



American Journal of
Food Technology

ISSN 1557-4571



Academic
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Finger Millet Polyphenols: Characterization and their Nutraceutical Potential

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Abstract: Finger millet, one of the important minor cereals contains 0.3-3% polyphenols. The millet is known for its health benefits such as hypoglycemic, hypocholesterolemic and anti-ulcerative characteristics, besides for its extremely good storage qualities. It is generally presumed that, the polyphenols of millet have major beneficial role in some of these health beneficial characteristics of millet. However, a very limited reports are available relating to the varietal variations with respect to the polyphenol contents, methods of isolation and characterization of the polyphenols and their nutritional implications. Recently, some information on the characteristics of millet polyphenols, their localization in the millet kernel and also on their nutraceutical ability has been generated. This review deals with the information published as well as under publication on the millet polyphenols in general and their isolation, characterization and also their nutraceutical potential in particular.

Key words: Finger millet, polyphenols, antioxidants, enzyme inhibition, anti-microbial properties

INTRODUCTION

Finger millet (*Eleusine coracana*) is one of the important minor cereals in the Indian subcontinent and many of the African countries. It is commonly known as ragi and forms staple for a large segment of population in the area under its cultivation. It is a small seeded caryopsis, largely spherical in shape with light brown to brick red colored seed coat. Of late a few white seeded millet have also been released but are not still popular.

The millet contains about 5-8% protein, 1-2% ether extractives, 65-75% carbohydrates, 15-20% dietary fibre and 2.5-3.5% minerals. It is also a very good source of micronutrients and phytochemicals such as dietary fibre, polyphenols, pigments and phytate (Hulse *et al.*, 1980). The whole grain millet is edible and the traditional foods are generally prepared from the whole meal. This indicates that, the millet phytochemicals including polyphenols are edible and do not cause any adversities on the human health. On the other hand, some of the known health benefits associated with the millet, such as its hypoglycemic (Lakshmi Kumari and Sumathi, 2002), hypocholesterolemic (Hegde *et al.*, 2002) characteristics and anti-ulcerative properties (Tovey, 1994) and also the excellent storage quality of the millet (Iyengar *et al.*, 1945) could be attributed to a large extent to its polyphenol contents. In recent years, the millet polyphenols have received a considerable interest, in view of their antioxidant and other nutraceutical properties.

The scientific information on the polyphenols of finger millet is scanty. According to Hulse *et al.* (1980) they are a set of phytochemicals with the molecular weight ranging from 150-30,000 Da, mainly consisting of phenolic compounds and their derivatives, flavonoids and tannins.

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Varietal Variations in Polyphenol Contents

Similar to many other cereals and grain legumes, varietal variations with respect to the polyphenol content of finger millet have been reported (Table 1). Ramachandra *et al.* (1977) analyzed 32 varieties of the millet comprising of both brown and white seed coat material from Indian and African source and reported that the white grain varieties contained lower levels of polyphenols (0.04-0.09%) than the brown grain varieties (0.08-3.47%). The total polyphenol contents of the Indian varieties ranged from 0.08-0.96% as chlorogenic acid equivalents and 0.04-1.05% tannins as catechin equivalents. On the other hand, in the African varieties, the corresponding values were 0.54-2.44 and 0.5-3.47%, respectively.

Rao and Prabhavathi (1982) in an unspecified variety of finger millet reported 0.36% tannin (catechin equivalents) where as McDonough *et al.* (1986) observed 0.55-0.59% total polyphenols and 0.17-0.32% tannins (catechin equivalent) in a small number (n = 3) of the millet varieties. Subsequently, Rao and Deosthale (1988) showed that, the tannin (catechin equivalents) contents in 12 number of brown colored millet varieties ranged from 0.35-2.4% but they did not detect tannins in the white (n = 3) varieties. Shankara (1991) analyzed a large number of finger millet varieties (n = 85) from the Indian state of Karnataka and reported a wide variability in the total polyphenol contents assayed as chlorogenic acid (0.06-0.67%), tannic acid (0.03-0.57%) and catechin (0.03-2.37%) equivalents. According to Sripriya *et al.* (1996) the total polyphenol contents of a brown variety of the millet (0.1%) was higher than the white variety (0.003%). Very recently Chethan and Malleshi (2006) analyzed five brown and two white varieties and reported 1.3-2.3% polyphenols as gallic acid equivalents in brown varieties and 0.3-0.5% in white varieties.

