Discussion
6. DISCUSSION

Many existing healthy eating guidelines helps the public to decide on a well-balanced daily diet to meet all the nutrient requirements. At the same time, the information regarding the associated toxins, the ways of mitigating or detoxifying them are scarce. The persistent population increase, changing lifestyle and increase in buying capacity and the so called “grab and go” and “everywhere instant” world demand continuous supply of processed and packaged foods comprising more ready to eat food, from food sector all over the world (Murugan et al., 2012). Hence, others prepare most of the foods we eat today and we are not aware about the minor ingredients they contain especially if they are toxic and their route of entry into the food as well as the ways of removing them. Search into the traditional food preparation may provide us some lead for these. Therefore, the current research was undertaken to ascertain the traditional use of culinary plants during oil frying.

Preparation of number of food items using the culinary groundnut oil are very common in the study area Thiruvannamalai, Tamilnadu, India due to the large-scale cultivation of groundnut and easy expulsion oil from them using the accessible local large scale and small-scale oil expellers. Therefore, the groundnut oil in the study area has a place in the healthy, balanced diet even though they are energy dense and contain a high proportion of fat. In addition, the local people use number of culinary plants while oil frying meant for curry preparation and storage. The ubiquitous presence of the aflatoxigenic fungi in all fields and stored agricultural commodities throughout India, especially in the study area, a place known for high temperature, moisture, unseasonal rains and flash floods, all the predisposing factors of aflatoxigenic fungal growth and its toxin production justified the need for this study.

Around a third of groundnuts produced are used as a food commodity globally. The groundnut oil is produced and used for the most part in India and China; there is little trade in this type of oil outside these countries (FEDIOL, 2008). India occupies an important place in the world concerning major oilseeds production. Edible oil economy of India is one of the world’s largest with 15,000 oil mills, 689 solvent extraction units, 251 Vanaspati plants and over 1,000 refineries employing more than one million people. The total market size is about Rs. 600,000 million.
Being the leading producer of oilseeds, contributes world’s 8-10% of oilseed and 6% edible oil production. It is the fifth largest producer of oilseeds in the world, behind US, China, Brazil, and Argentina (MFPI, 2011). Its major edible oil source is the groundnut oil. During the year 2009-2010, its production is around 12.49 lakhs ton extracted from the produced 54.29 lakhs tons of groundnut (DVVO&F, 2011).

Groundnut or peanut oils are most commonly used for frying and cooking, as well as in salad dressings. It is suitable for deep-fat frying as it has a high smoke point (229.4°C), which allows the food to cook quickly and develop a crisp coating without absorbing too much oil. Highly aromatic peanut oil and peanut extracts are used for producing baked goods, desserts, sauces, breakfast cereals, frozen dairy products and flavourings, because of the strong nut flavour and aroma. Production of peanut oil is the fifth highest of all the plant oils worldwide (Foster et al., 2009). One study has suggested that peanut oil incorporation into the diet had positive effects on markers of cardiovascular diseases (CVD) risk, compared with a lower-fat diet with no peanut oil. Clinical studies have also shown consistent reduction in total cholesterol and LDL in subjects who consume peanuts and peanut oil (Sanders, 1982).

In India the edible oil quality is defined mainly by organoleptic parameters such as flavour, odour, as well as colour for expeller-pressed unrefined vegetable oils; and the physicochemical characteristics such as FFA value, saponification value, iodine value, Bellier turbidity test, refractive index, unsaponifiable matter, in addition to FA composition for refined vegetable oils. However, more emphasis is placed on the PUFA content and natural antioxidants present as minor components in the oil (Shiela et al., 2004). In the current study, the physicochemical characters of the culinary groundnut oil are analysed to determine nature and quality as per standard methods.

