

Inhibitory Activity of *Polyalthia longifolia*, *Anaphalis lawii* and *Gnidia glauca* against *Colletotrichum capsici* and Urinary Tract Pathogens

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Abstract

The present study was conducted with an aim of determining inhibitory effect of extracts from different parts of *Polyalthia longifolia* (leaf, ripe and unripe pericarp), *Anaphalis lawii* (leaf and flower), *Gnidia glauca* (leaf, bark and flower) against *Colletotrichum capsici* and urinary tract pathogens. The shade dried plant materials were extracted using methanol. Antifungal effect of extracts was evaluated against *Colletotrichum capsici* isolated from anthracnose of chilli by Poisoned food technique. Antibacterial activity of extracts was determined against five antibiotic resistant bacteria isolated from urinary tract infections by Agar well diffusion assay. The extracts caused marked inhibition of mycelial growth of *C. capsici* as indicated by reduced diameter of fungal colonies on poisoned plates. Extracts of *P. longifolia* inhibited *C. capsici* to higher extent followed by *A. lawii* and *G. glauca*. All extracts from selected plants inhibited test bacteria but to varied extent. Susceptibility was higher in case of Gram positive when compared to Gram negative bacteria. *Staphylococcus aureus* and *Escherichia coli* were inhibited to higher extent among Gram positive and Gram negative bacteria respectively. Among plants, *P. longifolia* caused higher inhibition of test bacteria. The extracts from the selected plants can be considered as promising sources of bioactive agents which can be used to treat urinary tract infections caused by antibiotic resistant bacteria and to control anthracnose of chilli. Further studies are to be carried in order to isolate and characterize bioactive principles from extracts and to determine their inhibitory potential against *C. capsici* and uropathogens.

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INTRODUCTION

Productivity of crops that are grown for the purpose of human consumption is at risk because of abiotic factors (such as lack or excess of water, extreme temperature etc), weeds, pathogens, insects and other animal. Such crop losses can be extensive and may be prevented, or reduced, by certain crop protection measures. These measures can be taken in fields (pre-harvest) and in storage (post-harvest). Among pathogens causing crop loss, fungi play an important and dominant role. In fields, fungal infection of plants leads to death of plants and significant crop losses. Many fungi also deteriorates crops after harvest (i.e., in storage conditions), contaminate stored commodity and produce mycotoxins (Oerke, 2006; Farooq *et al.*, 2010). Synthetic chemicals are extensively used as fungicides to prevent and control fungal infections of plants. However, these agents suffer from several drawbacks such as high cost, toxic effects on non-target organisms including humans, biomagnifications in ecosystem, emergence of resistant strains of pathogens etc. Plant extracts, components from plants and plant

based formulations appear as promising alternatives for prevention and control of phytopathogenic fungi. Plants possess several bioactive compounds which have pronounced effect on pathogens. Many studies have shown potential of plants against phytopathogenic fungi (Chutia *et al.*, 2009; Fathima *et al.*, 2009; Farooq *et al.*, 2010; Dileep *et al.*, 2013; Mohammadi *et al.*, 2013; Vivek *et al.*, 2013a).

Urinary tract is a system meant to collect, store and release urine. This system includes kidneys, ureters, bladder and urethra. Infection caused by microorganisms anywhere in the urinary tract is referred as Urinary Tract Infection (UTI). These infections are one of the most common infections in both community and hospital settings and are known to affect people of all age groups in both sexes. UTIs are more common in females than in males. It affects millions of people worldwide and forms a serious health problem. It is often the leading cause of Gram-negative bacteraemia in hospitalized patients. UTIs

are caused by a number of pathogens. Majority of UTIs are caused by a single bacterial species, some may be polymicrobial. *E. coli* is considered as the predominant cause of UTIs. *Klebsiella pneumoniae*, *Enterobacter* sp., *Pseudomonas aeruginosa*, *Proteus* sp., *Enterococcus* sp., *Staphylococcus aureus* and *Streptococci* are other bacteria causing UTIs. Antibiotics are regularly used to treat UTIs. Many bacterial strains causing UTIs acquired resistance against most commonly used antibiotics owing to uncontrolled usage. Antibiotic resistance in uropathogens is a global problem and is creating a serious threat for successful treatment of UTIs (Kyabaggu *et al.*, 2007; Amin *et al.*, 2009; Okonko *et al.*, 2010; Beyene and Tsegaye, 2011; Humayun and Iqbal, 2012; Shifali *et al.*, 2012; Manasa *et al.*, 2013). The objective of the present study was to study the effect of extracts from various parts of the plants *viz.*, *Polyalthia longifolia* Thw., *Anaphalis lawii* (Hook.f) Gamble and *Gnidia glauca* (Fresen) Gilg against *Colletotrichum capsici* isolated from anthracnose of chilli and drug resistant bacteria strains isolated from urinary tract infection.