This information on the polyphenol contents of the millet gives an indication that, considerable variations exist among different genotypes of the millet. However, the values have to be taken on their face value, because the method of extraction, as well as the method of assay and also the standards used, vary considerably among different reports.

Extraction and Methods for Assay

Finger millet polyphenols have not been investigated in detail and hence, in the absence of clear information about the nature of its phenolics, the methods normally followed for the assay of sorghum polyphenols is followed for the extraction and assay of the millet polyphenols also.

Table 1: Total polyphenols and Tannin content of finger millet

	Total phenols	Reference standard	Tannin	Reference standard	Reference
Indian brown					
(N = 13)	0.08-0.96%	Chlorogenic acid	0.12-1.05%	Catechin	Ramachandra <i>et al.</i> (1977)
(N = 01)	-	-	0.36%	Catechin	Rao and Prabhavati (1982)
(N = 85)	0.03-0.57%	Tannic acid	0.03-2.37%	Catechin	Shankara (1991)
	0.06-0.67%	Chlorogenic acid	-	-	
(N = 1)	0.1%	Chlorogenic acid	-	-	Sripriya <i>et al.</i> (1996)
(N = 12)	-	-	0.35-2.39%	Catechin	Rao and Deosthale (1988)
(N = 1)	Free 0.77%	Gallic acid	-	-	Subba Rao and
	Bound 0.57%	Gallic acid	-	-	Muralikrishna (2002)
(N = 3)	0.55-0.59%	Chlorogenic acid	0.17-0.32%	Catechin	McDonough <i>et al.</i> (1986)
(N = 5)	1. 3-2.3 %	Gallic acid	-	-	Chethan and Malleshi (2006)
African brown					
(N = 10)	0.54-2.44%	Chlorogenic acid	0.46-3.47%	Catechin	Ramachandra <i>et al.</i> (1977)
Indian white					
(N = 6)	0.06-0.09%	Chlorogenic acid	0.04-0.06%	Catechin	Ramachandra <i>et al.</i> (1977)
(N = 1)	0.003%	Chlorogenic acid	-	-	Sripriya <i>et al.</i> (1996)
(N = 2)	0.03-0.05%	Gallic acid	-	-	Chethan and Malleshi (2006)

N, indicates the number of varieties used for the studies

Table 2: Percentage of the finger millet polyphenols extracted by different solvents

Solvents	Extraction at ambient condition		Extraction by refluxing	
	Pure	Acidified with 1% HCl	Pure	Acidified with 1% HCl
Water	0.17 (7.4)	0.34 (14.8)	0.25 (10.9)	0.45 (19.6)
Acetone	0.30 (13.0)	0.50 (21.75)	0.90 (39.0)	1.04 (45.2)
Propanol	0.24 (1.0)	0.58 (25.2)	0.86 (37.3)	1.23 (53.5)
Ethanol	0.30 (13.1)	0.74 (31.3)	1.02 (44.3)	1.38 (60.0)
Methanol	0.45 (19.6)	0.90 (39.5)	1.38 (53.9)	2.30 (100)

Average of two independent determination, Values in the parenthesis indicate percentage of the total assayable polyphenols

Careful scrutiny of the literature on the extraction and method of assay followed for the millet polyphenols reveals that, the majority of the workers used 1% HCl in methanol as solvent for extraction of the millet polyphenols, but the duration as well as the mode of extraction differed considerably (Table 2). Ramachandra *et al.* (1977) treated the millet meal for 24 h with 1% HCl-methanol solvent system with occasional swirling at ambient conditions, but Rao and Deosthale (1988) used 1% HCl methanol at ambient condition, on the other hand Sripriya *et al.* (1996) refluxed the millet with only methanol at 60°C for 2 h whereas Shankara (1991) used 1% HCl methanol with refluxing for 30 min. Subba Rao and Muralikrishna (2001; 2002) used 70% ethanol and 1M sodium hydroxide containing sodium borohydrate under nitrogen atmosphere to extract free and bound phenolics respectively. The recent studies from our lab (Chethan and Malleshi, 2006) indicated that, the extractability of the millet polyphenols with different polar solvents varies considerably and acidifying the organic solvents enhances the levels of extraction (Table 2). They also observed that, 1% HCl-methanol solvent and refluxing the material with small aliquots of solvent in sequence is most suitable among the different solvents tested for complete extraction of the polyphenols. However, for extraction, a large volume of solvent (about 6.5 L for 100 g of the millet meal) is needed, probably because of the rigid attachment of the phenols with other seed coat biochemical constituents.

