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Introduction
The present investigation deals with synthesis of some new benzoxazine and quinazolinone derivatives bearing a bulky moiety at position -2 in order to study the stability and reactivity of their nucleus towards different nucleophiles. Here we report reactions of 6,8-dibromo-2-(3,4-dichlorophenyl)-4H-benzo[d][1,3]oxazin-4-one (1) with nitrogen and carbon nucleophiles, aiming to synthesize condensed and non-condensed heterocyclic systems involving quinazoline moiety due to its significant biological activities as anticonvulsant (Dandia et al., 2005) as well as antihistaminic agents, (Amine et al., 1996), inhibition of cathepsin (Gutschow et al., 2002), besides other antihyperglycemic activities (Ram et al., 2003) and in continuation of other investigations directed towards the synthesis and reaction of some benzoxazine and quinazoline derivatives (Ma et al., 2006; Zheng et al., 2006).

Materials and Methods
Melting points are uncorrected. IR spectra were recorded on a Perkin-Elmer 1600 spectrophotometer using KBr wafer technique. The 1H-NMR spectra 200 MHz were determined on a Jeol JNM-EX200 spectrometer using TMS as internal reference (chemical shifts are expressed as δ, ppm). Micro-analytical data (C, H, N) were obtained from the Microanalytical Center at Cairo University. The physical data are listed in Table 1.

Abstract. The reaction of 3,4-dichlorobenzoyl chloride with 3,5-dibromo anthranilic acid yielded benzoxazine derivative (1), whose reaction with primary and secondary amines such as benzyl amine, p-chloroaniline, p-anisidine, p-toluidine, piperidine and morpholine in boiling ethanol yielded six (3,5-dibromo-2(3,4-dichlorobenzoylamino)-N-substituted benzamides (2-7). Reaction of the derivative (1) with hydrazine hydrate (1:1 molar proportions) gave the unexpected product 6,8-dibromo-2-(3,4-dichlorophenyl)-3-(2-(3,4-dichlorobenzoylamino)3,5-dibromobenzamido) quinazolin-4-one (8).

Keywords: benzoxazine derivatives, quinazoline derivatives, 3-thia-1-azabutane-2,4-dione

6,8-Dibromo-2-(3,4-dichlorophenyl)-4H-benzo[d][1,3] oxazin-4-one (1). To a solution of 3,5-dibromoanthranilic acid (0.01mol) in dry 50 ml pyridine, 3,4-dichlorobenzoyl chloride was added dropwise with stirring. The reaction mixture was heated on water bath for 2 h, and then poured onto acidified cold water. The separated solid was filtered off, dried and crystallized from benzene to give compound 1 as yellow crystals. IR: 1773 cm-1 (C=O lactone), 1622 cm-1 (C=N) and 1600 cm-1 (C=C); 1H NMR (DMSO): δ 7.63-7.90 (m, 3H) and 8.07-8.45 (m, 2H).

3,5-Dibromo-2-(3,4-dichlorobenzoylamino)-N-benzyl benzamide (2).

3,5-Dibromo-2-(3,4-dichlorobenzoylamino)-N-(4-chlorophenyl) benzamide (3).

3,5-Dibromo-2-(3,4-dichlorobenzoylamino)-N-(4-methoxyphenyl) benzamide (4).

3,5-Dibromo-2-(3,4-dichlorobenzoylamino)-N-(4-methylphenyl) benzamide (5).
Synthesis of Some 2-Methyl-3-(Arylthiocarbamido) Quinazol-4-Ones and 2-Methyl-3-(Arylidencarboxamido) Quinazol-4-Ones as Potential Antimicrobial Agents

B. D. Gupta
Department of Chemistry, Northern India Engineering College, Lucknow 226001, India

(received July 23, 2007; revised June 10, 2008; accepted June 12, 2008)

Abstract. Some quinazolone derivatives of 2-methyl-3-(arylthiocarbamido) quinazol-4-ones (2) and 2-methyl-3-(arylidencarboxamido) quinazol-4-ones (3) have been synthesized and assayed for their possible antibacterial activity against Bacillus subtilis, Bacillus cereus, Salmonella aureus, Salmonella luteae and antiviral activity against Gomphrena mosaic virus. Some of these compounds show notable activity.

Keywords: quinazol-4-ones, antibacterial activity, antiviral activity

Introduction

Quinazolone derivatives exhibit a wide range of activity such as dopamine receptor (Srivastva et al., 1987) anthelmintic (Gupta et al., 1988; Alaimo, 1972) anti-inflammatory (Alagarsamy et al., 2003), antimicrobial (Pandey et al., 2004; Alagarsamy et al., 2000) CNS depressant (Saksena and Khan, 1989; Kacker and Zaheer, 1951) neuroleptic (Mukerji et al., 1980) hypotonic (Gujral et al., 1955) and analgesic (Ram et al., 1990). Pharmacological activity of this class of compounds is beyond any doubt, thus it was decided to synthesize some new title quinazolones in order to study their antibacterial and antiviral activities.

