Introduction

1. INTRODUCTION

Herbs are nature's gift to mankind and an herbal renaissance is blooming across the world. Medicinal herbs contain substances known to modern and ancient civilizations for their healing properties. They have been important to mankind, both socially and economically, for thousands of years. Their contribution is important to people that do not have access to modern medicines and moreover, modern pharmaceuticals rely heavily on the same active principles, natural or synthetic. Most of the modern medicines have originated from plant metabolites. Herbal medicines have become more popular in recent years because it is believed that these do not show many side effects, adverse or toxic, compared to synthetic medicines. Biological scientists have played equally significant role in introducing meaningful screening and testing models for evaluation of new agents. The natural products obtained from plants and animals are used as such in its original form or will be modified using lead as the basic molecule in the compound. The lead compounds are modified accordingly to show affinity and efficacy (Farooqi and Kumar, 2003).

India has a vast number of medicinal plants and several thousands have been claimed to possess medicinal properties. Medicinal plants are of considerable interest for ethno-botanical community as they are recognized to possess certain valuable medicinal properties in its different parts. Medicinal plants grow throughout the Indian subcontinent. Besides Ayurveda, Siddha and Unani system of medicines also use several plant materials to prepare medicines. The use of medicinal plants in the management of various illnesses is due to their phytochemical constituents (Yakubu

et al., 2007). The classical Indian system of medicine uses over 1500 plant species. There are about 200 plant species of global interest and about 50 of them can be prioritized on the basis of their proven safety and efficacy over 1000 years. World Health Organization (WHO) estimates that 4 billion people, 80% of the world population, presently use herbal medicine for some aspect of primary health care. Herbal medicine is a major component in all indigenous people's traditional medicine. WHO notes that of 119 plants-derived pharmacological medicines, about 74% are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures. Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value. Phytochemicals isolated from medicinal plants can be used as agents in the synthesis of drugs.

Allium cepa

Allium, commonly called as onion is a large genus containing about 400 species, are perennials that are cultivated for food worldwide and it is one of the most common vegetable adding flavor and taste to all preparative (Fritsch and Friesen, 2002). There are many varieties of onion. Most onion bulbs are white, yellow or red. The green stems and leaves are hollow and can reach 3 feet (1m) in height (Farooqi and Kumar, 2003). The plants bear small flowers that are usually white or purple. The fleshy bulb that grows below the ground is used medicinally as well as for food. Onions are members of the lily family (Vamshi Sharath Nath *et al*., 2010).

Distribution

Onions are distributed throughout temperate regions of the world including Europe, Asia, North America and Africa (Khare, 2002).

Scientific classification

Domain : Eukarya

Kingdom : Plantae

Division : Angiosperms

Class : Monocots

Order : Asparagales

Family : Alliaceae

Genus : *Allium*

Species : *cepa*

Binomial nomenclature: *A.cepa*

Morphological features

Bulbs 1-3, not rhizomatous, mostly depressed-globose, varying in size from cultivar to cultivar, 5-8 x 3-41 cm; outer coat enclosing 1 or more bulbs, yellowish brown, red or white, membranous, without reticulation; inner coat white to pink, cells obscure to quadrate (Wang *et al.*, 1998). Leaves persistent, 4-10, sheathing proximal 1/6-1/4 scape; blade fistulose, usually semicircular in cross section, 10-50cm x 4-20mm. Scape persistent, solitary, erect, fistulose, inflated below middle, 30-100 cm x 3-20 mm. Umbel persistent, erect, compact, to 500-flowered, globose, bulbils occasionally found; spathe bracts caduceus, 2-3, 3-4 veined, ovate, apex acute to

acuminate. Flowers stellate to companulate to urceolate, 3-7mm; tepals erect to spreading, white to pink with greenish midveins, withering in fruit, margins entire, apex obtuse or acute, outer ovate, inner oblong; stamens exserted ; anthers white; pollen white; ovary crestless; style linear; stigma capitate, unlobed; pedicel 10-50 mm. Seed coat not known (Halliwel and Guteridge,1992).