For assay of the polyphenols including tannins most of the reports indicate use of Vanillin-HCl method of Burns (1971), Folin-Dennis method of Swain and Hillis (1959) and Folin-Ciocalteu method of Singleton *et al.* (1995). The Vanillin-HCl method involves the condensation of the aromatic aldehyde vanillin with monomeric flavanols and their oligomers to form a red adduct that absorbs at 500 nm, but Folin-Ciocalteu method is based on the oxidation of phenols with a mixture of phosphotungstic acid and phosphomolybdic acids in alkaline conditions leading the formation of blue colored compounds, the concentration of which is read at 760 nm. Ramachandra *et al.* (1977) used Folin-Dennis method to estimate the total phenols using chlorogenic acid as a reference standard but for assay of tannins, they followed Vanillin HCl method using catechin as reference standard. But in contrast to this, McDonough *et al.* (1986) used vanillin-HCl method for the assay of tannin and Folin-Ciocalteu method for total polyphenols. Chethan and Malleshi (2006) also used Folin-Ciocalteu method for the estimation of total polyphenols using gallic acid as standard.

The large variability in the polyphenols reported for the millet could be the reason for the different methods followed for the assay.

Distribution of Polyphenols in the Millet

A limited information on the distribution of polyphenols in the millet kernel indicates that, in its kernel, about 90% of phenolics is concentrated in the seed coat and the rest of it is distributed in the endosperm cell walls. Fulcher *et al.* (1972) observed intense auto-fluorescence in the cell walls of the millet indicating the presence of phenolic acids, probably ferulic acid as a constituent of the cell wall. McDonough *et al.* (1986) also substantiated the observation of Fulcher *et al.* (1972) with the aid of

fluorescence microscopy by observing intense fluorescence in the testa and mild fluorescence in the endosperm cell walls. They largely attributed fluorescence to the ferulic acid content. Similar observations were also made by Chethan and Malleshi (2006) using fluorescence microscopy (Fig. 1). Shobana *et al.* (2006) identified the phenolics in the endosperm cell walls of the millet by staining the phenolics with FeCl_3 (Fig. 2). Their histochemical observations were corroborated and also by chemical estimation of polyphenols of the seed coat rich and endosperm rich fractions prepared by milling the millet (Table 3). Guided by this observations, they suggested a protocol for preparation of polyphenol rich fraction of the millet (Fig. 3). According to Ramachandra *et al.* (1977) dehulling of the high tannin finger millet varieties reduced their polyphenol contents by 80% indicating the concentration of the phenolics in the seed coat. Similarly, Malleshi (2003) reported that, decorticated finger millet after hydrothermal treatment contained only 0.067% polyphenols as against 0.24% in the native kernel.

Processing of Millets

The information on the status in of polyphenols of malted millet has received the attention since, the millet is used for malting to a significant extent (Malleshi and Desikachar, 1986). Rao and Deosthale (1988) reported 0.91% tannins in ungerminated millet which decreased by about 72% on 72 h germination, whereas Sripriya *et al.* (1996) reported 35% decrease in the total polyphenols on germination but 34% increases on fermentation. A two fold decrease in all the major phenolic acids after 96 h of germination was recorded by Subba Rao and Muralikrishna (2002). The decrease in the bound phenolics may be due to the action of esterases developed during germination which is known to act on various phenolic acid esters linked either to arabinoxylans or other non-starch polysaccharides. A three fold decrease in protocatechuic acid content but marginal loss in caffeic acid upon 96 h of malting was reported by these workers. Similar observations was made by Lakshmi kumari and Sumathi (2002) also. Malleshi (2003) reported that hydrothermal treatment and decortication of finger millet decreases polyphenols by 74.7%. According to Chethan *et al.* (2006) nearly 44% of the polyphenols of the millet decrease during the first 24 h of germination and about 80% of it is lost after 120 h of germination. Chethan and Malleshi (unpublished data) reported that, hydrothermal treatment to the millet lowers its polyphenol content hardly by 10%.

