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Original Research Article

Antibacterial activity of leaf and tuber extract of orange, purple flesh antioxidants rich sweet potato (*Ipomoea batatas* (L.))

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Abstract

Sweet potato (*Ipomoea batatas* (L.), the fifth most important food cum vegetable crop is rich in starch, minerals and vitamins supplements. This tuberous vegetable provides food to small and marginal farmers in sweet potato growing countries. Recently β-carotene rich orange flesh sweet potato and anthocyanin rich purple flesh sweet potato are gaining special attention as cheap source of food cum antioxidants. Central Tuber Crops Research Institute (CTCRI) at Bhubaneswar harbours a sizable collection of sweet potato germplasm. Progressive screening and evaluation resulted in identifying high starch (21%) white flesh, β-carotene rich (14 mg 100g^{-1}) orange flesh and anthocyanin rich (90 mg 100 g^{-1}) purple flesh sweet potato. Post harvest evaluation on storability of these genotypes was found to be longer by 2-3 months than popular and local cultivars. Disease and pest incidence was also found to be minimum (5-10%) in these varieties. In the present study, the high valued sweet potato tested for their yield performance across Odisha. Further, freshly harvested tubers and leaf extracts of purple, orange and white flesh sweet potato were studied for antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus.* Of the different leaves and tubers extract used for disc diffusion methods significant zone of inhibition was recorded with the extracts of purple and orange flesh tubers. Such inhibition was observed against both the pathogenic microbes. These results are encouraging to work further on edible therapeutics, food and other valuable traits of purple and orange flesh sweet potato.

Key words: Sweet potato, antibacterial activity, *Escherichia Coli, Staphylococcus aureus*

INTRODUCTION

Sweet potato is an important food crop provide energy (194 MJ ha^{-1} day^{-1}), vitamins (Vit E – 4.6 mg (100g^{-1}, 8-14 mg) 100 g^{-1} β-carotene) in most of the improved sweet potato varieties developed at Regional Centre of CTCRI (Mukherjee, A., 2013; Mukherjee, A. and Naskar, S. K., 2012). Besides high starch rich white flesh sweet potato, β-carotene rich orange flesh and anthocyanin rich purple flesh sweet potato are used up now as cheap source of antioxidants. Globally there is growing interest in exploiting plants for their medicinal purposes to meet the highly demanding needs of the explosive population. This is ascribed to the high disease incidence, emergence of

multi drug resistance in certain pathogens and ineffectiveness of the present drugs against non pathogenic diseases. Plants are used as food, flavours, cosmetic, ornamental, fumigants, insect deterrents and medicine. Most of the plants have the capacity to defence against a number of infectious diseases in almost all parts of the world. They provide a myriad of natural products, which are used in extensive applications in combating the diseases. In this context, β-carotene rich orange and anthocyanin rich purple flesh sweet potato can play pivotal role.

In many parts of India, herbal medicine is used for treating various diseases. India possesses a vast number of medicinal plants (Srinivasan *et al.,* 2001). These medicinal plants are one of the best resources for the discovery and development of novel bioactive substances (Tomoko *et al*., 2002). Studies indicated that medicinal plants contain substances like peptide, unsaturated long chain fatty acids, aldehydes, alkaloids, essential oils, phenols and water or ethanol soluble compounds. These compounds are potentially significant in therapeutic applications against human and animal pathogens including bacteria, fungi and viruses (Iwu *et al*., 1999; Khan *et al.,* 2003).

Plant derived new drugs are discovered and developed throughout the world (Stuffness and Douros., 1982), as they provide basic structure for manufacturing new synthetic drugs (Nair *et al*., 2005). The cost of production of synthetic drugs is also high and they produce adverse effect compared to plant derived drugs. Hence much attention has been paid recently, to the biologically active compounds derived from plants used in herbal medicine (Essawi, 2000). Therefore, over the past 20 years, there has been an increased interest in the investigation of natural materials as sources of new antibacterial agents. As a result, some natural products have been approved as new antibacterial drugs which necessitate an urgent need to identify novel substances that are active towards pathogens with high resistance (Barbour, 2004).

Anthocyanin and β-carotene rich sweet potato were found to be a powerful source of antioxidants besides their food and nutrition values. In addition, as such anthocyanin also show antimicrobial, antidiabetic and anticancerous effect. Sweet potato rich in anthocyanin and beta carotene thus can serve as a potent source of future antimicrobial, anticancerous and antidiabetic drugs friendly to health and environment.