Materials and Methods

Melting points were determined in open glass capillary and are uncorrected. IR spectra (ν max in cm⁻¹) were recorded on a Perkin Elmer-157 spectrometer and ¹H NMR (60 MHz) spectra on Varian EM 360 spectrometer.

2-Methyl-1,3-benzo [d] oxazin-4-one called acetanthranil was obtained essentially by the method of Zentmyer and Wagner (1949).

2-Methyl-3-(4-chlorophenylthiocarbamido) quinazol-4-one (2, R=4-Cl). (Scheme I) Acetanthranil (1.6 g) and 4-chlorophenylisocyanate (1.8 g) in methanol (20 ml) were heated together upto 3 h. The reaction mixture was cooled. The solid thus obtained was washed with dil. Na₂CO₃ followed by dil. HCl and the product was finally crystallised from ethanol; yield 75%. m.p. 168°C; MS: m/z: M⁺ 344; IR(KBr) cm⁻¹ : 1150, 1595, 1570, 1440, (Ar-H), 1620 (C=N), 1660 (C=O), 3250 (NH); ¹H NMR (DMSO-d₆): δ 2.1 (s, 3H, CH₃), 7.0-7.5 (m, 8H, Ar-H), 8.2 (s, 2H, NH); Anal. found: C 55.61; H 3.64; N 16.12. calcd: C 55.73; H 3.79; N 16.25% (Table 1).

2-Methyl-3-(4-chlorophenylthiocarbamido)-quinazol-4-one (3, R=4-OCH₃). Acetanthranil (1.6 g) and 4-methoxybenzaldeydesemicarbazide (1.8 g) in presence of excess of acetic anhydride (10 ml) were heated together on a water bath for 3 h. The reaction mixture was cooled. The solid thus obtained was washed with dil. Na₂CO₃ followed by dil. HCl and the product was finally crystallised from ethanol; yield 79%. m.p. 166 °C; MS m/z : M⁺ 336; IR (KBr) cm -1: 1595, 1570, 1460 (Ar-H), 1650 (C-NH), 1670 (C=O), 3400 (NH); ¹H NMR (DMSO-d₆): δ 2.1 (s, 3H, CH₃), 6.1 (s, 1H, N=CH), 7.2-7.6 (m, 8H, Ar-H), 8.6 (s, 1H, NH); C 55.61; H 3.64; N 16.12. calcd: C 55.73; H 3.79; N 16.25% (Table 1).

E-mail: bdgupta_chem@rediffmail.com
Some Physical Characteristics and Nutritional Composition of the Seeds of Wild Pepper (*Erythrococca anomala*, Benth)

E. A. Akande*, G. O. Adegokeb and F. C. Mathookoc

*Department of Food Science and Engineering, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria

bDepartment of Food Technology, University of Ibadan, Ibadan, Oyo State, Nigeria

cDepartment of Food Science and Technology, Jommo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

(received January 31, 2008; revised March 26, 2008; accepted April 04, 2008)

Abstract. Study of physical properties and nutritional components of whole and powdered wild pepper seeds (*Erythrococca anomala*, Benth) revealed that the seeds have good parameters for machineability. The contents of moisture, ash, protein, lipid and carbohydrate and major and trace minerals were found in functional quantities, while the heavy metals were negligible or absent. Thus the seeds are potential source of nutrients and can be used as additive in food product development.

Keywords: *Erythrococca anomala*, nutritional composition, product development, seed machineability

Introduction

Seeds are abundantly found in nature and are good and cheap sources of foods. They have multiple nutritive values and are also known to contain reasonable quantities of edible oils and fats. The satiety value, flavour enhancing and hunger delaying abilities are the particular attributes of fats. Moreover, seeds are cheap source of protein, known to be very important for the normal body functions in animals. Considering the shortage of food nutrients in human diet and reliance of the country on imports of food products from foreign countries, lot of efforts have been focussed on the exploitation of locally available natural raw materials for food production. For example, work has been done on bitter kola (*Garcinia kola*) (Daramola and Adegoke, 2007), African oil bean seed (*Pentaclethra macrophylla*) (Ajibola, 2005), African breadfruit seeds (*Treculia africana*) (Omobuwajo, 2002) etc.