Vernacular names

English : Onion

Malayalam : Cyvannulli

Telugu : Erragadda,

Hindi : Pyaj

Tamil : Venkayam

Kannada : Niruli

Sanskrit : Polanduh

Red onion

Red onion has a dark red skin and is mildly sweet. It is very low in its pungent odor but has a saturated source of dietary fiber, vitamin B6, folate, potassium and manganese. Red onion has the richest concentration of flavonoids and phenols which possesses anti-oxidant and anti-proliferative activity.

White onion

White onion is a type of dry onion that has a pure white skin and a sweet, mild white flesh. Its skin is soft and white in colour, very low in saturated fat, cholesterol

and sodium. They are also a good source of dietary fibre, vitamin B6, folate, potassium and manganese and an excellent source of vitamin C. It contains phosphorous, calcium and iron. Bulbs are used as stomachic, tonic, anthelmintic, antispasmodic, diuretic, expectorant, febrifuge and hypotensive.

Small onion (Sambar vengayam)

Small onion is a perennial with leaves that are 3-5 cm long over topping the inflorescence, arising from solitary or clustered egg-shaped bulbs with brown or gray fine-textured coats. It is also called “chinna vengayam” or “sambar vengayam” in Tamil and is commonly called shallot in English. It is abundantly grown in India.

Chemistry of *Allium cepa*

Nutritional value per 100 g (3.5 oz)

Energy : 166 kJ (40 kcal)

Carbohydrates : 9.34 g

Sugars : 4.24 g

Dietary fibre : 1.7 g

Fat : 0.1 g

Saturated fat : 0.042 g

Monounsaturated fat: 0.013 g

Polyunsaturated fat: 0.017 g

Protein : 1.1 g

Water : 89.11 g

Vitamin A : 0 µg (0%)

Thiamine (Vit.B1) : 0.046 mg (4%)

Riboflavin (Vit.B2): 0.027 mg (2%)

Niacin (Vit.B3) : 0.116 mg (1%)

Vitamin B6 : 0.12 mg (9%)

Folate (Vit.B9) : 19 µg (5%)

Vitamin B12 : 0 µg (0%)

Vitamin C : 7.4 mg (12%)

Vitamin E : 0.02 mg (0%)

Vitamin K : 0.4 µg (0%)

Calcium : 23 mg (2%)

Iron : 0.21 mg (2%)

Magnesium : 0.129 mg (0%)

Phosphorus : 29 mg (4%)

Potassium : 146 mg (3%)

Sodium : 4 mg (0%)

Zinc : 0.17 mg (2%)

Ethnomedical uses

A.cepa is used in treatment of cold, allergies, toothaches, laryngitis and coughing. Homeopaths make a tincture of onion to treat a variety of conditions including diarrhea, facila paralysis, hay fever, hernia, laryngitis, pneumonia and trauma (Patil and Patil, 2007). Onion has been used for healing both internal and external wounds. Internally, onion has been recommended to treat bronchitis,

whooping cough, asthma and other respiratory problems. It is believed to help loosen congestion in the lungs and expand the airways (Patil and Patil, 2007).

Diversified uses of *A.cepa*

Onions were rubbed to firm up the muscles to relieve hair loss and snake bite by the Roman Gladiator. Onions were also prescribed by doctors in ancient times to help infertility in North American women (Kartikar and Basu, 1975 and Kokate, 2003). It has also been found that alliums can prevent the growth of malignant cells. In other words, they are anti-carcinogenic and can help to prevent the growth of cancerous cells in animals. It has been documented that in areas of high garlic and onion consumption, rates of stomach cancer are relatively low. There is mounting evidence that all members of the onion family have a positive effect in lowering incidences of heart disease. More than its nutritional value, onions play a vital role as anti-carcinogenic and cardioprotective agent. Its distinctive flavour, smell and texture makes it blend with many meals and forms the base ingredient in many recipes (Nadkarn, 1976 and Kokate, 2003). 3-mercapto-2-methylpentan-1-ol in onion was found to have an antioxidant potency that inhibits peroxynitrite induced diseases (Shah and Gopal, 1988).