Though the physicochemical characteristics of the analysed groundnut oil samples showed extensive variation, they are within the PFA and BIS permissible limits. Even if much variation is observed in the organoleptic characters, but they are all characteristic one. The other contaminant directly related to health that deserves attention is the natural toxin, the mycotoxins. The groundnuts are easily prone to fungal attack and the numbers of reports on the associated aflatoxin contamination in
India are available. Hence, the fungal aflatoxin threat associated with groundnut oil cannot be neglected like other stored products. In addition, it is troublesome one in most of the locally manufactured or expelled oil samples. Certain species of *Aspergillus*, mainly *A. flavus* produces the fungal toxin aflatoxin B₁ (AFB₁), a common food contaminant all over the world, mostly in the regions where hot and humid climates favour the growth of these fungi and where food is improperly stored (Partanen *et al.*, 2010). The available published and oral reports emphasise that the groundnut oil derived from contaminated products should be essentially free from aflatoxin and that alkali refining should render such oil free of trace amounts of aflatoxin. However, all these reports fail to give any laboratory or factual evidence to support the statement that refining eliminates aflatoxin from the oils derived from contaminated raw materials. In addition, it must be emphasized that, since these earlier statements were made, the sensitivity of the analytical test has increased by a factor of number of times. Thus, the sensitivity of the earlier methods, availability of methods, sophisticated instruments made what was regarded as a negligible or nonmeasurable amount in the earlier period may not be so regarded today. It is apparent from the facts presented above, that there has been a need for an objective and definitive investigation on the fate of aflatoxin during the vegetable oil refining (Parker and Melnick, 1966). Due to the loss of raw crude oil’s nutty aroma during refining and the resultant odourless oil, limited or not at all refining is applied to groundnut oil. Also highly aromatic peanut oil and peanut extracts are used in products such as baked goods, desserts, sauces, breakfast cereals, frozen dairy products and flavourings, because of the strong nut flavour and aroma (Foster *et al.*, 2009). Hence, there is every chance for the carryover of aflatoxins in groundnut oil even after their processing and packing. The existence of potential aflatoxin contamination of both groundnut and its product groundnut oil necessitates monitoring for the presence of aflatoxin at all levels from production to end use.

Due to the harmful effect of aflatoxins on human and animal health and their consequence in international food trade, the aflatoxigenic fungal food contamination has received worldwide attention. Many workers (Blesa *et al.*, 2003; Gurses, 2006; Moss, 2002; Rustom, 1997) observed the presence of aflatoxin B₁ in peanuts. Bankole and Eseigbe (2004) already reported the presence of aflatoxins in Nigerian dry roasted
groundnuts. They reported the persistence of aflatoxin B₁, B₂, G₁ and G₂ in these Nigerian dry groundnuts even after roasting. Though many reports are available on the groundnuts aflatoxin contamination, very little information is available on the aflatoxin-associated mycoflora of its product groundnut oil and the associated quality problems. This study, for the first time reports the wide occurrence of aflatoxin contamination in unrefined groundnut oil marketed in Thiruvannamalai, Tamilnadu, India. The results of this study revealing the aflatoxin presence in the marketed groundnut oils sold in Thiruvannamalai, Tamilnadu, the Southern part of India corroborates with those reports of Dwarakanath et al. (1969). They reported the varying aflatoxin content of unrefined oil from 0.02 to 0.26%. They also found it’s the absence in the refined and hydrogenated peanut oil samples. Also Idris et al. (2010) gave an account on the Sudanese edible oils aflatoxin contamination. In this study, the collected oil samples preliminary qualitative aflatoxin determination was carried out by thin layer chromatography. Earlier Oliveira et al. (2000) employed TLC for aflatoxin detection in eggs and Var et al. (2007) used the same for the detection and determination of aflatoxin in Turkey helva samples.

Fungal contaminants present in peanut kernels are a concern to processors and consumers due to their association with quality deterioration and mycotoxin production. Among the number of groundnut oil isolates, the fungi like A. flavus, A. niger, Fusarium solani, F. Oxysporum, Rhizopus arrhizus, Rhizoctonia solani, Sclerotium rolfsii, Pythium ultimum, P. myriotylum and Verticillium alboatrum are the important fungi-causing seed rots and seedling diseases of groundnut (Subrahmanyan and Ravindranath, 1988). Whereas the F. solani, F. oxysporum, Macrophomina phaseolina, R. solani, S. rolfsii, and A. niger jointly attack groundnut and causes groundnut pod rots (Faujdar Singh and Oswalt, 1992). The other isolates might be contaminants entered during manufacturing.