MATERIALS AND METHODS

Collection and Identification of Plants

Details of family, parts used and place of collection of plants used in this study are shown in Table 1. The plant specimens were authenticated by Dr. Vinayaka K.S, Department of Botany, Kumadvathi First Grade College, Shikaripura, Karnataka. The leaf, flower, bark and pericarp materials were shade dried and powdered.

Table 1: Plants used in this study.

Plant name	Family	Part used	Place of collection
<i>P. longifolia</i>	Annonaceae	Leaf, ripe pericarp & unripe pericarp	SRNMNC campus, Shivamogga, Karnataka
<i>A. lawii</i>	Compositae	Leaf and flower	Talakaveri, Kodagu district, Karnataka
<i>G. glauca</i>	Thymelaeaceae	Leaf, flower and bark	Haniya, Hosanagartaluk, Shivamogga, Karnataka

Extraction

For extraction, a known quantity (25g) of each of the shade dried and powdered plant material was transferred into separate containers containing 100ml of methanol (HiMedia, Mumbai) and mixed well. The containers were left for overnight. The contents were filtered through 4-fold muslin cloth followed by Whatman No. 1 filter paper. The filtrates were concentrated under reduced pressure (Kekuda *et al.*, 2010).

Antifungal Activity of Extracts of Selected Plants

The inhibitory effect of extracts was tested by Poisoned food technique against *C. capsici* isolated previously from anthracnose of chilli (Kambar *et al.*, 2013). Here, petriplates containing PDA medium poisoned with plant extracts (1mg extract/ml of medium) were inoculated with the spore suspension of test fungus by point inoculation method. The inoculated plates were incubated at 28°C for 5 days. The colony diameter of test fungus in each plate was measured in mutual perpendicular directions on 5th day. The inhibitory effect of extracts was recorded in terms of inhibition of mycelial growth (%) and was calculated using the formula:

$$\text{Inhibition of mycelia growth (\%)} = (C - T / C) \times 100$$

where C is colony diameter in control plate and T is the diameter of colony in poisoned plates.

Antibacterial Activity of Extracts of Selected Plants

The antibacterial activity of extracts of selected plants was determined by Agar well diffusion assay against five antibiotic resistant uropathogens *viz.*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis* (Manasa *et al.*, 2013). The test bacteria were seeded into sterile Nutrient broth (HiMedia, Mumbai) tubes and incubated at 37°C for 24 hours. The broth cultures were swabbed aseptically on sterile Nutrient agar (HiMedia, Mumbai) plates with the help of sterile cotton swabs. Later, wells of 6mm diameter were punched in the inoculated plates using sterile cork borer, 100µl of extracts (20mg/ml of 25% dimethyl sulfoxide [DMSO]), standard antibiotic (Chloramphenicol, 1mg/ml) and DMSO (25%, in sterile water) were added into labeled wells and the plates were incubated at 37°C for 24 hours in upright position. The zone of inhibition was measured using a ruler.

Statistical Analysis

The experiment was conducted in triplicate. Results are represented as Mean±Standard deviation.

RESULTS

The result of effect of poisoning of medium with extracts from selected plants on mycelial growth of *C. capsici* is shown in Table 2; Figure 1 and 2. The diameter of colonies of test fungus on poisoned plates was lesser when compared with fungal colony diameter on control plate indicating antifungal effect of extracts. Extracts of *P. longifolia* caused inhibition of *C. capsici* to higher extent followed by *A. lawii* and *G. glauca*. In case of *P. longifolia*, unripe pericarp extract displayed higher inhibitory activity (>80% inhibition) against *C. capsici* than ripe pericarp and leaf extracts. Among extracts of *A. lawii*, leaf extract was more inhibitory (>50% inhibition) when compared to flower extract. Among extracts of *G. glauca*, leaf extract showed higher inhibitory activity (>30% inhibition) followed by bark and flower extract.