Antimicrobial activity

Onions have been shown to possess antibacterial and antifungal properties. Volatile oil of onion has been shown to be highly effective against Gram positive bacteria, dermatophytic fungi, growth and aflatoxin production of Aspergillus fungi genera including *Aspergillus niger*, *Brettanomyces anomalus*, *Candida albicans*,

C.lipolytica, *Cladosporium werneckii*, *Fusarium oxysporium*, *Geotribum candidum* and *Saccharomyces cerevisiae*. Aqueous extract or the juice of onion has been reported to inhibit *in vitro* growth of *Escherichia coli*, *Serratia marcescens*, *Streptococcus* species, *Acetobacillus odontolyticus*, *Pseudomonas aeruginosa* and *Salmonella typhosa* (Bison and Verma, 1994). Petroleum ether extract of onion inhibited the *in vitro* growth of *Clotridium paraputrificum* and *Staphylococcus aureus*. Welsh onion extracts have been reported to exert more inhibitory activity towards aflatoxin production than the preservatives sorbate and propionate at pH values near 6.5, even at concentrations 3-10 folds higher than maximum level used in foods (Vamshi Sharath Nath *et al*., 2010). Organosulphur compounds have been reported to be responsible for antibacterial effects of onion extract against oral pathogenic bacteria causing dental caries. In addition to inhibitory effects against pathogenic bacteria, onions have been found to promote beneficial microorganisms (Vamshi Sharath Nath *et al.,* 2010).

Antioxidant activity

Onions are known to contain anthocyanins, and the flavonoids quercetin and kaempferol (Wang *et al.,* 1998). However, anthocyanin pigments, concentrated in the outer shell of red onions, are only minor constituents of the edible portion. Kaempferol, while detectable in certain onion varieties, is present in much smaller quantities than quercetin. Therefore, quercetin is the major flavonoid of interest in onions. Mechanisms of action include free radical scavenging, chelation of transition metal ions, and inhibition of oxidases such as lipoxygenase (Udayan and Venkatesh,

2005). Extracts from the outer scales of onion have exhibited potent free radical scavenging activities. The homogenate fresh onion and hot water extract of fresh aerial parts of *A.cepa* exhibit significant inhibition of lipid peroxidation (Vamshi Sharath Nath *et al.,* 2010). The antioxidative effects of onions have been associated with a reduced risk of neurodegenerative disorders, many forms of cancer, cataract formation, ulcer development and prevention of cardiovascular diseases by inhibition of lipid peroxidation and lowering of low density lipoprotein (LDL) cholesterol levels (Fremont *et al.,* 1998; Aviram *et al.,* 1999 and Kaneko and Baba, 1999). Another antioxidant effect of onions and its extracts includes the reduction of rancidity in cooked meat. Protection from arachidonic acid metabolites and lipoxygenase activity is important in prevention of vascular diseases.

Quercetin has been shown to not only directly inhibit the lipoxygenase enzyme, but also suppress consumption of α-tocopherol and preserve human serum paraoxonase which are potent antioxidants against lipid peroxidation (Halliwel and Guteridge, 1985). It has been demonstrated that the novel 3-mercapto-2-methylpantan-1-ol (3-MP), of which four possible diastereoisomers can occur in varying amounts in *A.cepa*, significantly inhibited peroxynitrite mediated tyrosine nitration and inactivation of α-1-antiproteinase. Moreover, 3-MP also inhibited peroxynitrite-induced cytotoxicity, intracellular tyrosine nitration and intracellular reactive oxygen species (Udayan and Venkatesh, 2005).

Immunosuppression

Inflammation is the part of the body's natural immune response to trauma. Thiosulfinates and capaenes responsible for the anti-inflammatory activities also cause inhibition of the immune response (Hedges and Lister, 2007). Quercetin also affects immunosuppression and has been shown to create a beneficial effect in aiding renal transplantation. Quercetin was shown to suppress both immune and non-immune injury responses, the key risk factors in chronic graft loss. This showed promising application as it was noted that current drugs and treatments may worsen harmful, non-immunological reactions (ischemia, hypertension and hyperlipidemia) to the transplant. Alternately, quercetin has been shown to prevent immunosuppression induced by UV exposure to mice (Steerenderg, 1998).

Neuro protective effect

Recently, it has been reported that administration of methanolic extract of outer scales and edible portion of *A.cepa* bulb to mice before cerebral ischemia and reperfusion, exhibit significant neuroprotection by markedly reducing cerebral infarct size, significantly decreasing the increase in thiobarbituric acid reactive substances (TBARS) concentration in brain mitochondria and supernatant fractions and preventing global cerebral ischemia reducing impairment of short-term memory and motor incoordination (Shri, 2008).