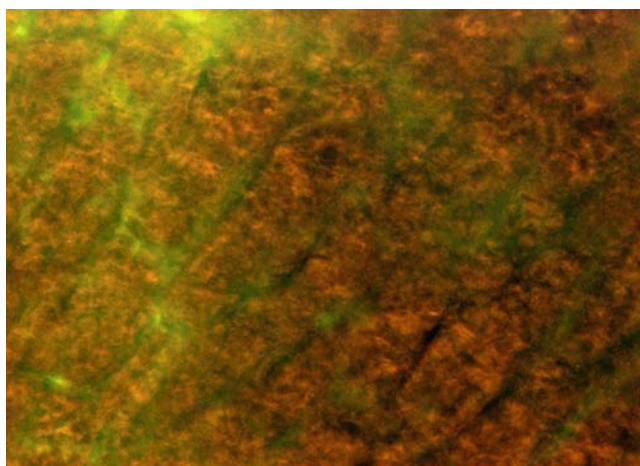


Fig. 1: Section viewed under fluorescence microscopy showing cell walls fluorescing

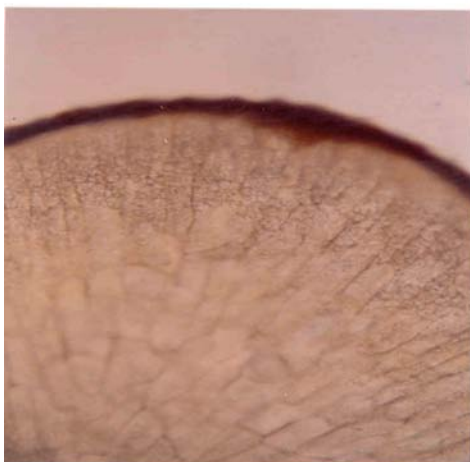


Fig. 2a: Section showing polyphenol stain in the endosperm cell walls

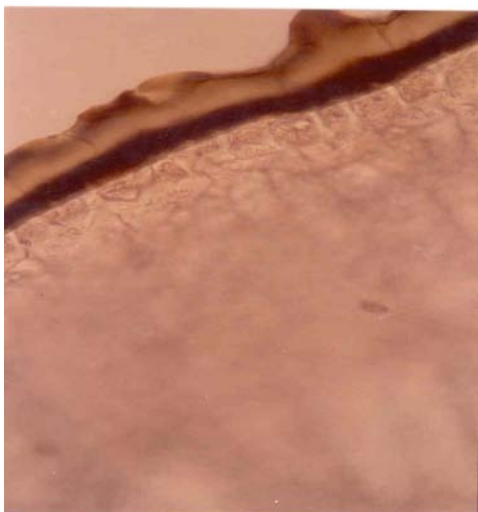


Fig. 2b: Section showing polyphenols concentrated in the seed coat

Characterization of Phenolics

Very little information is available on the characterization of millet polyphenols and the total profile of the millet phenolics has not been established till date (Table 4). Hilu *et al.* (1978) characterized the flavonoids by HPLC and identified orientin, isoorientin, vitexin, isovitexin, saponarin, violanthin, lucenin-1 and triclin. McDonough *et al.* (1986) identified ferulic ($405 \mu\text{g g}^{-1}$), coumaric ($67 \mu\text{g g}^{-1}$), gentisic ($53 \mu\text{g g}^{-1}$), cinnamic ($35 \mu\text{g g}^{-1}$), caffeic ($15 \mu\text{g g}^{-1}$), vanillic ($15 \mu\text{g g}^{-1}$), protocatechuic acid ($14 \mu\text{g g}^{-1}$), p-hydroxy benzoic ($09 \mu\text{g g}^{-1}$), syringic ($07 \mu\text{g g}^{-1}$) and sinapic acid ($04 \mu\text{g g}^{-1}$) as component phenolics in the millet. Sripriya *et al.* (1996) hypothetically assumed that catechin is the major phenolic, whereas, Subba Rao and Muralikrishna (2002) identified gallic, vanillic, coumaric and ferulic acids as free phenolics and ferulic, caffeic and coumaric acids as bound phenolic

