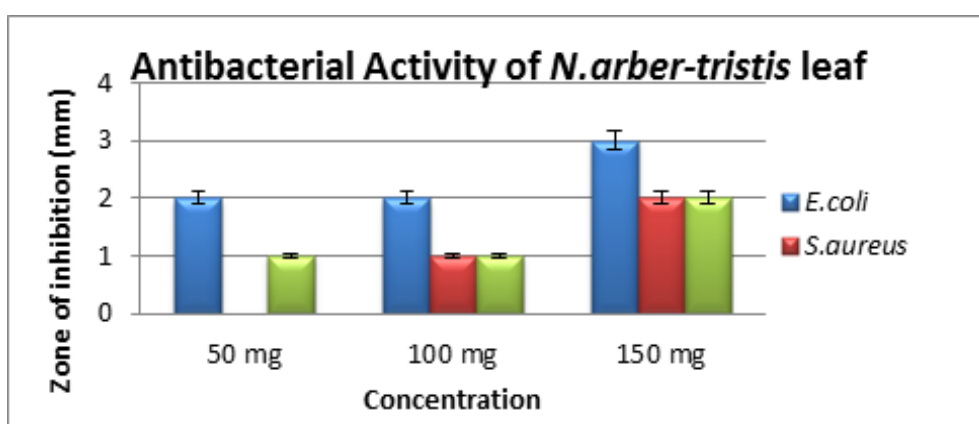


Figure 7: Antibacterial Activity of Chloroform Extract of *N. arbor-tristis* Plant leaf.

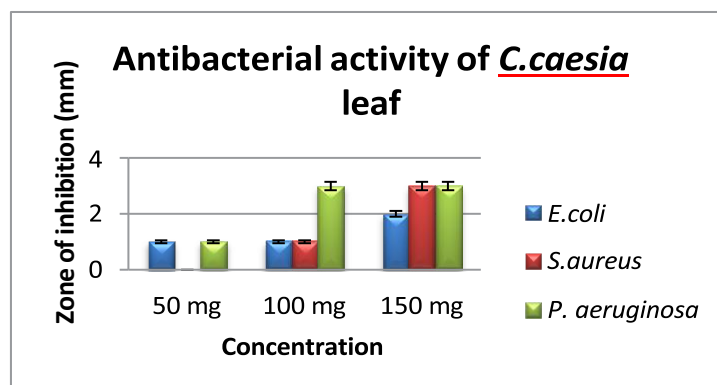


Figure 8: Antibacterial Activity of Chloroform Extract of *C.caesia* Plant leaf.

Table 2. Antifungal activity of chloroform extract of plant leaf.

NAT, Leaf Chloroform		Concentration		
		50 mg	100 mg	150 mg
<i>R. stolonifer</i>		1 mm	1 mm	2 mm
<i>A. niger</i>		1 mm	3 mm	5 mm
<i>F. oxysporum</i>		1 mm	1 mm	2 mm

Table 3. Antifungal activity of chloroform extract of plant leaf.

<i>C.caesia</i> leaf, Chloroform		Concentration		
		50 mg	100 mg	150 mg
<i>R. stolonifer</i>		2 mm	2 mm	4 mm
<i>A. niger</i>		0 mm	1 mm	2 mm
<i>F. oxysporum</i>		1 mm	2 mm	2 mm

Table 4. Antibacterial activity of chloroform extract of plant leaf.

NAT, Leaf, Chloroform		Concentration	
		100 mg	150 mg
<i>E. Coli</i>	50 mg 2 mm	2 mm	3 mm
<i>S. aureus</i>	0 mm	1 mm	2 mm
<i>P. aeruginosa</i>	1 mm	1 mm	2 mm

Table 5. Antibacterial activity of chloroform extract of plant leaf.

C. Caesia Leaf, Chloroform		Concentration	
		100 mg	150 mg
<i>E. coli</i>	50 mg 1 mm	1 mm	2 mm
<i>S. ureus</i>	0 mm	1 mm	3 mm
<i>P. aeruginosa</i>	1 mm	3 mm	3 mm

reported in *Nyctanthes arbor-tristis* leaves extract are tannin, Cardiolglycosides, phenols, steroids, flavonoids and in *Curcuma caesia* leaves extracts extract cardiolglycosides, flavonoids, Cynogenic glycosides, alkaloids, reducing sugar are present.

Since the plant is rich in a wide variety of secondary metabolites such as tannins, phenols, steroids, flavonoids and cardiolglycosides, it could be used to produce more effective's antimicrobial agent to demonstrate the extract from the leaves of *Nyctanthes arbor-tristis* and *Curcuma caesia* leaves extracts as effective as modern medicine to combat *E. coli* and *pseudomonas*. Now due to biologically and pharmacological screening of these medicinal plant using the modern tool may leads to some new safe and interesting drug. Which could be further exploited for isolation and characterization of the novel phytochemical drugs used in the treatment of infectious diseases especially in the conditions of drug resistant microorganisms.

CONCLUSION

With all these wide spectrum antibacterial properties, *N. arbor-tristis* and *C. caesia* can be considered an effective antimicrobial agent to treat infectious diseases against human pathogen. The results of the phytochemical analysis of these medicinal plants showed that the terpenoids, Phenols, reducing sugar, flavonoids and alkaloids were found to be present in mentioned medicinal plants. The study supported scientifically the ethno pharmacological use of the plant as an antimicrobial and antifungal agent and could account for some of the variations observed in the ethnopharmace *gastrointestinal* preparation methods. The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of the new drugs for curing of various diseases. Further comparative study of bioactive compounds present in both plant extracts revealed their probable pharmaceutical use in the treatment of diseases in human being.

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