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Active phytochemical and antibacterial potentiality of *in-vitro* regenerated plantlets of *Canscora decurrens* (Dalzell)

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Abstract

The present paper deals with *in vitro* produced phytochemicals of *Canscora decurrens* (Dalzell) such as alkaloids, flavanoids, phenols, steroids, anthracene glycosides and triterpenoids etc for their antibacterial activities against human pathogenic strains *viz. Staphylococcus aureus, Bacillus subtilis, Rhodococci sp., Escherichia coli, Proteus vulgaris, Pseudomonas sp, Salmonella sp. and Bacillus stearothermophilus*. The investigation suggests that the plant may be used in therapeutic treatments of gastrointestinal disorders, diarrhoea and skin diseases.

Keywords: Phytochemical, regeneration, *Canscora decurrens*, herbal plant, antibacterial.

Introduction

The medicinal value of herbal plants lies in their bioactive principles as plant constituent that produce definite physiological action on the human body (Akinmoladun *et al.,* 2007). Some of them are alkaloids, essential oils, flavonoids, tannins, terpenoid, saponins, phenolic compounds (Edeoga *et al.,* 2005). These natural compounds formed the foundations of modern prescription drugs as we know today (Goh *et al.,* 1995).

There have been a continuous search for especially the native plants or their extracts and many of these herbal remedies proved successful (Gangadevi *et al*., 2008; Indira Iyer *et al*., 2009; Mungole *et al*., 2009; Varaprasad Bobbarala *et al*., 2009).

Canscora decurrens (Dalzell) is an herb belonging to the family Gentianaceae found in tropical and subtropical Africa, Asia, Australia and China. Economically, some species of Gentianaceae are cultivated as ornamental plants and many species yield bitter principles used medicinally and in flavorings. Species of Gentianaceae have been used by people of different countries like India, China etc., as medicine to cure various diseases such as diarrhoea and other stomach ailments. In fact, many plants of Gentianaceae have been known for their antibacterial activity. *Swertia corymbosa* leaves, traditionally used in Indian medicine as an antidote for poisoning, diarrhoea and as stomach wash in cattle. Hexane, chloroform and methanol extracts show antibacterial activity against a wide range of microorganism, that cause diarrhoea (*E. coli, Salmonella*, *V. cholerae* & *Staphylococcus aureus*) (Iqbal *et al.,* 2006). *Canscora diffusa* (Vahl), extracts was found to be effective against both gram-positive and gram-negative bacteria (Mahida & Mohan, 2006). *Canscora decurrens* belongs to the same category and therefore it was chosen to study. It is already being utilized in traditional medicine as Shankhapushpi in central India. Present paper is an effort to bring about knowledge of different ethno medicinal uses, chemical constituents and pharmacological activities of *in vitro* regenerated plant parts to elucidate the actual unexplored medicinal value.

Material and methods

Canscora decurrens plantlets were *in vitro* regenerated by standard tissue culture techniques in the plant tissue culture laboratory of SFS Center of biotechnology, Seminary hills, Nagpur. Leaves and roots were separated from the plantlets growing on the tissue culture media and washed repeatedly in distilled water to get rid of the media. The roots and leaves were then oven dried overnight and ground to a fine powder. The powdered form of these plant parts are stored in airtight glass containers, protected from sunlight until required for analysis.

Preliminary phytochemical screening

Extract preparation: Preliminary phytochemical screening of plant was done according to the standard procedures adopted by the various workers (Amarsingham *et al.,* 1964; Das & Bhattacharjee, 1970; Gibbs, 1974; Harborne, 1998; Chhabra *et al.* 1984; Trease & Evans, 1985; Danial, 1991). Accordingly, the extracts were prepared viz. petroleum ether, chloroform, acetone, ethanol and water. Simple chemical tests were conducted for the chemicals such as, alkaloids, anthocyanins, anthocyanidins, anthraceneglycosides, aminoacids, coumarins, flavanoids, saponins, gums and mucilage, steroids triterpenoids, volatile oils, fatty acids, emodins, carotenoids and tannins.

Antibacterial assay

The test microorganisms used for the antibacterial activity screening were selected pathogenic bacteria, *Staphylococcus aureus*, *Bacillus subtilis, Rhodococci* sp., *Escherichia coli, Proteus vulgaris*, *Pseudomonas sp*., *and Salmonella sp.* obtained from the culture collection of Center for Biotechnology, SFS College, Seminary hills, Nagpur, India and Veterinary College, Nagpur, India. All

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bacterial species were maintained on nutrient agar medium for 36 h. Old bacterial cultures were inoculated into nutrient broth and incubated at 37 ±2°C on rotary shaker at 100 rpm. After 36 h incubation the bacterial suspension were used for further tests. The modified agar well diffusion method (Perez *et al.,* 1990) was used to evaluate the antibacterial activity. Petri dishes and nutrient agar medium was sterilized by autoclaving. To sterilized nutrient agar medium 10 ml of one day old bacterial cultures were added. The medium was stirred well and poured in petri plates and allowed to solidify at room temperature. With the help of a 5 mm cork borer two wells were punched. To each well 50 µl extract was poured. The plates were incubated at 37°C for 48 h. Finally the plates were observed for clear zone of inhibition and the diameter of zone of inhibition extending laterally around the well was measured in mm with zone scale of 1 mm or more considered positive inhibition.