Wild pepper (*Erythrococca anomala*, Benth) is an indigenous plant whose seeds are popular among the local people in the Western part of Nigeria, owing to the benefits associated with them. Its seeds are locally known as Iyere (Yor), Monsoro (Hausa) and Osunrisa (Ghana). They are usually of reddish to yellow colour on ripening, while deep brown after drying. The plants are creepers, found clustering around the stems of cocoa and kolanut trees, proliferating freely in parts of Southern Nigeria. The seeds of *E. anomala* are aromatic, pungent and medicinally used in treatment of sore throat, mouth infections, preparation of herbal soups for women and new mothers etc. The seeds are also used as food additive (in flavouring ‘kunnu’), preparation of pepper soups, cooking of rice and meat. Recently, the seeds of *E. anomala* have been employed in perfumeries in the northern Nigeria.

Since, no work has been rendered on determining the physical characteristics and the nutritional potential of the seeds, the present study was undertaken to establish the attributes of the seeds of wild pepper (*E. anomala*, Benth).

Materials and Methods

The physical characteristic including sphericity index, aspect ratio and density (kernel density, bulk density and density ratio) were determined using the methods of Maduako and Faborode (1990) and Mohsenin (1986).

Properly dried seeds of *E. anomala* were milled using Shromadzu grinding machine (AGG -270 F 005028F4) and sieved using a 6 mm mesh size. The powdered seeds were then made into 50 packs and kept for further analysis.

The moisture content was determined by drying method, protein by Kjedhal digestion method, and crude fat by Soxhlet extraction, according to AOAC (1990). The ash content was determined using Toyo Seisokusho Muffle Furnace (KL420: 00004023021) at 550 °C for one h.

Minerals analysis. Use of atomic absorption spectrophotometer, AAS-324-75603-84 & 2P88887GM, was made for the minerals analysis.

*Author for correspondence; E-mail: felemma@yahoo.com*
Relative Study of the Colour Fastness of Cotton, Woolen and Silk Fabrics Dyed With Walnut Bark Dye

BBushra Khalid*, Azra Yaqub, Lubna Liaquat and Mohammad Sohaib

Applied Chemistry Research Centre, PCSIR Laboratories Complex, Lahore-54600, Pakistan

(received February 19, 2008; May 27, 2008; accepted June 12, 2008)

Abstract. Natural walnut dye was extracted from walnut bark and applied to cotton, woolen and silk fabrics with the same depth of colour and colour fastness was assessed. Walnut dye had good saturation on all the three fabrics and its colour fastness properties ranged between good and excellent.

Keywords: walnut bark dye, Juglan regia, natural dye, colour fastness

Introduction

Natural and synthetic dyes are used for dyeing of fabrics and pottery. Some synthetic dyes such as disperse dyes and azo amine dyes have harmful effects on human beings causing allergy, cancer etc., and are anti-environment (IARC, 1975; Scott, 1952). Natural dyes are less allergenic, non-toxic and environment friendly and can be used in textile, pharmaceutical, food and cosmetic industry safely (Ali et al., 2007). Shades produced with most of the natural dyes are not bright, so mordants are used to produce fast and bright colours (Gulrajani and Gupta, 1992), while some dyes are substantive and can be directly applied on the textile fabrics, wool and leathers, without any need of mordants.

The drawback associated with natural dyes is that there are no suitable standard shade cards and standard test procedures relating to their extraction and other dyeing properties. A lot of work is, therefore, in progress to improve poor reproducibility and lack of desirable properties of natural dyes (Ali et al., 2007).

The present work is concerned with the extraction of natural dye from the Juglan regia (walnut), dyeing of various fabrics (cotton, woolen and silk) with it and then studying the fastness properties.

Juglan regia (walnut) belongs to the family Juglandaceae. It is a slow growing tree in northern parts of Pakistan. It is planted mainly for timber and nuts. The husk is smooth and nuts are easy to split (Cannon and Cannon, 1994). Green hulls or rinds of walnut were used for dyeing. The roots, inner bark referred to as walnut bark, was also used even though it had less potency of colour than the rind (Rita, 1971). Fruit is excellent for eating and baking. It is often used in confectionery and ice cream.

Materials and Methods

Instruments. D400 IR dyeing machine (SDL Atlas England); Launderometer (Roaches), Perspirometer kit (SDL Atlas England); oven, Ci 3000 + Xenon; weatherometer (Atlas England); water bath; grey scales for staining (ISO 105 A03); grey scale for change in shade (ISO 105 A02); crockmeter (SDL Atlas England); multifiber (DW).