A. flavus infection of groundnuts occurs under both preharvest and postharvest conditions. Preharvest infection by A. flavus and consequent aflatoxin contamination are important in the semi-arid tropics (SAT), especially when end-of-season drought occurs. Drought stress may increase susceptibility to fungal invasion by decreasing the moisture content of the pod and seed, or by greatly lowering the physiological activity of the groundnut plant (Waliyar, 1997). Commonly the yellow mold fungus of
groundnut *A. flavus* causing is found in the seeds of both rotten and apparently healthy pods. Many strains of this fungus aflatoxins producing capability renders the seed unacceptable due to their high toxicity for human or animal consumption. Aflatoxin contamination in groundnut can occur in the stems of seedlings, pods, and seeds. The fungus is capable of invading groundnut seeds before harvest, during postharvest drying, and during storage (Faujdar Singh and Oswalt, 1992).

In this present study, the potential aflatoxin producing fungi *A. flavus* MTCC 10680 capable of growing under xerophilic conditions was isolated from groundnut oil samples. Earlier many researchers reported the *A. flavus* contamination in groundnut and its products and other foods like milk. Okpokwasili and Molokwu (1996) reported the presence of *A. flavus* as a predominant one in groundnut oil due their oils and oil seeds high lipolytic nature. They also found the frequent occurrence of *Aspergillus* in other vegetable oils like palm kernel oil, maize and groundnuts. They pointed that these *Aspergillus* survive in these oils due to their heat resistant conidia producing ability.

Magnoli *et al.* (2007) explained the survival of the storage fungi *A. flavus* in peanut at different months of storage. They also added that during the first and second month of storage, the *A. flavus* level was similar but during the subsequent months, its percentage increases. In the last month, the percentage increases to 50. This study enlightened the storage fungi multiplication in the stored peanuts. In the present study, the xerophilic fungal isolation from the groundnut oil warrants due attention for these toxigenic storage fungi.

These aflatoxins have turn out to be recognized as ubiquitous contaminants of the human food supply throughout the economically developing world since their discovery 50 years ago. The public health impact of aflatoxin exposure is omnipresent. Because of a wide range of exposures leading to acute effects, the adverse toxicological consequences of these compounds in populations are quite varied, including rapid death, and chronic outcomes such as hepatocellular carcinoma. Besides, up-and-coming studies describe a variety of general adverse health effects associated with aflatoxin, such as impaired growth in children. Aflatoxin exposures have also been established to multiplicatively amplify the risk of liver cancer in
people chronically infected with hepatitis B virus (HBV) illustrating the harmful impact that even low toxin levels in the diet can pose for human health (Kensler et al., 2010). India accounts the largest reported outbreak of aflatoxicosis. During 1974, 200 villages in Western India (Banswada and Panchamahals districts of Rajasthan and Gujarat resp.) an outbreak of hepatitis resulted in 305 cases and 106 deaths due to aflatoxicosis was reported (Krishnamachari et al., 1975). The outbreak, which lasted for 2 months, was confined to tribal population taking maize as staple food. Analysis of *A. flavus* contaminated (6.25 - 15.6 ppm) maize samples showed that affected people might have consumed between 2000 - 6000 µg kg\(^{-1}\) (ppb) aflatoxins daily over a period of 1 month. In South India, Canara district of Karnataka, hepatomegaly in children was found to have correlation with aflatoxin contamination. During 1994, in Ranga Reddy district of Andhra Pradesh, more than 200,000 broiler chickens died in after eating aflatoxin contaminated feed groundnut cake. Earlier Chittoor district of Andhra Pradesh state recorded heavy chicks’ mortality due to aflatoxicosis. The groundnut cake contaminated with aflatoxin at a level of 3590 µg kg\(^{-1}\) was implicated in these. Another outbreak of aflatoxicosis in commercial poultry farms was reported in the same district with 100% mortality. Aflatoxins (1400 - 3600 µg kg\(^{-1}\)) were found in samples of maize and groundnut cake fed to the birds during the outbreak. Egg production dropped from 85 - 40% during an outbreak of aflatoxicosis in poultry during October 1985 in and around Warangal, Andhra Pradesh. Post-mortem examination of dead birds revealed liver lesions of varying severity in all birds examined. An amount of 600-µg kg\(^{-1}\) aflatoxin was detected in these feed samples. Occurrence of aflatoxicosis in poultry in Mysore state, India was also reported earlier. The disease was first recognised at the Government Poultry Breeding Unit, Hebbal, Bangalore in 1966 wherein 2219 chicks died in one week. Subsequently, several sporadic incidences were found in various poultry farms in the state. The disease was predominant in younger stocks, possibly due to increased percentage of protein in the form of toxic groundnut cake (Reddy and Raghavender, 2007). In all outbreaks of aflatoxicosis reported throughout India, either the raw material or the by-product groundnut cake is implicated and not the contaminated groundnut main product oil.