Role of free radicals

Free radicals are molecules which is produced when the body metabolites food or by environmental exposure like tobacco smoke and radiation. Free radicals can damage cells and thus play an important role in the onset and development of many degenerative diseases like arthritis, heart diseases, neuro degenerative diseases and cancer.

By definition a free radical is any atom (e.g. oxygen, nitrogen) with at least one unpaired electron in the outermost shell, and is capable of independent existence. A free radical is easily formed when a covalent bond between entities is broken and one electron remains with each newly formed atom (Karlsson, 1997). Free radicals are highly reactive due to the presence of unpaired electrons. Any free radical involving oxygen can be referred to as reactive oxygen species (ROS). Oxygen centered free radicals contain two unpaired electrons in the outer shell. When free radicals steal an electron from a surrounding compound or molecule a new free radical is formed in its place. In turn the newly formed radical then looks to return to its ground state by stealing electrons with antiparallel spins from cellular structures or molecules. Thus the chain reaction continues and can be "thousand of events long." (Goldfarb, 1999). Oxygen acts as the terminal electron acceptor within the electron transport chain (ETC). The literature suggests that anywhere from 2 to 5% (Sjodin *et al.*, 1990) of the total oxygen intake during both rest and exercise have the ability to form the highly damaging superoxide radical via electron escape. During exercise, oxygen consumption increases 10 to 20 fold to 35-70 ml/kg/min. In turn, electron

escape from the ETC is further enhanced. Thus, when calculated, 0.6 to 3.5 3ml/kg/min of the total oxygen intake during exercise have the ability to form free radicals (Dekkers *et al*., 1996). Onions (*A. cepa*) have higher radical scavenging activities than garlic (*A. sativum*). Red onion is more active than yellow onion (Nuutila *et al.,* 2003). In onions, both total phenolic and flavonoid content are strongly correlated with total antioxidant activity (Yang *et al.,* 2001).

Antioxidants

Antioxidant means "against oxidation." Antioxidants work to protect lipids from peroxidation by radicals. Antioxidants are effective because they are willing to give up their own electrons to free radicals. When a free radical gains the electron from an antioxidant it no longer needs to attack the cell and the chain reaction of oxidation is broken (Dekkers *et al.,* 1996). After donating an electron an antioxidant becomes a free radical by definition. Antioxidants in this state are not harmful because they have the ability to accommodate the change in electrons without becoming reactive. The human body has an elaborate antioxidant defense system. There are two lines of antioxidant defense within the cell. The first line, found in the fat-soluble cellular membrane consists of vitamin E, beta-carotene, and coenzyme Q (Kaczmarski *et al.,* 1999). Of these, vitamin E is considered the most potent chain breaking antioxidant within the membrane of the cell. These also include vitamin C, glutathione peroxidase, superoxide dismutase (SD), and catalase. Antioxidants have also received increased attention by nutritionists and medical researchers for their potential effects in the prevention of chronic and degenerative diseases (Liu, 2003).

Substances that neutralize the potential ill effect of free radicals are generally grouped in the so-called antioxidant defense system. Such a system encompasses many substances (Cutler, 1984 and Heffner and Repine, 1989), which are often called by such generic names as antioxidants, free radical scavengers, chain terminators or reductants. Antioxidants terminate these chain reactions by removing free radicals intermediates and inhibit other oxidation reactions by being oxidized themselves.

Herbs as potent antioxidants

Drugs derived from plants are used in the treatment of various diseases including cancer (Sivalokanathan *et al*., 2004). They provide the necessary health security to the rural populations (Anbuselvam *et al*., 2007). Generally the medicinal plants are considered as potent antioxidants (Farnsworth *et al*., 1985). In recent years, a number of workers have accumulated enormous evidences revealing that enrichment of body systems with natural antioxidants can prevent the diseases caused by free radical mediated oxidative stress.

Phenols

Plant polyphenols are natural antioxidants and are candidates in exerting the protective effects of vegetables and fruits against some forms of cancer and cardiovascular diseases (Arts and Hollman, 2005). There is substantial genetic variation in the content of phenolics among fruit and vegetable cultivars. The levels of phenolic antioxidants appear to be sensitive to environmental conditions both

before and after harvest (Kalt, 2005). Polyphenols are probably the most abundant antioxidants in the diet (Scalbert *et al.*, 2005).