Chemicals. Detergent ECE (without optical brightener), sodium per borate, l-histidine monochloride monohydrate, sodium dihydrogen orthophosphate, distilled water, sodium
**Quantitation of Fatty Acids by GLC and Separation of Omega-6 Nutraceutical Fatty Acid From *Carthamus tinctorius* L. Seed Oil Cultivated in Pakistan**

Rubina Saleem*, Ambrat*, Zahra Yaqeen* and Tehmina Sohail*

*a Applied Chemistry Research Centre, PCSIR Laboratories Complex, Karachi - 75280, Pakistan
b Pharmaceutical Research Centre, PCSIR Laboratories Complex, Karachi - 75280, Pakistan

(received July 13, 2007; revised June 8, 2008; accepted June 15, 2008)

**Abstract.** The GLC analysis of *Carthamus tinctorius* (safflower) yielded average hexane extracted oil content of 28% (25-30%); the oil contained high level of linoleic acid (74%). Monounsaturated fatty acid, oleic acid amounted 12.94%, while the saturated fatty acids like palmitic acid and stearic acid were 9.43 and 1.81%, respectively. Iodine value of linoleic acid was found to be 160.1 while its purity was 93.1%.

**Keywords:** *Carthamus tinctorius*, linoleic acid, omega-6 fatty acids

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**Introduction**

*Carthamus tinctorius* (safflower) is an annual species of the family compositae. This crop is adapted to dry land or irrigated cropping system. It is also known as false saffron, thistle saffron, cartame, saffron baturd in English, while Arab calls it Kazhirah (Eckey and Miller, 1954; George, 1892) and in Pakistan (Sindh) it is commonly known as Kushumba, Khusakdana or Powariji bij (Jafri, 1966). Seeds usually mature in September, about four weeks after the end of flowering (Oelke et al., 1989) with a seed oil content between 30-45%.

The plant is cultivated in California, Middle East, Africa and India (Eckey and Miller, 1954). It is an indigenous crop in Pakistan and is cultivated in Gilgit, Hunza, Kotri and Mirpur (Jafri, 1966). Normally two varieties of *C. tinctorius*, are found, one that produces oil with high amount of monounsaturated fatty acid (oleic acid) and the other with high concentration of polyunsaturated fatty acid (linoleic acid). *C. tinctorius* is a valuable source of one of the most important nutraceutical fatty acid, omega-6 (linoleic acid), an essential fatty acid which cannot be synthesized by the body but plays an important role in the control of joint ailments, skin, hair, nail and scalp disorders like eczema, acne, psoriasis etc.

The present study was made on the seeds of *C. tinctorius* variety producing linoleic acid and growing in Pakistan. The aim of the study was to assess the fatty acid composition of *C. tinctorius* in order to separate the linoleic acid quantitatively using urea adduct separation technique. This procedure is frequently applied to obtain polyunsaturated or branched chain fatty acids in concentrated form. Toxicity studies were also carried out to check the feasibility of using the oil as a source of essential fatty acid. Such studies based on the application of urea complexation technique have not yet been reported by any author in Pakistan.

**Materials and Methods**

All the reagents (analytical and GC) used, were purchased from E-Merck/Sigma/Aldrich. Pure standards of fatty acid methyl esters were obtained from Supelco Chemicals Co.

**Extraction of oil.** Seeds (one kg) were taken from the seed cultivars, cleaned to remove admixtures then grinded in an electrical grinder and fed to a soxhlet extractor fitted with a 2 litre round bottom flask and a condenser. The extraction was done on a water bath for 4-6 h with 1.5 litre *n*-hexane. Then the solvent was distilled off under vacuum in rotary evaporator. The oil was dried over anhydrous sodium sulphate, filtered and weighed. The procedure was performed in triplicate.

**Fatty acid composition.** Fatty acid methyl esters were prepared according to the standard IUPAC method (IUPAC, 1987) and analyzed on a Perkin-Elmer gas chromatograph model Clarus 500, fitted with a polar capillary column SP 2340 (60 m x 0.25 mm) and a flame ionization detector. Oxygen free nitrogen was used as a carrier gas at a flow rate of 3.5 ml/min.

Other operational conditions were as follows: initial oven temperature 70 °C for 5 min, increase in temp @ 10 °C/min to 180 °C and then @ 3 °C/min to 220 °C, held for 8 min; FID temperature: 275 °C, injector: 250 °C.

A sample volume of 1.0 μl was injected and the total analysis time was 37 min. Fatty acid methyl esters were identified by
Major Ion Chemistry of Groundwaters From the Peshawar Intermontane Basin, NWFP, Pakistan

M. Asim Yousafzai
Department of Geology, University of Peshawar, Peshawar, Pakistan

(Received April 21, 2008; revised June 12, 2008; accepted June 15, 2008)

Abstract. Investigation of spring and well waters of the Peshawar Intermontane basin and its surroundings in the Himalayan belt of Pakistan revealed that their temperature, pH, total dissolved solids and electrical conductivity values fall within the permissible range of drinking and irrigation water except the waters in vicinity of exposed faults. Minerals in the spring water are in the order of Ca > Mg > (Na + K) with bicarbonate as the dominant anion, whereas in the well water, the order is reversed, with sulphate as the dominant anion.

