Original Research Article

Effects of different extracting solvents on non-phenolic phytochemical profiles of selected Nigerian spices and spice-treated foods

ABSTRACT

The aim of this research was to investigate the impact of extraction solvents on the non-phenolic phytochemical profiles of selected spices (*Ocimum viride*, *Monodora myristica*, *Monodora tenuifolia* and *Tetrapleura tetraptera*) and spice-treated foods in southern part of Nigeria. The spice samples were processed into powder for antioxidant screening. The spice extracts were obtained from the samples using 5 extracting solvents [distilled water, 95 % methanol, acetone / hexane (1:1 v/v), n-hexane / methanol / acetone (2:1:1, v/v/v) and acetone / water / acetic acid (70:29.5:0.5, v/v/v)]. Water extracts were obtained from beef, pork and fluted pumpkin leaves. The alkaloid, saponin, oxalate and phytate components of the spice extracts and the spice-treated foods were evaluated using standard methods. The laboratory analyses were performed at analytical laboratory, National Centre for Energy Research and Development, University of Nigeria, Nsukka during the 3rd quarter of 2020. The percentage yield of the extracts were low (0.32 - 0.96 %) and varied widely among extracting solvents, spices and spice-treated foods. *M. myristica* and *T. tetrpleura* had the highest yield, 0.96, in methanol extracts. Phytochemical contents differed significantly ($p < 0.05$) among spices, extracts of the same spice and among spice-treated foods. Spices had high contents of oxalate (2.0 – 7.0 mg/100g), alkaloid (0.8 – 5.76mg/100g) and phytate (2.14 – 3.88mg/100g) but relatively low content of saponin (0.03 – 0.736 mg/100g). Methanol alone or in combination with other solvents extracted higher amounts of phytochemicals (0.96 %) than other solvent mixtures from the spices. Phytochemical contents of spice-treated foods were in the order: vegetable > rice > pork > beef.

***Keywords**:* Spices. Nigerian spices. Phytochemicals. Extraction solvents, non-phenolic compounds.

1. INTRODUCTION

In Nigeria, a high proportion of the rural and urban population resort to some natural food ingredients due to their availability. Spices are a large group of such natural ingredients, and include dried seeds, fruits, roots, rhizomes, barks, leaves, flowers and any other vegetative substances used in a very small quantity as food additives to colour, flavour or preserve food [1].

Spices are fragrant, aromatic and pleasant. Only very small fractions of dry matter of the spices, the phytochemicals are responsible for these flavouring, colouring, preservative and health-promoting characteristics [2,3]. Phytochemicals are either phenolic or non-phenolic. Phenolic phytochemicals have at least one phenol ring in their structures and are usually good hydrogen donors, exhibiting antioxidant activities in plants, foods and human. Non-phenolic phytochemicals are numerous, belonging to diverse homologues with different structures and properties, performing numerous functions in both plants and animals. Common non-phenolic phytochemicals include alkaloids, trypsin inhibitors, oxalate, hemogglutinin, saponin and phytate. These compounds constitute antinutritional factors when they exceed recommended maximum limit in foods. However, traditional home cooking drastically reduce them to allowable levels in food. Most non-phenolic phytochemicals have been associated with antimicrobial activities and numerous physiological activities in mammalian calls [4, 5].

Both epidemiological and clinical studies have proven that phytochemicals present in plants are mainly responsible for reduced incidence of chronic and degenerative diseases among populations whose diets are high in plant foods [6]. Phytochemicals are health-promoting and are of many disease-preventive [1, 7]. Many phytochemicals can have profound physiological effects, mimicking body hormones and suppressing development of diseases in the body [8, 9, 10]. As a result there has been an increased search for phytochemicals with antimicrobial potency for preservation and heath promoting of consumers. Many plants containing alkaloids have diuretic, antispasmodic, anti-inflammatory and analgesic effects [11]. Phytochemical compositions of many spices of international trade like rosemary, garlic and ginger have been evaluated for these important constituents since such information is important for more practical and industrial applications. Unfortunately there are no documented reports on phytochemical composition of many Nigerian spices.

Some Important Uses of Selected non-phenolic Compounds

Alkaloid

Alkaloids have diverse therapeutic and nutritional benefits. Their presence in plants such as *Moringa oleifera* made the plant to be useful in the treatment of hypertension. Many plants containing alkaloids and flavonoids have diuretic, antispasmodic, anti-inflammatory and analgesic effect [12, 13]. The high alkaloid content of these spices could account for their popular use in the traditional treatment of hypertension. These secondary metabolites have been associated with numerous physiological activities in mammalian cells in various studies [4, 5, 14, 15].

Oxalate

The oxalic acid content of vegetables has been used as an index of their toxicity since a high content of it would lower the nutritive value of food [13]. Presence of oxalic acid in plants contributes to antioxidant properties and hence the therapeutic potentials of the spices. Oxalic acid content in food would be an index of toxicity level of the food. However, oxalate at low level advantageously confers antioxidant activity in both food and human. Dietary oxalate has also been shown to complex with calcium, magnesium and iron, forming insoluble oxalate salts which cause oxalate stone [16]. Oxalic acid chelate radical-initiating divalent metals thereby reducing incidence of oxidative degenerative diseases in human.