Fungi grow over a wide range of environmental conditions and grow on stored grain flourish most rapidly between 25\(^{\circ}\) and 35\(^{\circ}\) C. This indicates that fungal growth
is particularly fast in the tropics. As fungi grow, they respire, produce heat and free water, often raise the moisture content and temperature, and accelerate the rate of spoilage. Many important storage fungi are xerophilic including the deuteromycotina *A. flavus* (Farrell *et al.*, 2007). The suspected aflatoxigenic *Aspergillus* spp., the seventeen *A. flavus* along with the other *Aspergillus* sp upon inoculation on the preliminary screening AFPA medium, only eleven of the aflatoxigenic isolates produced bright orange yellow on the reverse. Rojas *et al.* (2005) also observed the yellow colour pigmentation on the reverse side of the aflatoxigenic AFPA medium cultured colonies and used them for screening. They also observed that the direct relation between the intensities of colour and fluorescence and absence of colour below the *Aspergillus* nonaflatoxigenic colonies. The UV light blue fluorescence of aflatoxigenic fungi was also observed by Rojas *et al.* (2005) who also noted its absence around non aflatoxigenic *A. flavus* strains. The MY50G media used in this study for xerophilic fungal isolation is a reasonable choice since the supplementation with glucose lowers the $a_w$ value lesser than 0.89 (Pitt and Hocking, 1997). The growth patterns like moderate fast growing, flat, floccose to granular, bright yellow to green occasionally yellow brown with reverse cream coloured or pinkish coloured, variable length, rough, pitted and spiny conidiophores and their uniseriate or biseriate are the typical characters helped the for taxonomic positioning of isolates into *Aspergillus* genus and mentioned by other workers (Gugnani, 2003; Hedayati *et al.*, 2007).

The aflatoxin producing xerophilic *A. flavus* was identified in the present study by both traditional morphological and emerging molecular methods, since molecular approaches offer additional rapid and objective identification than do traditional phenotypic methods. Earlier number of authors have reported the use of parts of the ITS region targets for the species level identification for the reason that they generally display sequence variation between species, but only minor, or no, variation within strains of the same species. Their application is wide spread in medical field since they aid in rapid identification (Hinrikson *et al.*, 2005; Balajee *et al.*, 2009).

Now a day’s interest in nucleotide sequence based fungal molecular systematics is increasing since they lack distinct morphological characters and the
associated problems in determining homologous characters among them (Al-Shohaibani et al., 2010). The molecular identification tool targeting the ITS region amplification and direct sequencing helps their easy and rapid direct identification. The approach used in this study, direct sequencing, followed by comparative reference sequence GenBank analysis, is measured to be one of the most dependable methods for the molecular identification of morphologically similar fungal species by number of workers (Henry et al., 2000; Hinrikson et al., 2005; Kumeda and Asao, 1996; Leema et al., 2010). Their evolutionary similarity with other reference fungal isolates ITS gene sequences deposited in the GenBank databases was determined. The phylogenetic analysis of the gene sequences of ITS gene sequences the consensus tree for these species indicating their close ancestry. During the present study, the food, environmental and clinical isolates were used for the comparative GenBank analysis. The sequence comparison of these isolates not only revealed their identity but also close similarity with their anamorphic forms.

Apart from the currently used ITS gene sequences, several other targets are also used for the molecular identification of Aspergillus sp by other authors. It includes the Yokojama et al. (1999) work based on the mitochondrial cytochrome b gene, a putative aflatoxin pathway regulatory gene (aflR), the DNA topoisomerase gene, the ß-tubulin gene, and various rRNA gene regions. But the most reliable target investigated is ITS region (Leema et al., 2010).