Phenolics are mostly synthesized from phenylalanine and the pathway is essentially chloroplastic and is known in its entirety (Parr and Bolwell, 2000). A key step in the biosynthetic route is the introduction of one or more hydroxyl groups into the phenyl ring, thus producing phenols. This means that phenols are derived from a common building block in their carbon skeleton, the phenylpropanoid unit C6-C3 that builds up the large variety of plant phenols, *e.g*. cinnamic acids, benzoic acids, flavonoids, proanthocyanidins, stilbenes, coumarins, lignans and lignins (Hollman, 2001).

These compounds may be synthesized in different cell types and during different stages of plant development and thus, the regulation of phenolic accumulation in plants is very complex. The biological functions of phenolics in plants include structural polymers, UV-screens, antioxidants, attractants such as colour and smell, defense responses and signal compounds within the plant (Parr and Bolwell, 2000).

Flavonoids

One of the most abundant groups of polyphenolic compounds in plants is the flavonoids. Over 4000 different flavonoids occurring in plants have been described (Hollman, 2001). Among the more ubiquitous flavonoids, over 50 different glycosides have been identified (Meltzer and Malterud, 1997). But the most common sugar is D-glucose (Meltzer and Malterud, 1997). The flavonoids are known to

control the level of auxins, one of the regulators of plant growth and differentiation. In food, the plants are add colour, texture and taste. Many of the biological roles played by the flavonoids are associated with their capacity to bind metals, *e.g.* iron and copper, which enhances the antioxidant and UV screening actions of flavonoids (Formica and Regelson, 1995). The flavonoid content in plants is strongly influenced by extrinsic factors such as variations in plant type and growth, season, climate, degree of ripeness, food preparation and processing (Aherne and O´Brien, 2002).

Quercetin

There are seven major flavonoid compounds in onions. Quercetin aglycone, *i.e.* with no sugar molecule attached, quercetin monoglucoside, quercetin diglucoside, isorhamnetin (a methyl ether of quercetin), isorhamnetin monoglucoside, rutin and kaempferol (Park and Lee, 1996). Quercetin diglucoside and monoglucoside account for up to 93% of the total flavonol content in onion (Lombard *et al.*, 2002). It has been shown that onion is one of the most quercetin-rich crops compared to kale, blackcurrants and broccoli, black grapes and apple (Higashio *et al.*, 2005). Quercetin content in onion can be doubled after harvest using UV light lamps (Higashio *et al.*, 2005). In onion, total quercetin content in the dry outer skins is significantly higher than that in the edible parts and a decrease is found from outer to inner parts on both fresh and dry weight basis. The total content of quercetin is higher in the upper part of an onion, compared with the lower part (root end) (Trammell and Peterson, 1976).

Pathogenic organism in human

Human pathogens are those organisms which cause disease in humans. Certain viruses, bacteria, protozoans, round and flatworms can live inside humans. Most bacteria that live on the skin or in the gut of humans are harmless and beneficial. Some are usually harmless, but can cause disease only under certain conditions. Some species of bacteria are known as pathogens but affect small numbers of humans. Some species are highly pathogenic and are lethal for a high percentage of the humans they infect. Some are highly contagious, but rarely cause death. Smallpox and malaria, diseases caused by other microbes, have killed more humans than bacterial diseases, but diseases such as tuberculosis, typhus, plague, diphtheria, typhoid, cholera, dysentery and pneumonia have taken a large toll on humanity. At the beginning of the twentieth century, pneumonia, tuberculosis and diarrhea were the three leading causes of death (Todar, 2008). But many new bacterial pathogens have been recognized in the past 30 years, and many "old" bacterial pathogens, such as *Staphylococcus aureus* and *Mycobacterium tuberculosis,* have emerged with new forms of virulence and new patterns of resistance to antimicrobial agents. The human mucous membranes are a reservoir for many pathogenic bacteria found in the environment (i.e. *Pneumococci, Staphylococci, and Streptococci),* some of which are resistant to antibiotics (Fischetti, 2003). The genus Salmonella is among the most common causes of food and water borne infectious diseases in the world (Baird-Parker, 1990). The organism has a wide host range which comprises most animal species including mammals, birds and cold-blooded animals in addition to human. A

number of studies in Nigeria have shown that Salmonella infections is endemic in many parts of the country (Katung, 2000 and Onunkwo *et al.,* 2001) and its endemicity increases especially in areas with low environmental hygiene (Chen *et al.,* 1985; Mara and Caincross , 1989; Bailey *et al.,* 1994 and Mbata *et al.,* 2006).