Keywords: Peshawar hydrochemistry, ground water, minerals, Pakistan

Introduction

Investigation was carried out to determine the field characteristics and major ion chemistry of spring and well waters. The study site covered the Peshawar intermontane basin (PIB) and its surroundings in the Himalayan foreland fold-and-thrust belt of Pakistan (Fig. 1). The area, located between latitudes 33.5°N-34.7°N and longitudes 71°E-73°E, is characterized by steep topography and V-shaped fluvial valleys in the north, which are drained by the river Indus entering from Indian Kashmir and the river Kabul entering from Afghanistan. Cold winters and warm, dry summers characterize the climate of the study area. June through August are the hot months, during which the daily mean maximum air temperature is about 40 °C. The mean annual potential evaporation ranges from 85 cm in the northern part of the study area to 130 cm in the centre of the basin. Snowfall occurs in the mountainous north during the cold months of December to February, when monthly mean minimum temperatures are several degrees Celsius below the freezing point.

Peshawar valley is home to two million plus inhabitants with ever growing demand for water for drinking, industrial and irrigation purposes. Every water usage (human, industrial, irrigation etc.) requires a set of hydrochemical characteristics, suited for that particular consumption. The hydrogeological data for the region is scattered and scanty (Shah and Tariq, 2007; Tariq, 2001) and this research is the first attempt to fill this gap. The broader study conducted for this research is the first of its kind in that it synthesizes field, laboratory and simulation data to bracket the hydrochemical speciation in the PIB. This paper presents the field and laboratory data for groundwater in the study area.

E-mail: masim@kent.edu

General geology. Information on subsurface geology of the study area has been derived from the lithological logs of boreholes drilled by WAPDA, Pakistan, and made available through various information releases. These data indicate that the quaternary sediments vary in thickness from few meters to more than 150 m. However, the total thickness of quaternary sediments is not known because none of the boreholes penetrates the bedrock. The coarseness of these sediments increases from north to south in the basin (Tariq, 2001). In the central part of the basin, the alluvial sediments consist of a relatively large proportion of fine-grained material, where the sandy silt is interbedded with discontinuous alluvial sand and thin gravel layers of various thickness. The main rock types in this area are slates, phyllites, various types of schists, paragneisses, sandstones, and quartzitic crystalline conglomerates, all of which are intruded by basic-to-acidic igneous rocks.

Hydrogeology. The study area can be divided into two hydrogeological provinces. The basin is bordered by mountains of the Lesser Himalayas on three sides while the southeastern side is a fluvial valley carved at the confluence of the Indus and Kabul rivers. In the mountainous region, the water table varies in depth in different intermontane valleys, suggesting hydraulic discontinuities. In fact, water table elevation varies considerably within the area, ranging from less than 100 m in the basin centre to more than 1600 m in the mountainous north in relation to mean sea level (Fig. 2). Abundant springs (both normal and high temperature) are present in this part of the study area, and locally they constitute an important source of drinking water in addition to dugwells. The outflow from these springs ranges from less than one l/sec to more than 2000 l/sec. The field investigations indicate that most of the springs have good quality water, and according to local authorities, there are no water-
The octadecenoic acid separated from the seed oils of *Nicotiana tabacum* L. and *Nicotiana rustica* by the application of argentation thin layer chromatography was oxidized by modified Von Rudloff’s technique. The liberated monofunctional and difunctional carboxylic acids were separated and identified by the application of thin layer chromatography and gas chromatography. The positional isomers determined in both species were cis-9-octadecenoic acid and cis-11-octadecenoic acid.

**Keywords:** isomers, monoenoic fatty acids, *Nicotiana tabacum*, *Nicotiana rustica*, seed oils

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Table 1. $R_f$ values of different fatty acid bands (as methyl esters) separated by the AgNO$_3$-TLC

<table>
<thead>
<tr>
<th>Band no.</th>
<th>Bands</th>
<th>Fatty acid</th>
<th>$R_f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>saturated acids</td>
<td>C$<em>{12:0}$ C$</em>{14:0}$ C$_{16:0}$</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C$<em>{18:0}$ C$</em>{20:0}$</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>monounsaturated acid</td>
<td>C$_{18:1}$</td>
<td>0.51</td>
</tr>
<tr>
<td>III</td>
<td>diunsaturated acids</td>
<td>C$_{18:2}$</td>
<td>0.30</td>
</tr>
<tr>
<td>IV</td>
<td>triunsaturated acids</td>
<td>C$_{18:3}$</td>
<td>0.18</td>
</tr>
</tbody>
</table>
Stimulatory Effect of Medium Ingredients on Alkaline Protease Production by Bacillus licheniformis N-2 and Compatibility Studies With Commercial Detergents