Qualitative and quantitative phytochemical screening

Quantitative phytochemical screening of flavanoids, phenolics, saponins and alkaloids was done according to the standard procedure adopted by Mallikharjuna *et al.* (2007). Qualitative analyses of various phytochemicals like alkaloids, flavanoids, phenolics and saponins were done by employing thin layer chromatographic technique as followed by Krishnaiah *et al.* (2007).

Table 1. Preliminary phytochemical screening

Tests with all five extracts						
Chemical	Part	P. ether	Chloroform	Acetone	Ethanol	Water
Alkaloids	Leaf	+	+	+	+	+
	Root	+	+	+	+	+
Coumarins	Leaf	+	+	+	+	+
	Root	+	+	+	+	+
Triterpenoids	Leaf	-	-	+	-	-
	Root	-	-	-	-	-
Phenolics	Leaf	+	+	+	+	-
	Root	+	+	+	+	-
Flavanoids	Leaf	+	-	-	-	-
	Root	+	-	-	-	-
Steroid	Leaf	+	+	+	-	-
	Root	+	+	+	-	-

Result and discussions

Phytochemical screening: A general screening was conducted to characterize chemical composition of *in vitro* regenerated *C. decurrens* leaf and root samples. It covered mainly nitrogenous compounds, isoprenoids and acetogenins. Screening for nitrogenous compounds was mainly concerned with alkaloids which are reputed to have dramatic physiological activities, mainly on central nervous system. Both the leaf and root samples showed positive results (Table 1). Phytochemical screening is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance, to make the best and judicious use of available natural wealth. A number of medicinal plants have been chemically investigated by several workers (Bhattacharya *et al.,* 1971; Ambashta *et al.,* 1986; Kokate *et al.,* 1998; Ram, 2001).

Table 2. Tests with alcohol & water extracts

Chemical	Part	Ethanol	Water
Anthocyanins	Leaf	-	-
	Root	-	-
Anthocyanidins	Leaf	-	-
	Root	-	-
Anthracene glycosides	Leaf	-	-
	Root	-	-
Tannins	Leaf	+	+
	Root	+	+
Cardiac glycosides	Leaf	-	-
	Root	-	-

Acetogenin screening included tannins, flavanoids, coumarins, emodins, anthocyanins, anthocyanidins, anthracene derivatives, phenolic acids and fatty acids. Tannins and coumarin were present in all the extracts of both leaf and root samples (Table 1 & 2). Tannins decrease the bacterial proliferation by blocking key enzymes at microbial metabolism (Geidam *et al.,* 2007). Tannins play important role such as potent antioxidant (Trease & Evans, 1985). Herbs that have tannins as their main component are astringent in nature and are used for treating intestinal disorders such as diahorriea and dysentery. Emodins, anthocyanidins, anthocyanins, anthracene glycosides and fatty acids were absent in both the samples (Table 2 & 3).

Flavanoid was found in the petroleum ether extract of both leaf and root (Table 1). Flavonoids were found to be extractable in all the solvent system. Agrawal and Tiwari (1991) reported flavonoids extracted from *Phyllanthus nirurii* using solvents- petroleum ether and methanol. However, Phanikumar (2003) could not detect flavonoids in petroleum ether extract from *Euphorbia nivulia*. Andrei *et al.* (2000) isolated flavonoids from the roots of *Trichosanthes tunicata* in hexane. While Zafar and Mujeeb (2002) extracted flavonoids form *Tephrosia purpurea* in 30% methanol. Dongarwar (1998) reported these compounds in petroleum ether, methanol and water from the *Tephrosia sp.* Phenolic acid was found to be present in all except water extract (Table 1). In the present study, phenolics were detected in both the parts of the plants. Phenolics have attracted a great attention in relation to their potential for beneficial effects on health. Over the last few years, several experimental studies have revealed biological and pharmacological properties of phenolics compounds, especially their antimicrobial activity (Narayana *et al.,* 1999), anti-inflammatory activity (Castillo *et al.,* 1989), antiviral, anti-inflammatory and cytotoxic activity (Chhabra *et al.,* 1984). It is a well documented that most medicinal plants are enriched with phenolic compounds and bioflavonoids that have excellent antioxidant properties (Shirwaikar *et al.,* 2003). Phenolics are active in curing kidney and stomach problems as well as

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helpful as anti-inflammatory in action (Zhu *et al.,* 1997). Choi *et al.* (1998) found eight types of phenolics form stem bark of *Cornus walteri,* while, Tiloo (1997) reported the isolation of phenolics from roots of *Phyllanthus simplex.*