Bacillus subtilis has been implicated in various food spoilage including ropiness in bread, production of CO_2 in canned meats, sliminess and coagulation in milk, etc. (Frazier and Westhoff, 1991). *Escherichia coli* is one of the main causes of both nosocomial and community-acquired infections in humans and one of the micro-organisms most frequently isolated from blood. *E. coli* in humans is a common inhabitant of the gastrointestinal tract. It can also cause various intestinal and extra-intestinal diseases (Donnenberg, 2002).The pathogenic isolates of *E. coli* have a relatively large potential for developing resistance (Williams and Heymann, 1998 and Lark *et al.,* 2001). The spread of microbial drug resistance is a global public health challenge, which impairs the efficacy of antimicrobial agents and results in substantial increased illnesses and death rate (Byarygaba, 2004 and WHO, 2006).

Trematodes

Trematodes, or flukes, are flat and leaf-shaped, and range in length from a few millimeters to 75 millimeters. A large number of gastro-intestinal trematode species (paramphistomes) have been described. They can infect all ruminants but young calves and lambs are the most susceptible. Not all species are pathogenic but clinical outbreaks of paramphistomiasis have been caused by *Cotylophoron cotylophorum*

(Asia), P. ichikawar, *Paramphistomum microboth rium* (Africa), *C. calicophorum* (Australasia) and *P. cervi* (Europe).

The life cycles of all flukes involve freshwater snails as an intermediate host. Flukes are contracted by ingestion of eggs or encysted (encased) larva from contaminated water, raw water plants (water chestnuts, water bamboo shoots, etc.) or inadequately cooked fish or snails. The eggs or larva mature into adult worms in the intestines. In many developing countries around the world, farmers, herders, pastoralists and occasionally veterinary surgeons use plant or plant products to treat cases of parasitism. The related available evidence mainly concerns gastrointestinal helminthes, but there is also evidence for effects on blood parasites and external parasites. In traditional societies there seems to be a number of plant remedies deemed suitable for each parasitic disease. For example, seeds or the foliage of plants such as garlic, onion, mint, walnuts, dill, or parsley have been used to treat animals that suffer from gastrointestinal parasitism, while cucumber and pumpkin seeds have been associated with the expulsion of tapeworms from the gastrointestinal tract (Guarrera, 1999).

The paramphistomosis is an infection caused by a trematode belonging to the suborder paramphistomata. Some of their species are parasitic of the rumen and reticulo of ruminant. One of the most frequent trematodes in the world is *C. cotylophorum*. It affects domestic and wild ruminants, especially cows, sheeps, goats and buffalos in tropical and subtropical regions (Nofre Sanchez, 2009).

The usefulness of plant products on human health and well being has been evidenced for decades. In recent years, the search for understanding the mode of action of plant with known antimicrobial properties has markedly increased which is proved to be safe and effective without side effects. India has a rich heritage of traditional system of medicinal plants. Among which the *Allium* species are of particular interest. It contains mainly sulfur based pharmaceutically interesting compounds. Particularly onion extracts (*A.cepa*) are reported to have different functional properties against fungi, bacteria and viruses.

The survey of literature reveals that different parts of the onion varieties have antimicrobial, anti-diabetic and anti cancer effects. However, using the bulb part of the differential extracts of onion varieties against helminthes, parasites and microbes has received only a little attention. Therefore, the present study was designed to assess the evaluation of antimicrobial and antihelminthic effects of different extracts of onion [Big (Red and white) and small onion] varieties. In account of their properties, wide usage, cost effectiveness, onions [(Big (Red and white) and small onion] were selected for the present investigation.

OBJECTIVES

- To evaluate preliminary phytochemical screening of different extracts of *A. cepa* varieties.

- To study antioxidant, antimicrobial and antihelminthic activity of different extracts of *A. cepa* varieties.

- To extend the efficacy of *A. cepa* to human health.