Table 2: Colony diameter (CD) of *C. capsici* on control plate and plates poisoned with extracts of selected plants

Plant	Part	CD in cm
Control	-	3.1±0.1
<i>P. longifolia</i>	Leaf	0.7±0.0
	Ripe pericarp	0.7±0.0
	Unripe pericarp	0.5±0.0
<i>A. lawii</i>	Leaf	1.5±0.1
	Flower	1.6±0.1
<i>G. glauca</i>	Leaf	2.1±0.1
	Flower	2.3±0.0
	Bark	2.2±0.0

Table 3 and Figure 3 show antibacterial effect of extracts from selected plants against antibiotic resistant uropathogens. The presence of zone of inhibition around the wells was considered positive for antibacterial activity. All extracts were found inhibitory against test bacteria but to a varied extent. The susceptibility pattern varied among bacteria. Gram positive bacteria were inhibited to high extent than Gram negative bacteria. *S. aureus* and *E. coli*

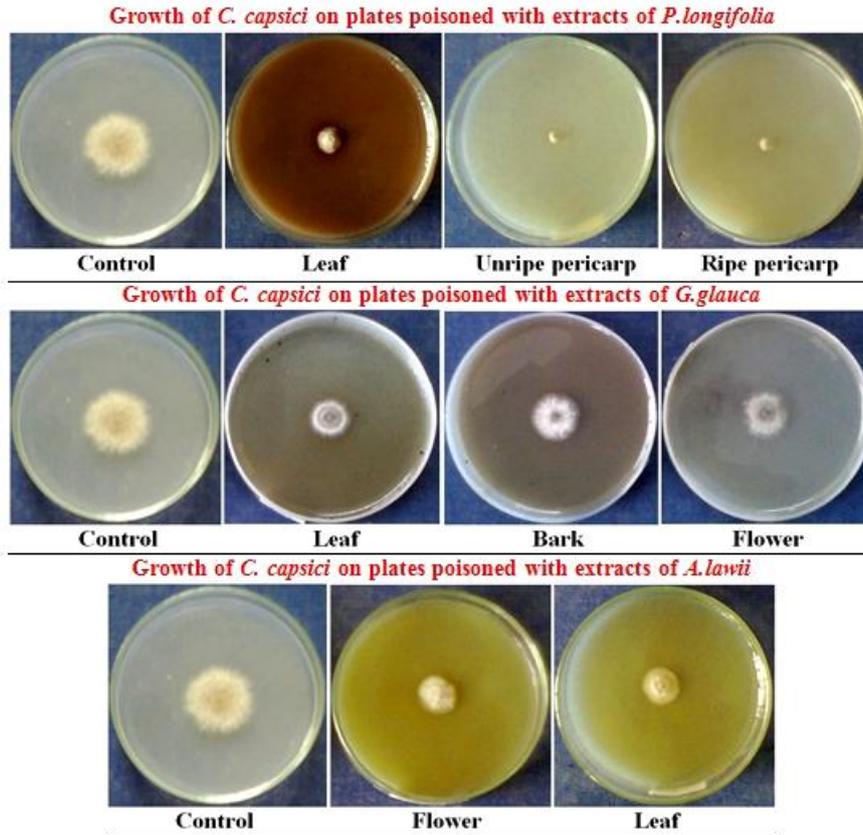


Figure 1: Growth of *C. capsici* on control plate and plates poisoned with extracts of selected plants.