Screening of isoprenoids was confined to steroids, triterpenoids, saponins, cardiac glycosides and carotenoids. Steroids were detected in all extracts except in alcohol and water extracts (Table 1). Steroids have been reported to possess anti-inflammatory activities (Chawala *et al.,* 1987) from only stem bark of *Phyllanthus flexosus*. Saponin which is widely well known to have expectorant activity was observed in water extract. Saponins were also found to be present in both the plant parts (Table 4). Recent studies at Toronto, Department of Nutritional Sciences, Canada, have indicated that, dietary source of saponins offer preferential chemical preventive strategy in lowering the risk of human cancer. Saponins are found in many plants and animals. Rao *et al.* (1984) and Sharma *et al.* (1984) carried out an extensive phytochemical analysis of plants for the presence of saponins. Triterpenoids, volatile oils and cardiac glycosides showed negative tests in both the samples whereas carotenoids showed positive result only in leaf sample. The result of qualitative and quantitative phytochemical screening has been given in Table 5 and 7 respectively.

Table 3.Tests with petroleum ether extracts

Chemical	Part	Pet. ether
Emodins	Leaf	-
	Root	-
Carotenoids	Leaf	+
	Root	-
Fatty acid	Leaf	-
	Root	-

Table 4. Tests with water extracts

Chemical	Part	Water
Gums & Mucilages	Leaf	+
	Root	+
Saponins	Leaf	+
	Root	+
Phlobatanin	Leaf	-
	Root	-
Chlorogenic acid	Leaf	-
	Root	-
Cyanogen glycosides	Leaf	-
	Root	-

Table 5. Quantitative phytochemical analysis

Compound	Plant part (mg/gm of sample)	
	Leaf	Root
Flavonoids	25	20
Phenolics	5	3
Saponins	135	120
Alkaloids	50	62

Antibacterial activity: All the extracts except the petroleum ether showed significant antibacterial activity against the test organisms (Table 6). Petroleum ether extract did not show antibacterial activity against any of the nine pathogenic bacteria. The chloroform extract did not show any activity against *E. coli*. Chloroform extract of roots of *Canscora decurrens* plantlets showed more activity as compared to the leaf extracts. The maximum antibacterial activity was seen against *Pseudomonas*, *B. subtilis* and *Rhodococci* sp. Acetone extracts of leaf and root showed the maximum activity against *Rhodococci* sp. Both the leaf and root extracts showed significant activity against all the test pathogens except *E. coli*. The ethanol extracts of *Canscora decurrens* shows absolutely no activity against *Salmonella.* Maximum activity was observed against *Proteus vulgaris*. Aqueous extract showed selective antibacterial activity against the test pathogens. Significantly high antibacterial activity was observed against *Staphylococcus aureus*, *Pseudomonas* sp. and *Rhodococci* sp. Whereas absolutely no antibacterial activity was observed against *Proteus vulgaris*, *Salmonella* and *E. coli*.

These observations suggested that the aqueous and organic extracts from the same plants differed in antibacterial effect. Antibacterial effect of the plant extracts on *E. coli, Pseudomonas* sp. and *S. aureus* suggest that this plant may have potential therapeutic value in the treatment of gastrointestinal disorders, diarrhoea and skin diseases. In addition, the effectiveness of plant was not due to one main active constituent, but the combined action of the chemical compounds (secondary metabolites) involved in it. This finding supports the traditional knowledge in selecting the most active medicinal plants to use in traditional medicine practices in the future. Further work is needed to isolate active principle from the plant and to carry out pharmaceutical studies.

Table 6. Screening of in-vitro regenerated Canscora decurrens for antibacterial activity

Bacteria	Zone of inhibition in mm (without 5 mm well diameter)									
	Pet. ether extract		Chloroform extract		Acetone extract		Ethanol extract		Water extract	
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Salmonella	-	-	2	3	-	2	-	-	-	-
Pseudomonas	-	-	6	8	4	9	3	4	-	-
Bacillus subtilis	-	-	6	8	3	11	5	4	9	10
Staphylococcus aureus	-	-	2	4	-	8	2	6	12	23
Proteus vulgaris	-	-	6	6	7	10	11	20	-	-
E.coli	-	-	-	-	-	-	4	5	-	-
Rhodococci	-	-	6	7	6	12	4	7	-	18
Bacillus stearothermophilus	-	-	6	8	2	11	4	2	16	14

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Table 7. Qualitative chemical screening for Thin Layer Chromatography

Chemical	Solvent system	Part	Rf values	Total bands	Spray reagent
Alkaloids	Chloroform: Methanol (15:1)	Leaf	ND	ND	Dragendorf's reagent
		Root			
Flavonoids	Chloroform: methanol (19:1)	Leaf	0.838	1	No reagent, UV light
		Root	0.812	1	
Phenolics	Chloroform: methanol (27:0.3)	Leaf	0.986,0.93,0.77,0.68,0.527 0.291,0.083,0.04	8	Folin-Ciocalteau reagent
		Root	0.986,0.93,0.77,0.506, 0.236,0.027	6	
Saponins	Chloroform: glacial acetic acid: methanol: water (64:34:12:8)	Leaf	0.53,0.639,0.932	3	Iodine vapors
		Root	0.879,0.969	2	

ND – Not detected

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