Multiple drug resistance has been reported due to indiscriminate use of commercial antimicrobial drugs used in the treatment of infectious diseases (Service, 1995). This is emerging as global problem due to use of higher dose with increased risk of drug toxicity. Moreover, excessive budget is currently spent on import of antibiotics manufactured abroad. Therefore, antibacterial activity of local medicinal plants should be studied to provide alternative and locally available antibacterial regimens. In this context, the antioxidants rich, herbaceous short duration creeper food crops like sweet potato rich in starch (21%), β-carotene (14 mg 100 g^{-1}) and anthocyanin (90 mg 100 g^{-1}) were studied for their yield performance and antibacterial activity during the present course of investigation. The results on antibacterial activity of orange and purple flesh tubers of sweet potato are communicated here for the first time.

MATERIALS AND METHODS

Explant sources

The high starch, high β-carotene and anthocyanin rich sweet potato developed by Mukherjee and Naskar 2012 were tested for yield performance and their biochemical contents following the standard package of practices and methodologies (Mukherjee, A. and Naskar, S. K., 2012). Based on yield performance and biochemical constituents, five out of six evolved varieties were used as source materials. Fresh leaves and tubers of white, orange and purple fleshed five sweet potato (Figure.1) varieties was collected from Central Tuber Crops Research Institute (CTCRI) farm, Bhubaneswar, Odisha. Those were already recommended for commercial cultivation in hilly, plain and costal areas of Odisha. Those are also being used in West Bengal, Chattisgarh, Jharkhand and North eastern region. The leaves and tubers of the four varieties were thoroughly washed with running tap water followed by rinsing with distilled water.

Preparation of Extract

The tubers and leaves were freeze-dried, milled to a coarse powder and extracted using 90% ethanol in the ratio 1:5 (w/v) in a shaker incubator for 24 hours. The extracts were centrifuged at 2,000 rpm for 20min and filtered by using Whatman filter paper No.55. The solvents were then evaporated using rotary vaccum evaporator. The extracts obtained were stored at $4^{o}C$.

Test microorganism

Pure cultures of the test organism *Escherichia coli* (pEX2717) and *Staphylococcus aureus* (wS-2) was collected from Central Tuber Crops Research Institute (CTCRI), Thiruvananthapuram and maintained on nutrient agar plates and slants. The cultures were streaked on sterile nutrient agar plates and maintained at

37ºC inside the incubator. Bacterial cultures were sub cultured after every 3 to 4 days.

Preparation of bacterial suspension

The suspension were prepared by transferring a loop of microorganisms from nutrient agar plates to 50ml nutrient broth for growing the pure bacterial culture in nutrient broth medium at 37ºC overnight.

Antibacterial assay

Disk Diffusion Method was used for the antibacterial assay using 20 µl of standardized suspension of tested bacteria (10^8 CFU ml^{-1}) spread on nutrient agar plates with the help of sterile glass spreader. Filter paper disc of 6 mm diameter was prepared by using Whatman no. 1 filter paper and sterilized. Discs were soaked in 10 µl prepared extract for overnight. The excess extract was drained and air dried. Using an ethanol dipped and flamed forceps the discs were aseptically placed over the nutrient agar plates seeded with the test microorganisms. A control plate was also maintained in each case without any test materials. Plates were incubated at 37ºC for 24 hours. The test was carried out in triplicate. Zones of clearance around each disc determine inhibition and the diameter of such zones were measured in millimetre (mm). After 24hours the diameter of zone of inhibition were measured in millimetre and results were recorded in terms of diameter of zone of inhibition. The mean and standard deviation of inhibition zones were calculated.