Oxidative deterioration of food lipid results in loss of sensory quality such as colour and flavour due the hydroperoxides formation, which subsequently decompose to form volatile aroma components. These compounds are often perceived as off flavours. On the other hand, excessive intake of lipid peroxides may lead to adverse health effects. Even if the antioxidant compounds are present in significantly lower concentration; it inhibits or delays the oxidation of substrates. This suggests the need to use antioxidants for retarding lipid oxidation in foods. Due to increasing demand of natural food, spice essential oils may be used as natural antioxidants and antimicrobial agents in food products. The spices cinnamon, mace, anise, dill, and prikhom essential oils are found to possess both the antimicrobial as well as antioxidant activity and several microorganisms including bacteria and fungi effective inhibition. They are
possible to be used in foods as natural preservatives (Nanasombat and Wimuttigosol, 2011).

Panda et al. (2010) who determined and reported the antifungal activity of crude, petroleum ether, chloroform, ethanol, methanol and aqueous extracts of Cassia fistula L. against Candida albicans, C. krusei, C. parapsilosis C. tropicalis and A. niger, A. flavus and A. fumigatus found that the more accurate method of the antifungal activity assessment is the broth dilution technique.

The aflatoxin content reduction in oils received heat treatment along with culinary plants might be due to the aflatoxin detoxification. Earlier Dwarakanath et al. (1969) also observed the disappearance of aflatoxins in unrefined groundnut oils during heat treatment. Das and Misra (2000) suggested that aflatoxin degradation reaction might occur through enzymatic activity and these enzymes produced products or by products reacts with aflatoxins. They also proved that horseradish peroxidase present in the plants could detoxify aflatoxin B₁. Velazhahan et al. (2010) observed the AFG₁ detoxification ability of leaves/seeds aqueous extracts of various medicinal plants. Hence, they suggested that the Ajowen (Trachyspermum ammi (L.) Sprague ex Turrill) which demonstrated the maximum degradation of 65% may provide a biologically safe method to protect or livestock feeds and other agricultural commodities from aflatoxin.

Gowda et al. (2003) study on aflatoxin production reduction by clove oil, turmeric, garlic and onion suggests that the possible mode of action of these compounds is by interfering the biosynthetic pathway of aflatoxin production without actually affecting the growth of the fungus, thereby resulting in partial or complete inhibition of aflatoxin production. However, the best anti-fungal compounds are those that inhibit both fungal growth and toxin production.

Irkin and Korukluoglu (2007) investigated the A. niger control with fungicidal concentration (MFC) doses of garlic (Allium sativum L.), Onion (A. cepa L.) and leek (A. porrum L.) aqueous, acetone and ethyl alcohol extracts. They exposed that the inhibitoriest plant was garlic, followed by onion and leek. They also found that effectiveness of inhibition was related to the extraction solvent. Natural products may regulate the cellular effects of aflatoxins and evidence suggests that aromatic organic
compounds of spices can control the production of aflatoxins. The use of spices in food products preservation has been traditional and they are cultivated in many countries such as India, Japan and Russia. Spices occupy a prominent place in the traditional culinary practices and are indispensable part of daily diets of millions of people all over the world. They are essentially flavouring agents used in small amounts and are reported to have both beneficial effect and antimicrobial properties. Their antimicrobial properties have been found mostly due to the presence of alkaloids, phenols, glycosides steroids, essential oils, coumarins and tannins (Atanda et al., 2007). Fazekas et al. (2005) study on cooking revealing the failure of Hungarian spices in decreasing the aflatoxin content cautions this approach and warrant more scientific details on this.

All the examined culinary plants exhibited mycelial as well as aflatoxin inhibitory activity. The inhibitory activity was found to be dose dependent one. While fungal growth inhibition due to increasing the concentration of the extract was observed, the more remarkable on the inhibition of aflatoxin production was also observed. It might be due to the presence of various bioactive compounds in their extract. For instance, the phytochemical analysis of A. sativum L. carried out by GC-MS revealed the presence 28 compounds in their extracts where as the GC–MS analyses of the Z. officinale extracts resulted in the identification of 80 components. Similarly the Gas chromatography mass spectroscopic analysis of A. cepa L. showed the presence of 16 compounds in their extracts. The phytochemical analysis of A. cepa var. aggregatum extract exposed the presence of 9 compounds. The different solvent extracts of M. koenigii expressed different concentrations of numerous phytochemicals. The isolation and identification of these bioactive ingredients associated with the medicinal characteristics of these culinary plants gets correlated with studies carried out on their antifungal activities. Already numerous workers reported the antifungal activities of these culinary plant extracts and their isolated compounds.