Muhammad Nadeem**, Javed Iqbal Qazi⁵, Shahjahan Baig⁴ and Quratulain Syeda⁴

¹Food and Biotechnology Research Center, PCSIR Labs. Complex, Lahore-54600, Pakistan
²Department of Zoology, University of the Punjab, New Campus, Lahore, Pakistan

(received April 11, 2008; revised June 3, 2008; accepted June 12, 2008)

Abstract. Suitable concentration of ingredients of the growth medium played a vital role in production of alkaline protease by Bacillus licheniformis. Maximum enzyme activity (875.05 PU/ml) was achieved when the bacterium was grown in the medium containing glucose (1%), soybean meal (1%), K₂HPO₄ (0.5%), MgSO₄.7H₂O (0.05%), NaCl (0.05%), CaCl₂.2H₂O (0.05%) at 37 °C on 24 h incubation period with agitation of 140 rpm in shake flask cultures. More than 1% glucose decreased the enzyme production. The protease had excellent stability with wide range of commercial detergents such as Ariel, Bonus, Bright Total, Surf Excel, Wheel and non-branded detergents, recommending its use as an effective additive in detergent formulation.

Keywords: medium ingredients, detergent compatibility, B. licheniformis N-2, alkaline protease

Introduction
Proteases are one of the most important groups of industrial enzymes used in pharmaceutical industry and in food industry for peptide synthesis, in leather industry for de-hairing and in detergent industry as an additive of detergent formulation (Joo and Chang, 2005; Pastor et al., 2001). Alkaline proteases are known to constitute 60-65% of the global industrial market among various types of proteases (Banerjee et al., 1999). Alkaline proteases are produced by a wide range of microorganisms including bacteria, mould and yeast. Currently, a large portion of commercially available proteases is derived from Bacillus strains because of their high pH and temperature stability (Gupta et al., 2002; Joo et al., 2002).

The fermentation medium form the environment in which the microorganisms live, reproduce and carry out their specific metabolic reactions to produce useful products. Two distinct biological requirements are considered in most of the industrial fermentation processes for medium design, where the product is something other than the cell mass itself. First, the nutrient has to be supplied to establish the growth of the microorganism. Second, proper nutritional conditions have to be provided to maximize the product formation. It is also well established that extracellular protease production in microorganisms is greatly influenced by media components, especially carbon and nitrogen sources, metal ions and physical factors such as pH, temperature, dissolved oxygen and incubation time (Nadeem, et al. 2006; Oberoi et al., 2001; Kuar et al., 2001; Razak, et al. 1994; Moon and Parulekar, 1993). The cost of the growth medium is another significant parameter for making the production process industrially viable. Approximately 30-40% of the production cost of the industrial enzyme is estimated to be accounted for by the cost of the growth medium (Gessesse, 1997). Therefore, selection of the right medium ingredients and their concentrations optimization have become the need of today for high yield of desirable enzymes by fermentation.

Considering these facts, the effects of different concentrations of carbon and nitrogen sources as well as metal ions concentrations were studied to maximize the yield of alkaline protease by locally isolated Bacillus licheniformis N-2. Compatibility studies of alkaline protease with different detergents were also conducted to observe its commercial exploitation as an additive in detergent formulation for laundry industry.

Materials and Methods
Microorganism and culture maintenance. A proteolytic strain identified as Bacillus licheniformis N-2 was isolated from decaying organic soil sample (Nadeem et al., 2007) and the culture was grown on nutrient agar slants at 37 °C for 24 h and preserved at 4 °C for one month. The preserved culture was revived on fresh nutrient agar slants after every one month for subsequent experiments.
Antidiarrhoeal Evaluation of Some Nigerian Medicinal Plants Used in Bini Traditional Folk Medicine

Flossy I. Obuekweab* and Ihuoma C. Onyejekwea
aDepartment of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria
bDepartment of Environment Health, Concordia University College of Alberta, 7128 Ada Boulevard, Edmonton, Alberta, Canada

(Received February 13, 2008; revised June 10, 2008; accepted June 12, 2008)

Abstract. Four medicinal plants namely; Vernonia amygdalina, Psidium guajava, Chromolaena odorata and Anarcadium occidentale, commonly used for the treatment of diarrhoea in Bini traditional folk medicine in Nigeria were tested against Escherichia coli, Staphylococcus aureus and Klebsiella aerogenes. The leaf extracts of P. guajava and A. occidentale completely inhibited the growth of all the organisms tested, while V. amygdalina inhibited the growth of K. aerogenes only. Metronidazole was used as the standard antidiarrhoeal drug. Glycosides were found in all the plant extracts. This study, favours the use of the leaf extracts of A. occidentale, P. guajava and V. amygdalina for the treatment of diarrhoea in Nigeria.