Table 3: Polyphenol contents in the milling fractions

Milling fractions	Polyphenols (%)
Whole meal	2.3
Refined flour fraction (-180 μ)	0.6
Seed coat fraction (+180 μ)	6.2

Table 4: Phenolic acids and flavonoids reported in finger millet

Phenolic acids	References
Hydroxy benzoic acids Gallic, protocatechuic, p-hydroxy benzoic, gentisic, vanillic, syringic	McDonough <i>et al.</i> (1986), Subba Rao and Muralikrishna (2002) and Chethan and Malleshi (2006)
Hydroxycinnamic acids Ferulic, caffeic, p-coumaric, cinnamic, sinapic	McDonough <i>et al.</i> (1986), Subba Rao and Muralikrishna (2002) and Chethan and Malleshi (2006)
Flavonoids Quercetin, orientin, isoorientin, vitexin, saponarin, violanthin, leucine, triclin	Hilu <i>et al.</i> (1978) and Chethan and Malleshi (2006)

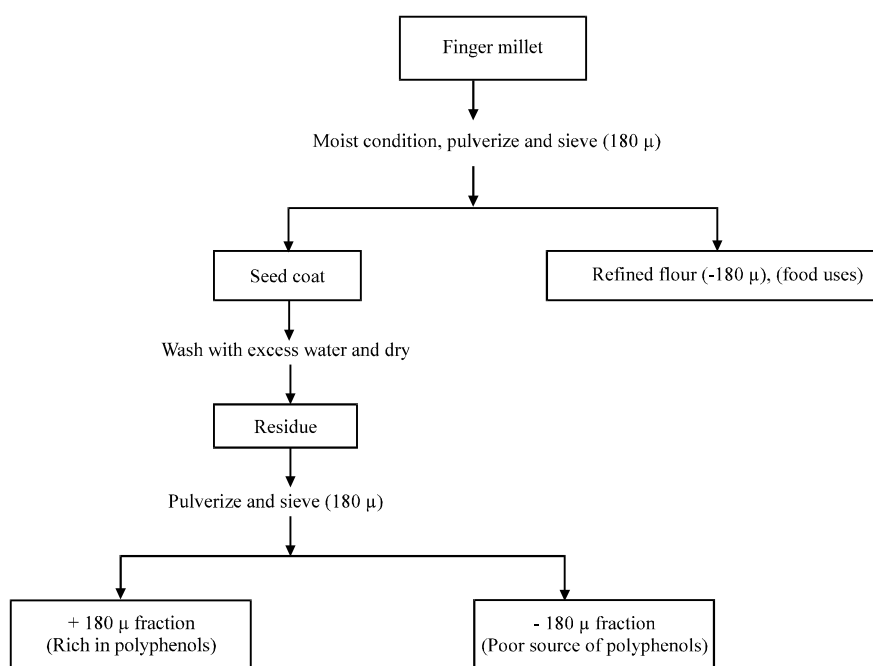


Fig. 3: Protocol for the preparation of the polyphenols rich seed coat fraction

acids. Very recently, Chethan and Malleshi (2006), made an effort to characterize the millet phenolics extracted in HCl-methanol solvent and fractionation by HPLC. They identified nine phenolic acids namely, the benzoic acid derivatives (gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, ferulic acid) and cinnamic acid derivatives (syringic acid, trans-cinnamic acid, p-coumaric acid) and also a flavonoid compound namely, quercetin (Fig. 4). Benzoic acid derivatives accounted for about 85% of the total phenolic compounds. They are probably the first to report quercetin, a flavonoid in finger millet.