RESULTS AND DISCUSSION

Yield and valued traits of sweet potato

In the present study, the white, orange and purple flesh sweet potato varieties evolved were tested for their yield and valued traits like starch, β-carotene and anthocyanin contents. Of the six varieties tested for yield performance in coastal (Balasore, Kendrapara, Nuapada and non-costal (Bhubaneswar, Mangarajpur) areas, white flesh varieties like ST10, Kalinga, Kishan, orange flesh ST14 and purple flesh ST13 gave higher yield than orange flesh check variety Gouri (Table 1). Among the white flesh varieties starch contents were observed to be more in ST10 and Kishan than Kalinga (Table 2). β-carotene was much higher in ST14 higher (11.8-14 mg 100 g^{-1}) as compared to Gouri (4.5-5.5 mg $100g^{-1}$), ST13 is the only purple flesh variety tested in the present study (Table 2). In fact, people in different developing countries suffer from vitamin A deficiency which causes night blindness, xerophthalmia and keratomalacia (Smith *et al*., 1996). Sweet potatoes (*Ipomoea batatas* (L.)) are rich in dietary fibre, minerals, vitamin A and antioxidants such as phenolic acids, anthocyanins, tocopherol and β-carotene (Woolfe, J., 1993). Besides acting as antioxidants especially carotenoids and anthocyanin contents provide sweet potatoes with their distinctive flesh colours like orange and purple. Sweet potato roots are one of the major food sources of carotenoids (Henkel Co., 1996). The carotenoids of orange-fleshed sweet potato storage roots are highly riched for vitamin A and β-carotene (Purcell, A. E., 1962; Purcell, A. E. and Walter, Jr. W. M., 1968; Simonne *et al*., 1993; Takahata *et al*., 1993). Thus development of orange and purple flesh sweet potato is gaining importance among the sweet potato growing countries. Research and development efforts in India resulted in evolving white, orange and purple flesh sweet potato (Figure1) rich in starch (21%), β-carotene (14mg $100g^{-1}$) and anthocyanin (90 mg $100g^{-1}$). Such nutritious crop was tested for their tuber yield across Odisha. Performance of orange and purple flesh varieties in different location are encouraging to combat food and nutritional crises in coastal backward areas (Mukherjee, A. and Naskar, S. K., 2012). This will have greater impact mainly for children and women the groups most vulnerable to vitamin A deficiency. Root and tuber crops including sweet potato is a major component of tribal food. Those are now being popularized in Jharkhand, Chattisgarh, Bihar and North Eastern States as source of supplementary food and nutrition especially in tribal dominated areas. Antibacterial activity of those varieties will no doubt open up new avenues as source of herbal medicine. These improved varieties can play significant role towards alleviation of food scarcity. Those varieties performed better than the locals and are being accepted well by farmers in tested areas. Sweet potato biotechnology (Mukherjee, A., 2002) research programme is being going on in all aspects incept its antibacterial activity. Hence the observation on antibacterial activity of high valued sweet potato was recorded in the present study.

Antibacterial activity

Five extracts derived from both leaves and tubers of different sweet potato varieties and their efficacy to inhibit the growth of *E. coli* and *S. aureus* was studied.

It was observed that ethanolic tubers extract of sweet potato (*Ipomoea batatas* (L.)) varieties ST-13 and ST-14 had shown significant inhibitory effect against *E. coli* and *S. aureus* than other varieties. Wherein others showed very less inhibitory effect and some of them showed no zone of inhibition at all (Table 3). On the contrary ethanolic leaf extract of sweet potato had shown no zone

Table 1. Tuber yield (t ha^{-1}) of white (W), orange (O), purple flesh (P) sweet potato across Odisha

Entry	Tuber yield (t ha^{-1}) in different areas of Odisha					Pooled Mean
	Farm(CTCRI) Bhubaneswar	Balasore	Kendrapara	Nuapada	Mangarajpur	
ST-10 (W)	24.77	23.31	24.16	24.03	23.81	24.01
Kishan (W)	21.25	20.42	21.34	20.81	20.32	20.82
Kalinga (W)	19.94	18.69	18.91	19.09	18.69	19.06
ST-14 (O)	21.59	21.29	20.87	20.54	21.58	21.17
ST-13 (P)	16.20	18.83	17.65	17.98	16.79	17.49
Gouri (O), [check]	16.77	16.05	17.61	15.20	17.84	16.69

Table 2. Bio-chemical constituents of high yielding white, orange, purple flesh sweet potato