Keywords: antidiarrhoeal drugs, medicinal plants, Nigeria, Bini folk medicine

Introduction

Nowadays, the value of medicinal herbs in combating diseases is being rediscovered and the herbal medicine trade has become a booming business worldwide. In India, for example, there are 46,000 licensed pharmacies manufacturing traditional remedies, 80% of which come from plants (Alok, 1991). In Africa, many plant species are reported to have medicinal value (George and Pamplona-Roger, 1997). These plant species are used for simple or complex pathological complications to psychological and mental illnesses.

Gastrointestinal disorders are one of the major health problems in developing countries and generally, plants are used in the treatment of diseases, indigenously. In sub-Saharan Africa in 1999, there were 8,181 deaths per day due to diarrhoea and traditional medicines were often the only affordable and accessible form of healthcare for the majority of this rural African population (Obuekwe and Obuekwe, 2002). Frequently, tannin-containing plants are used to treat diarrhoea. The bark and leaves of cashew (Anarcadium occidentale) are a rich source of tannins—a group of phytochemicals with documented biological activities. Cashew fruit has exhibited antibacterial activity against the Gram-negative bacteria Helicobacter pylori, now considered to be the cause of acute gastritis and stomach ulcers (Heirrich et al., 1992). Its effectiveness against leishmanial ulcers has also been documented in the same report. An infusion of the bark and leaves is an astringent and a mouthwash, used in toothache and is given internally in dysentery (Database entry for Cajueiro -A. occidentale). The natural rainforest remedy for diarrhoea is standard decoction of leaves and twigs of A. occidentale (Indian Medicinal Plants- A. occidentale, 2001). The antiulcerogenic effect acute, subacute toxicity as well as the genotoxic effect of a hydroethanolic extract of the cashew (A. occidentale L.) leaves have also been investigated (Konan and Bacchi, 2007; Konan et al., 2007).

Psidium guajava (guava) is a common shade tree or shrub in the tropics. The Tikuna Indians decoct the leaves as a cure for diarrhoea (People and Plants online - working paper 1: African Medicinal Plants). Much of its therapeutic activity is attributed to the flavonoids, which have demonstrated some antibacterial activity. Quercetin is thought to contribute to the antidiarrhoeal effect of guava. It is able to relax intestinal smooth muscles and inhibit bowel contractions. The effective use of guava in diarrhoea, dysentery and gastroenteritis can also be related to guava’s documented antibacterial properties. Bark and leaf extracts of the tree have exhibited in-vitro toxic action against numerous bacteria (Theunissen, 2002).

A range of medicinal plants with antidiarrhoeal properties have been widely used; but the effectiveness of many of the antidiarrhoeal traditional medicines have not been scientifically evaluated. This study investigates the potential antidiarrhoeal properties of the leaf extracts of four Nigerian medicinal plants P. guajava, A. occidentale, V. amygdalina and C. odorata against E. coli, S. aureus and K. aerogenes.

Fresh leaves of the plants. P. guajava, A. occidentale, V. amygdalina and C. odorata were collected locally from Benin City, Nigeria.
Aquaculture is a low energy expenditure and protein yielding in comparison to other agriculture sectors. Fish has long been recognized as high quality of food for human consumption. Fish lipids have great nutritional significance owing to their protective role against the cardiovascular diseases as fish is rich in unsaturated fatty acids. Moreover, the lipids provide energy 9.3 cal/g twice than that of protein (McGraw Hill, 1977). Fish has attained great nutritional significance, in recent years, as the best source of proteins and healthy oils. Farmed Labeo rohita possesses higher nutritional as well as commercial value as compared to that of wild fish (Mahboob et al., 2004).

During dressing of fish, head, fins, scales and skin are discarded which, with better management, can be put to better use with economic benefits (Choi and Regenstein, 2000). Fish skin and scales that are discarded as dressing losses are an important source of protein, lipids and minerals (Iqbal, 2002).

Fish collagen in general has more amino acid contents than mammalian collagen (Grossman and Bergman, 1992). Extraction of gelatin has been reported from cod (Gudmunsson and Hafsteinsson, 1997), Tilapia (Grossman and Bergman, 1992), shark skin, lung fish skin, carp skin (absent in the reference). Jamilah and Harvinder (2002) extracted gelatin from the skin of black and red Tilapia and determined the physiochemical characteristics. Catla catla (local common names: Taylee, Theila) is one of the major fresh water carps, native to the subcontinent Indo-Pak and neighboring countries of Bangladesh, Nepal and Myanmar. It is the 2nd most important species of the Rohu, consumed by the people, and an important contributor to the aquaculture production. According to (Choi and Regenstein, 2000) the discarded portions of fish such as head, fins, skin, scales can be used for poultry feedings. Head is used as food in different parts of the world but mainly it is lost during dressing.