The millet polyphenols irrespective of the extraction solvent, appear to be colored and the color varies from pink to dark red. The millet phenolics are pH sensitive and are stable in acidic pH but highly unstable in the alkaline pH range. However, the stability of the phenolics is reversible to

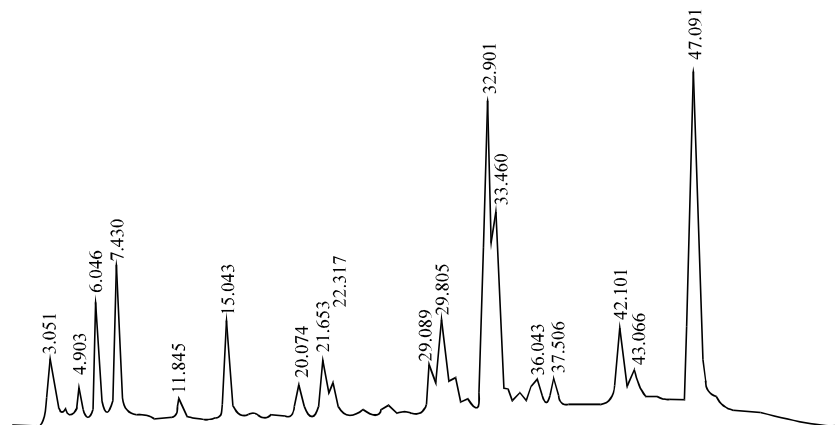


Fig. 4: HPLC chromatogram of native millet polyphenols. Polyphenols were fractionated on μ -Bondapack, C-18 column, using 0.1% tri-fluoroacetic acid: methanol at a flow rate of 1.0 mL min^{-1} phenolics identified based on elution time. 3.1: gallic acid; 6.04: proto-catechuic acid; 7.4: p-hydroxy benzoic acid; 11.8: vanillic acid; 5.0: p-coumaric acid; 20.07: syringic acid; 22.3: ferullic acid; 32.9: trans-cinnamic acid; 43.06: quercetin

a large extent. The mechanism of their stability or structural changes has not been studied. The phenolics are heat stable but are pH sensitive. They may precipitate by raising the pH to highly alkaline state (Fig. 5).

Antioxidant Properties

The antioxidant properties of millet polyphenols has received the attention of many researchers. Sripriya *et al.* (1996) investigated the antioxidant properties of polyphenols extracted with methanol which was able to quench about 77% of hydroxyl radicals. According to them, the 2, 2-diphenyl-1-picrylhydrazyl DPPH radical quenching ability of finger millet was 94% whereas its fermented as well as germinated and also germinated and then fermented sample showed only 22, 25 and 10% quenching, respectively. This showed that processing the millet reduces its free radical quenching capacity. The major antioxidant principle reported is catechin.

The effect of malting on phenolic antioxidants were studied by Subba Rao and Muralikrishna (2002). They reported that, the antioxidant activity of mixed free phenolic acids was higher compared to that of mixed bound phenolic acids. They reported an increase in the antioxidant activity coefficient from 770 to 1686 in the case of free phenolic acids and a decrease from 570 to 448 upon 96 h of malting in bound phenolic acids. They compared various, naturally occurring phenolic acids such as caffeic, coumaric, ferulic, gallic, gentisic, protocatechuic, syringic and vanillic acids with the synthetic antioxidants such as BHA and BHT and it was observed that, the antioxidant activity of the millet polyphenols was slightly lower than the synthetic antioxidant compounds. Cinnamic acid derivatives such as ferulic, caffeic and coumaric acids exhibit higher antioxidant activities (AACs) than their corresponding benzoic acid derivatives namely, vanillic, protocatechuic and p-hydroxy benzoic acids. Among the cinnamic acid derivatives, caffeic and ferulic acids were prominent compared to coumaric acid. Gallic acid, which has three -OH groups was stronger than protocatechuic acid, gentisic and syringic acids with respect to their antioxidant properties. Finger millet polyphenols exhibited the antioxidative properties effectively on super oxide, hydroxyl and nitric oxide radicals (Bindu and Malleshi, 2003). Asharani *et al.* (2006) have shown that the millet contain $199 \pm 77 \text{ } \mu\text{g}/100 \text{ g}$ and $4 \text{ mg}\%$ carotenoids and Vitamin E, respectively and exhibited $15.3 \pm 3.5 \text{ TE g}^{-1}$ the total antioxidant activity.