Bio-chemical constituents	ST-10 (W)	Kishan (W)	Kalinga (W)	ST-14 (O)	ST-13 (P)	Gouri (O)
Dry matter content (%)	27.4- 29.7	32.0-34.0	29.6	27-29	24-25.5	22-27
Starch content (%) (extractable)	20.8- 21.2	13.5-14.6	12.0-12.5	18.8-19.7	16.5-17	9.8-11.5
β-Carotene / anthocyanin content (mg 100 g^{-1})	-	-	-	11.8-14 (β-carotene)	85-90 (anthocyanin)	4.5-5.5 (β-Carotene)
Total sugar content (%)	3-3.7	3.0-3.5	2.5-3.3	2-2.4	1.9-2.2	4.8
Cooking quality	Excellent, soft and mealy	Good, sweet and mealy	Excellent	Good and mealy	Fair, non mealy	Good, Non-mealy

Table 3. Antimicrobial activity [zone of inhibition (Z.I.)] of ethanolic extracts of Sweet Potato [*Ipomoea batatas* (L.)] tubers at 200mg/ml.

Test organism	Z .I (mm) of different varieties of sweet potato				
	ST-10	ST-13	Kishan	Kalinga	ST-14
Escherichia coli	ND	10.4 ± 0.62	ND	ND	7.8 ± 0.38
Staphylococcus aureus	ND	10.8 ± 0.28	ND	ND	6.9 ± 0.16

Table 4. Antimicrobial activity [zone of inhibition (Z.I.)] of ethanolic extracts of Sweet Potato (*Ipomoea batatas* (L.)) leaves at 200 mg/ml.

Test organism	Z .I. (mm) of different varieties of sweet potato				
	ST-10	ST-13	Kishan	Kalinga	ST-14
Escherichia coli	ND	ND	ND	ND	ND
Staphylococcus aureus	ND	ND	ND	ND	ND

*Z.I. (Zone of Inhibition): Mean inhibition zone ± SD
*SD: Standard deviation
*ND: Antibacterial activity is not detected

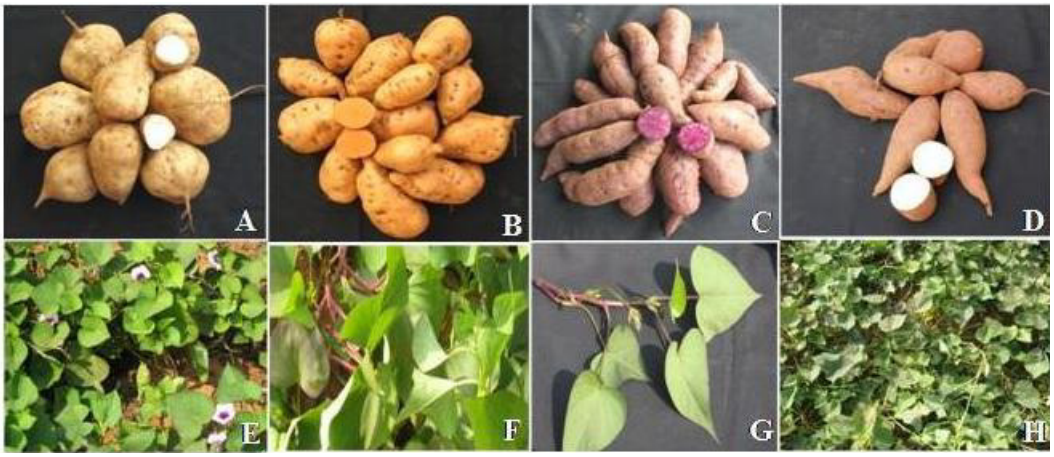

Figure 1. (A-H): Explant sources, A (ST-10), B (ST-14), C (ST-13), D (Kalinga) tubers of white, orange and purple flesh sweet potato, and E to H leaves of respective varieties.

of inhibition against *E. coli* and *S. aureus* (Table 4). The antimicrobial properties found for these extracts could be due to the presence of polyphenols like β-carotene and anthocyanin in the extracts at different levels.

These findings on antibacterial activity of orange and purple flesh tubers can lead to the development of potent antibiotic drugs to combat multi-drug resistant and sensitive bacteria. The rich source of anthocyanin in evolved sweet potato variety ST-13 can also be an ideal candidate for future anticancer and antidiabetic drugs. Further antioxidant activity of β-carotene and anthocyanin of these improved varieties can have several implications in delaying the process of ageing and can address the issues of food scarcity and malnutrition in disadvantageous areas and vulnerable section of the society in sweet potato growing countries.

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