Tansey and Appleton (1975) showed the antifungal activity of garlic against fungi. They reported that growth inhibition of many of fungi by garlic bulb aqueous extract. Caporaso et al. (1983) reported the antifungal activity of A. sativum. They found that garlic extracts with the concentration 1:100 is effective in inhibiting fungi
and the active ingredient is allicin. Garlic compounds such as allyl sulfides affect aflatoxin B1 carcinogenicity (Berges et al., 2004). The zingiberene compound in Z. officinale, diallylthiosulphatesulphonate in A. sativum, 1 propenyl cystine sulfoxide in A. cepa are the substances highly antimicrobial in action (Ceylan and Fung, 2004). Gowda et al. (2004) recorded the fungal growth inhibition and about 76% A. parasiticus aflatoxin biosynthesis reduction by the onion extracts. The fungistatic activities of allicin, thiosulphonates and other compounds of onions against A. niger, Rhodotorula nigricans, Penicillium italicum, P. cyclopium, A. flavus, Cladosporium macrocarpum, A. fumigatus, A. alutaceus, A. terreus and Penicillium. A. niger, Tryptophyton gypseum and Microsporon audouini are reported (Irkin and Korukluoglu, 2007). Tagoe et al. (2011) enumerated the antifungal properties of ginger, garlic and onion soluble extracts. They had shown the growth inhibition of A. niger, A. flavus and Cladosporium herbarum. Zingerone, shagols and gingerols were found to be responsible for the antifungal activity of ginger. Allicin present in garlic have high antifungal activity. Finally they concluded that the use of ginger, garlic and onion in controlling C. herbarum could help preserving meat.

M. koenigii extracts showed effective inhibition of growth and toxin elaboration by A. flavus. Rahman and Gray (2005) reported the antimicrobial activity of extract of M. koenigii stem bark against selected Gram positive, Gram negative bacteria A. niger and Candida albicans. They showed the presence of benzoisofuranone derivatives and MIC of these compounds ranged between 3.13 – 100 µg/ml. GC-MS analysis of extracts of Murraya leaves using different solvents revealed the presence of caryophylenone, phytol, isophytol, caryophyllene oxide and Hexadecanolic acid are commonly present in all solvents extracts of M. koenigii. Noor Haslizawati Abu Bakar et al. (2007) reported the presence of mahanimbine, girinimbine, murrayanine and murrayafoline in the extracts of M. koenigii stem bark. Chowdhury et al. (2008) studied the chemical composition of essential oils of leaves of M. koenigii. They reported the presence of 39 compounds in essential oil when analysed in GC-MS, the major compound is 3-carene, caryophyllene, caryophyllene oxide. Pande et al. (2009) studied the pharmacognostic and phytochemical properties of M. koenigii leaves extract. They reported the presence of alkaloids, flavonoids, volatile oil and phytosterols. Manvi and Sarin (2010) reported the antimicrobial
activity of *M. koenigii* leaves. They also reported that essential oil of *M. koenigii* leaves extracts showed potential antifungal activity against *A. niger*, *C. albicans*, *C. glabrata* and *T. ruburam*.

*M. koenigii* (L.) Spreng (Rutaceae) is usually cultivated for its aromatic leaves which are on the whole used for natural flavoring in curries and sauces. It was originated in Tarai regions of Uttar Pradesh, India and is now widely found in all parts of India and it adorns every house yard of Southern India and is also cultivated in Sri Lanka, China, Australia and the Pacific islands. This plant is also distributed in Andaman islands and throughout Central and Southeast Asia. Parts of the plant have been used as raw material for traditional medicine formulation for example ayurvedic and unani medicine in India. The plant is used in Indian system of medicine to treat various ailments. *M. koenigii* leaves and roots can be used to cure piles and allay heat of the body, thirst, inflammation and itching. The aromatic leaves, which retains their flavor and other qualities even after drying, are slightly bitter, acrid, cooling, weakly acidic in tastes and are considered as tonic, antihelminthic, analgesic, digestive, appetizing and are widely used in Indian cookery for flavouring food stuffs. The plant is credited with tonic, stomachic and carminative properties. The green leaves are used to treat piles, inflammation, itching, fresh cuts, dysentery, vomiting, burses and dropsy. The green leaves are also eaten raw as a cure for diarrhea and dysentery; bruised and applied externally to cure eruptions; given as a decoction with bitters as a febrifuge; and in snake bite. Moreover its leaves have a potential role in the treatment of diabetes. Hypoglycemic action on carbohydrate metabolism was reported in rats fed with *M. koenigii* leaves (Mohd Dikui, 2009).