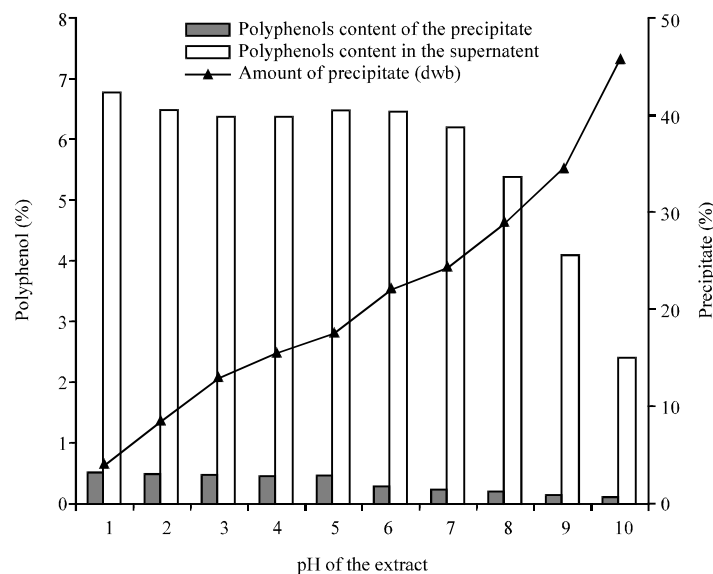


Fig. 5: Polyphenol contents of the supernatant and the precipitate formed at different pH

They identified the isomers of these and reported that, the total antioxidant activity of whole meal of finger millet is considerably higher than other millets. Vishwanath (unpublished data) determined the antioxidant activity of the polyphenols extracts from finger millet seed coat and whole meal and reported that the seed coat extract exhibits about 5 times higher activity compared to whole meal assayed in terms of reducing power assay and the β -carotene bleaching method.

Enzyme Inhibitory Activity

Chethan *et al.* (2006) conducted the investigations on the mode of inhibition of the millet polyphenols on its malt amylases and noted that in the presence of the crude extract, the K_m remained constant and the maximum velocity decreased indicating mixed non-competitive type inhibition. They also noticed that, among gallic, vanillic, ferulic, quercetin, trans-cinnamic, syringic, protocatechuic and p-hydroxy benzoic acids isolated from the millet, only gallic, vanillic, quercetin and trans-cinnamic acid exhibited uncompetitive type of inhibition. The inhibitory constant (K_i) for the crude polyphenol extract was 66.7 μg , but the dissociation constants (K_i') of phenolic compounds were in the range of $4.6 \times 10^{-7}\text{M}$ - $7.3 \times 10^{-7}\text{M}$. The kinetic studies of amylase inhibition by the phenolic compounds indicated the presence of secondary binding sites in malted finger millet amylase, similar to other cereal amylases.

The millet polyphenols have also been reported to exhibit the inhibitory activity on aldose reductase and snake venom phospholipases A_2 (PLA₂) (Chethan, unpublished data). It was observed that 100 μg of polyphenol extract inhibits 60% of the snake venom PLA₂ activity. In a separate study on possible anti-cataractogenesis of the polyphenols it was observed that, the millet phenolic acids exhibit inhibitory potency against marker enzyme for cataract formation. Quercetin showed positive high potency against the marker enzyme and competes with the substrate for the active site.

Antimicrobial Activity

Chethan and Malleshi (unpublished data) studied the antimicrobial activity of polyphenols from ungerminated and germinated finger millet on most pathogenic bacterial strains, *Escherichia coli*,