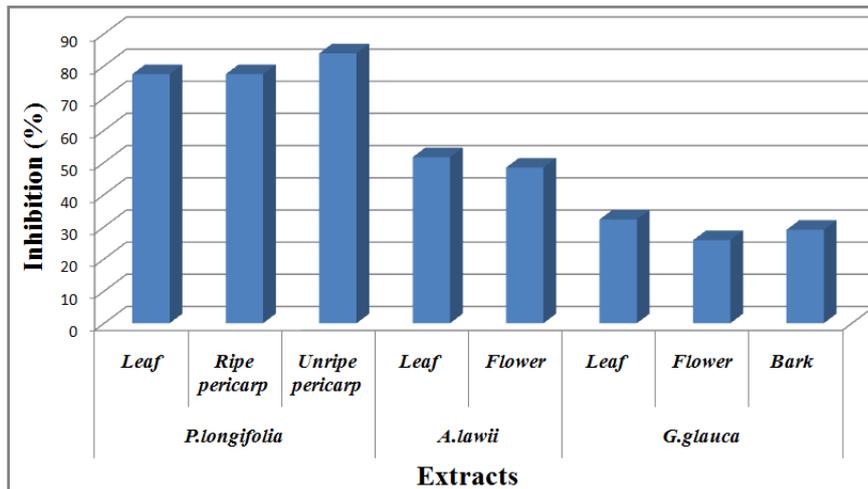


Figure 2: Extent of inhibition of *C. capsici* (%) by extracts of selected plants.

Table 3: Antibacterial activity of extracts against urinary tract pathogens.

Plant	Part	Zone of inhibition in cm				
		<i>Ec</i>	<i>Kp</i>	<i>Pa</i>	<i>Sa</i>	<i>Ef</i>
<i>P.longifolia</i>	Leaf	1.5±0.1	0.8±0.0	0.8±0.0	2.0±0.1	2.0±0.0
	Ripe pericarp	1.7±0.1	0.8±0.0	1.0±0.1	2.1±0.0	2.4±0.1
	Unripe pericarp	1.9±0.1	1.1±0.1	1.2±0.0	2.5±0.0	2.4±0.1
<i>A.lawii</i>	Leaf	1.6±0.1	0.8±0.0	1.0±0.0	2.1±0.0	1.8±0.1
	Flower	1.2±0.0	0.8±0.0	0.8±0.0	1.6±0.1	1.2±0.0
<i>G.glauca</i>	Leaf	0.8±0.0	0.8±0.0	0.8±0.0	1.7±0.0	1.2±0.0
	Flower	0.8±0.0	0.8±0.0	0.8±0.0	1.0±0.1	0.8±0.0
	Bark	1.2±0.0	1.1±0.0	1.1±0.0	1.9±0.1	1.4±0.0
Standard	-	1.9±0.2	1.4±0.1	1.8±0.0	3.9±0.2	3.8±0.2
DMSO	-	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Ec- E.coli; Kp- K.pneumoniae; Pa- P.aeruginosa; Sa- S.aureus; Ef- E.faecalis

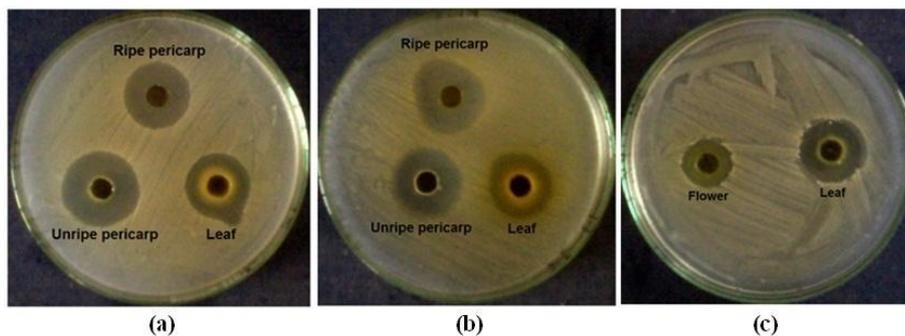


Figure 3: Inhibition of (a) *S. aureus* by *P. longifolia*; (b) *E. faecalis* by *P. longifolia*; (c) *S. aureus* by *A. lawii*.

were inhibited to higher extent among Gram positive and Gram negative bacteria respectively. *K. pneumoniae* was inhibited by extracts to least extent. Overall, *P. longifolia* displayed high inhibitory potential among plants selected. Among different parts of each of the selected plants, unripe pericarp extract of *P. longifolia*, leaf extract of *A. lawii* and bark extract of *G. glauca* caused higher inhibition of test bacteria. Reference antibiotic caused higher inhibition of test bacteria when compared to plant extracts. Here also, Gram positive bacteria exhibited higher susceptibility than Gram negative bacteria. DMSO did not cause inhibition of test bacteria.