Present study is an effort towards determining the composition of the wild and farmed varieties of Catla catla. The study is based on 21 farmed and 21 wild Catla catla of three different weight categories, (450-800 g), (850-1200 g) and (1250-1600 g), procured from Fish Hatchery Satiana Road, Faisalabad and Head Trimu, respectively, which were transported live to Fisheries Research Laboratory, of GC University (Faisalabad Pakistan) for the analysis.

After washing and dissecting the selected heads were weighed on electrical balance. The samples were then minced and immediately oven dried (65-70 °C) for 24 h on less than 100 mg/Hg to determine moisture contents (loss in weight was calculated as moisture percentage). Total nitrogen was determined by the automatic analyzer made by Tecator of Sweden, based on Kjeldahl’s method. Fat contents were determined by Soxhlet apparatus.

Fish head normally contains only traces of nitrogen free extract (carbohydrates) in the form of sugars, sugar phosphate and glycogen. Total carbohydrates (%) were estimated as nitrogen free extract by subtracting the total amount of proteins, lipids, ash and water.

Ash contents were calculated as percentage of the weight of the sample after ashing it at 450 °C for 12 h until while ash was obtained.

The results are summarised in Table 1. The moisture content in head of the wild Catla catla was recorded as 63.06 ± 0.46 percent and in farmed fish, were as 54.91 ± 0.53. The wild variety had the highest moisture contents. Protein contents of wild and formed C. catla were 14.77 ± 0.37% and 19.92 ± 0.44%,
Introduction

*Harpagophytum procumbens* DC. (Pedaliaceae), commonly known as Devil’s claw, is a perennial herbaceous plant native to the arid steppes. It is virtually restricted to the southern part of the African continent, occurring mainly in South Africa, Namibia and Botswana, where it is known locally not only as Devil’s claw, but also as grapple plant, wood spider and harpago.

*H. procumbens* occurs in areas with low annual rainfall (150-500 mm/year) on deep sandy soils of the Kalahari. It is found in savannah vegetation dominated by *Acacia* sp., but does not compete well with grasses. In fact, *H. procumbens* is most often found in areas where grass cover is less than 25% and where the herb cover is less than 20%. It is most abundant in open, trampled and overgrazed areas (Hachfeld, 2002), where it has clumped distribution (Raimondo and Donaldson, 2002).

In an area between the northern Cape and north west provinces of South Africa, densities were estimated at 50 plants/ha in the dense grasses of a well managed farm, 150 plants/ha on unharvested overgrazed communal land near the village of Madibeng and only 11 plants/ha on harvested communal land near Madibeng (Stewart and Cole, 2005).

*H. procumbens* is a weedy, tuberous plant with creeping annual stems up to 2 m long. The above-ground stems emerge after the first rains and die back in winter and during droughts. Stems grow from a persistent succulent primary tuber, called “mother tuber” by harvesters, the tap root of which can extend to a depth of 2 m. A number of secondary tubers, called “babies”, emanate from the primary tuber via fleshy roots. The secondary tubers are up to 25 cm long and 6 cm thick (Schneider, 1997). The secondary tubers contain up to 46% stachyose, a photosynthetic storage product, which is thought to be an adaptation to drought conditions (Stewart and Cole, 2005).

The leaves are opposite, blue-green and usually have several lobes. The flowers are tubular and deep mauve-pink with yellow and white throats. They are open for one day and are pollinated by bees (Von Willert and Sanders, 2004). The flat woody capsules, which give the plant its scientific and common names, bear two rows of curved appendages studded with curved spines (*Harpagophytum* literally means grapple hook plant). The fruit is dispersed by animals (Ernst et al., 1988), as it attaches readily to fur and wool, and is also wind dispersed to some degree, as a breeze can carry a fruit some distance from the parent plant.

Seeds have a high degree of dormancy (Stewart and Cole, 2005; De Jong, 1985), which may be an adaptation to drought (Ernst et al., 1988). Ernst et al. (1988) estimated that only 20-25% of the seeds in a fruit establish soil contact in a given year, suggesting that this may be an adaptation to animal (or wind) dispersal. The same authors also estimated that seeds may remain viable in the seed bank for more than 20 years, by virtue of their low respiration rate.

The thick, fleshy, tuberous secondary tap roots of *H. procumbens* are usually dried and used in south African traditional medicine.