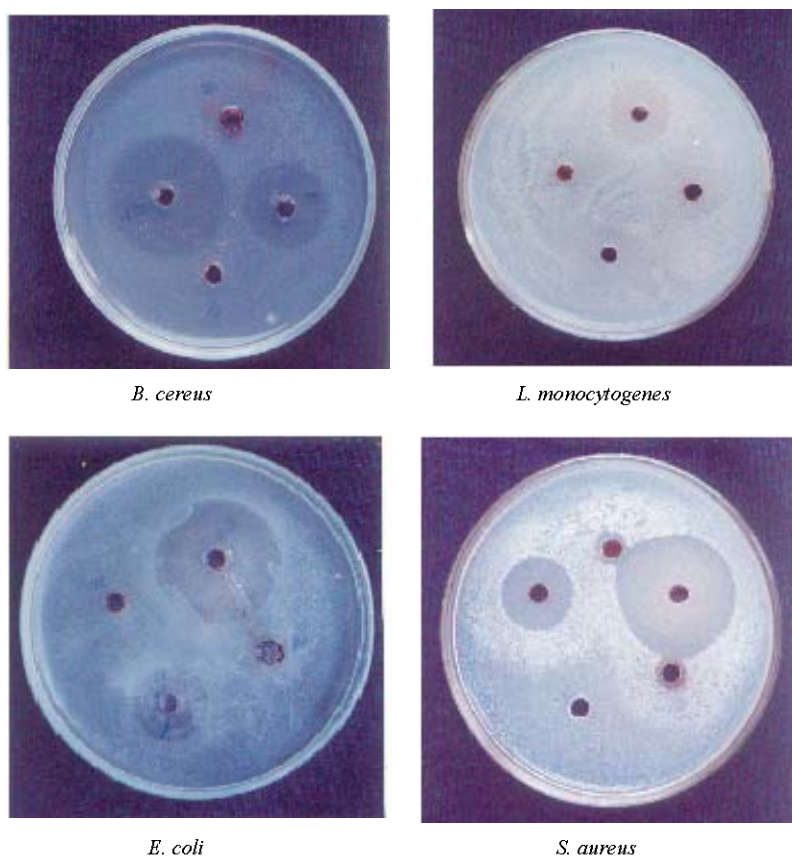


Fig. 6: Anti-microbial activity of finger millet polyphenols on some micro-organisms

Staphylococcus aureus, *Listeria monocytogenes*, *Sterptococcus pyogenes*, *Pseudomonas aeruginosa*, *Serrtia marcescens*, *Klebsiella pneumonia* and *Yersinia enterocolitica* and observed difference in the inhibitory activity (Fig. 6). The phenolic compounds fractionated from finger millet, such as quercitin inhibited the growth of all the pathogenic bacteria, whereas gallic, caffeic, protocatechuic, para-hydroxy benzoic acid showed their activity restricted only to few bacterial strains.

Nutritional Aspects

Polyphenols are known to possess inhibitory activity on the digestive enzymes. Ramachandra *et al.* (1977) studied the effect of the millet tannin content on its protein digestibility and observed that high tannin finger millet exhibit low protein digestibility. Dehulling had the effect of removing most of the phenolics from finger millet grain with concomitant increase *in vitro* protein digestibility. Tannins were found to be associated mostly with the glutelin fraction of finger millet protein. Rao and Deosthale (1988) studied the role of tannins in brown and white millet and reported that after extraction of tannins, the ionisable iron in brown millet raised by 85%, on the other hand addition of tannins extracted from brown millet to white millet lowered its ionisable iron by 52-65%.

Health Benefits of Finger Millet

Although, epidemically, the health benefits of the millet with respect to diabetes, CVD and duodenal ulcer have been known, the role of polyphenols towards these have not been investigated. But a few reports cover the matter with some relevance to these and the same have been referred here.

Hegde *et al.* (2002) reported the effects of methanolic extracts of finger millet on glycation and cross linking of collagen and identified that the oxidation of glycated collagen or glyoxidation may be the critical factor responsible for collagen cross linking and attendant complications in *Diabetes mellitus*. About 3 mg of the methanolic extract of the millet was found to inhibit glycation similar to 125 mg of aminoguanidine and 1 mg of the well known synthetic antioxidant butylated hydroxyanisole. The study on cross linking of collagen, analysed by pepsin digestion and CNBr digestion strongly suggested the protective role of the methanolic extracts of the finger millet.

The detailed investigations on the millet polyphenols with respect to structural characteristics of the component phenolics and their antioxidant property, inhibitory activity on digestive enzymes, anti-microbial activity on the intestinal microflora may be helpful towards identifying the pharmaconutritional characteristics of finger millet as well as its milling fractions.

ACKNOWLEDGMENTS

The authors acknowledge with thanks the support received from Dr. V. Prakash, Director, CFTRI, Mysore. One of the authors S.C. thanks the council of Scientific and Industrial Research (CSIR), New-Delhi, India for the award of Senior Research Fellowship.

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