DISCUSSION

Chilli, belonging to the genus *Capsicum* (Solanaceae), is an herbaceous, annual, dicotyledonous, flowering plant grown in tropical and subtropical regions as a commercial crop. It is grown for consumption, nutritional and economy purposes. It is used as spice (ripe and dried form) and vegetable (green fruit). The production of chilli is influenced by several diseases caused by fungi, bacteria and viruses. These diseases lead to considerable reduction in productivity. Among various diseases of chilli, anthracnose (both pre-harvest and post-harvest) is the most important disease resulting in yield loss (up to 50%) and deterioration of fruit quality. The anthracnose disease is caused by *Colletotrichum* species. Among various species implicated in causing anthracnose of chilli, *C. capsici* is the most important pathogen (Ushakiran *et al.*, 2006; Anand *et al.*, 2007; Ratanacherdchai *et al.*, 2007; Than *et al.*, 2008; Narasimhan and Shivakumar, 2012; Masoodi *et al.*, 2013; Kamar *et al.*, 2013). In the present study, we investigated antifungal effect of extracts of different parts of *P. longifolia*, *G. glauca* and *A. lawii* against *C. capsici* isolated from anthracnose of chilli by poisoned food technique. This technique is widely used to evaluate antifungal activity of a variety of samples including plant extracts (Chutia *et al.*, 2009; Farooq *et al.*, 2010; Rakesh *et al.*, 2013; Kamar *et al.*, 2013). Extracts of *P. longifolia* exhibited higher inhibition of mycelial growth of *C. capsici* followed by *A. lawii* and *G. glauca*.

The discovery of antibiotics from microorganisms is an important milestone in the field of chemotherapy. The subsequent use of the antibiotics revolutionized the field of medicine and saved a large number of individuals from infections by pathogens. This success is challenged by the emergence of microorganisms that have developed resistance against antibiotics and resistant microbial strains are continuously appearing because of their ability to transmit and acquire resistance genes. Wide spread use of antibiotics appears to be the major selective force for development of antibiotic resistance. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Mycobacterium*

tuberculosis, *Enterococcus faecalis*, *Escherichia coli* are among the most important antibiotic resistant pathogens (Cooke, 1976; Carmeli *et al.*, 1999; Demain and Sanchez, 2009; Davies and Davies, 2010; Onanuga and Awhowho, 2012). Antibiotic resistance is common in urinary tract pathogens. These resistant bacterial strains make the successful therapy often difficult (Kyabaggu *et al.*, 2007; Beyene and Tsegaye, 2011; Humayun and Iqbal, 2012; Manasa *et al.*, 2013). Hence, there is need for development of alternate strategy to combat UTIs caused by drug resistant uropathogens. Plants are one among the most suitable alternatives with activity even against drug resistant pathogens. Many studies have shown the potential of plants and their components to inhibit urinary tract pathogens (Peneira *et al.*, 2004; Sahoo *et al.*, 2008; Kannan *et al.*, 2012; Manasa *et al.*, 2013). In this study, we evaluated the potential of extracts from different parts of *P. longifolia*, *G. glauca* and *A. lawii* to inhibit antibiotic resistant uropathogens. Extracts of *P. longifolia* and *A. lawii* showed marked antibacterial activity than extracts of *G. glauca*. Inhibitory activity of extracts was marked against Gram positive bacteria when compared to Gram negative bacteria. Similar results are observed in a previous study of Vivek *et al.* (2013b) where extract of *A. indica* displayed stronger inhibition of Gram positive urinary tract bacteria than Gram negative bacteria. In Gram negative bacteria, the presence of an outer membrane might act as an additional barrier (Lodhia *et al.*, 2009; Nalubega *et al.*, 2011).

CONCLUSION

The present study evaluated antimicrobial activity of extracts from different parts of three selected plants. Extracts from *P. longifolia* and *A. lawii* displayed good activity. It is evident from the results that the plants contain active principles having inhibitory activity against urinary tract bacteria and anthracnose causing fungus. These plants can be used to control anthracnose in chilli and to treat urinary tract infections. Isolation and characterization of bioactive principles from the plants and determination of their inhibitory potential are to be conducted.

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