Already the antifungal and antibacterial activities of *M. koenigii* were reported by many authors. For example undiluted essential oil exhibited strong antibacterial and antifungal activity when tested with microorganisms. Even the crude leaf extracts of *M.koenigii* leaf plant are reported to possess antibacterial activity (Mohd Dikui, 2009).

Enzymes and vitamins like heat-unstable and biologically active compounds might be lost during cooking, and the relative activities could be decreased as a result of food preparation. Superoxide anion radical-scavenging abilities, of the juices
prepared from carrots, tomatoes and onions, decreased considerably in the process of boiling, but the juices of vegetables rich in chlorophylls still retain strong abilities (Bao et al., 2004).

The culinary plants found to have both health benefits and antimicrobial active phytochemicals. The presence of more bioactive compounds validates not only the medicinal properties of the selected culinary plants but also their antimicrobial preservative potential. At least 50,000 compounds have been isolated from plants, and one approximation notes the total number of plant metabolites likely exceeds 200,000. Interactions between phytochemicals, and even between different plants used in combination, form the basis of therapeutic use in traditional healing paradigms such as traditional Chinese medicine (TCM) and Ayurveda. The failure of reckoning true synergy between specific isolated phytochemicals within a herb or herbal extract owing to the plenty of uncharacterized phytochemicals in whole plants and many herb extracts, and the possibility of many complex interactions, provides the explanation for the net activity, as other unknown constituents may have a contributory role (Jordan et al., 2010).

Valerio et al. (2007) suggested that for carrying out predictive toxicological studies the computational methods can be useful when experimental data are insufficient, unreliable, unavailable, or inconsistent between studies. This approach reduces animal testing, facilitates, the review process and also has applicability for the toxicological evaluation of chemically identified individual components of botanical mixtures, or chemicals of natural origin that have not been subjected to in vivo testing. This latter application is of particular relevance for materials from plant-derived, natural products that are difficult to synthesize by chemical means, or may be present in a complex mixture of such products (e.g. botanical extract), or are not the principal pharmacologically active constituent in a mixture and should still be considered for potential toxicological effects including carcinogenic risk.

As Schilter et al. (2003) stated the interactions between constituents will be extremely important when data for highly purified preparations are used to assess relatively unpurified material, prediction their interaction is important. Already Yang et al. (2004) have discussed the possibility of using in silico methods for the
investigation of simple chemical mixtures. Most of these interaction data are the
derivatives of *in vitro* studies. Naturally, such studies should be followed up with *in vivo*
analysis. This is not at all times practical due to the wide complexity of herbs and
their extracts phytochemicals (Rietjens *et al.*, 2008) and is limited by the complex
combinations of plant chemicals (Jordan *et al.*, 2010). Hence assessment of potential
interactions between classes of compounds, or the major compounds involved may
give answer to the dearth of information on potential matrix effects in medicinal herbs
(Rietjens *et al.*, 2008), and the use of omics technologies to determine potential global
effects of interactions in mixtures (Jordan *et al.* 2010).

The culinary plant phytochemicals molecular docking studies revealed that
they have many potential compounds like hexadecanoic acid, guanosine, gingerol,
zingerone, 2-undecanone as well as isophytol could interact with the aflatoxin
synthesis regulatory proteins. Hence the obtained results indicated that the culinary
plants *A. sativum, Z. officinale, A. cepa L., A. cepa* var. *aggregatum* and *M. koenigii*
have many aflatoxin synthesis ver1 protein interacting phytochemicals which may
play a role in aflatoxin biosynthesis gene regulation and there by their inhibition.

Hence from the obtained results and above discussion, it can be interfered that
the culinary plants may provide natural antifungal safer preservative alternatives
protecting the culinary oils from pathogenic and saprophytic storage fungi mediated
spoilage. It also established the storage fungi associated threats of culinary groundnut
oil. Our results advocate that these culinary plants can be used alone or in conjunction
with other substances to reduce the risk of xerophilic aflatoxigenic fungi in culinary
oils. It may ensure their safety in addition to imparting aroma, flavour and
naturaceuticals to culinary oil with societal acceptability.