Saponin

Saponins possess carbohydrate moieties attached to tetraprenoid or steroidal aglycones [17]. Saponins constitute a key ingredient in traditional Chinese medicine and are responsible for many of the attributed biological effects. They reduce uptake of glucose and cholesterol through intra-lumenal physicochemical interaction during food transition in the gut. This could confer chemo-protection against heart diseases.

Phytate

Phytate is a natural plant inositol hexaphosphate constituting about 1.5% of many plants [18]. Phytate is a very stable and potent chelating food component that is considered to be an antinutrient by virtue of its ability to chelate divalent metals and prevent their absorption [19]. However, it has also been shown to have anticancer and antioxidant activity. It forms an iron chelate that suppresses lipid oxidation by blocking iron driven hydroxyl radical generation. Metal phytate complexes are highly insoluble over a wide range of pH and as a result inhibit iron-related hydroxyl radical formation by forming an inactive iron-chelate [20]. Presence of phytate in foods has been associated with reduced mineral absorption due to the structure of phytate with high density of negatively charged phosphate groups which can complex with many mineral ions, causing non-availability for intestinal absorption. However, presence of phytate in high fibre foods may reduce the incidence of breast cancer and cardiovascular diseases. Phytates are stable compounds that chelate excess divalent metals and control their excess absorption, thereby lowering the incidence of cancer in human [19, 21].

Phytochemical composition of cooked spice-treated food extracts

One would presume at this point that spices have maximum thresholds for photochemical contents in food. This could be attributed to low contents (2.5% and 7.5%) of these spices in the food and also the likely effect of heat in destroying part of these phytochemicals [22]. Phytochemicals are plant metabolites and are richly found in fruits and vegetables. Presence of these photochemical in cooked food extracts confirms that they contribute to preservative and health promoting quality of menu.

The aim of this study was to evaluate the effect of different extracting solvents on the non-phenolic phytochemical composition of M*onodora tenuifolia, Monodora myristica, Ocimum viride* and *Tetrapleura tetraptera,* which are commonly consumed spices in southern part of Nigeria.

2. MATERIALS AND METHODS

2.1 Materials

The spices *Tetrapleura tetraptera (*Schum & Thonn*)* [23], *Monodora myristica* (Gaertn) [24], *Monodora tenuifolia (*Benth) [25] and *Ocimum viride* (Willd) were purchased from commercial stockers at Ogige main market Nsukka in Enugu State, Nigeria. These are the major indigenous spices in the area. Fresh beef and pork (thigh muscle) were purchased from meat sellers at the Ikpa market, Nsukka, Nigeria. The beef and pork were frozen (4^{O}C) overnight and thawed the following day before use. All the reagents used were of analytical grade. The water used was deionized water obtained from Service and Training Centre (STC) unit of the University of Nigeria Nsukka, Nigeria. The methanol, acetone and ethanol were products of SIGMA-ALRICH® INC, St Louis, MO 63178, USA but purchased from Lavans Scientific Ltd, number 70 Enugu road, Nsukka, Enugu state, a Nigerian government-approved dealer on laboratory reagents. The laboratory analyses were performed at analytical laboratory, National Centre for Energy Research and Development, University of Nigeria, Nsukka.

2.2 The extracting solvents

Five solvent systems comprising distilled water, 95 % methanol, acetone / hexane (1:1 v/v), n-hexane / methanol / acetone (2:1:1, v/v/v) and acetone / water / acetic acid (70:29.5:0.5, v/v/v) were appropriately prepared based on the solvency of the constituent solvents and solubility characteristics of the extracting phytochemicals.

2.3 Preparation of spice samples for antioxidant screening

The method described by Effraim et al. [26] was used to prepare the spice samples. Fresh leaves of *Ocimum viride* were washed, air-dried under shade for 12 h and then oven-dried at 55^{o}C for 8 h. The leaves were plucked from their stalk, packed in black polyethylene bag and stored in a cilia gel packed glass desiccator till the next day. The *Tetrapleura tetraptera* fruits

were washed, drained and oven-dried at 55^{o}C for 12 h. These were crushed into coarse powder using wooden mortar and pestle. It was stored in 250 ml air-tight glass vial and stored in the laboratory cabinet. The *Monodora myristica* and *Monodora tenuifolia* seeds were toasted on a hot sand bath at 100-120^{o}C for 7 minutes. These were cracked to recover the nibs from seed coats. The nibs and dried leaves of *Ocimum viride* were milled into coarse powder using a laboratory hammer mill (Betch 5657 GMbH, Germany).

2.4 Preparation of spice extracts

One gram (1 g) of each of the spice powder was macerated and homogenized in 50 ml of the extracting solvent [distilled water, methanol, acetone, hexane, (1:1, v/v), n-hexane/methanol/acetone (2:1:1), v/v), acetone/water/acetic acid (70:29.5:0.5, v/v/v)] for 3 minutes in 100 ml amber bottles. The bottles were tightly corked and placed in boiling water bath for 10 minutes to inactivate inherent enzymes. These were cooled under running tap water within 10 minutes, manually homogenized by rocking them on the laboratory bench for 5 minutes. These were rested for 15 h in the laboratory cabinet until the next day. Each of the extract mixture was shaken and filtered through cheese cloth. The filtrates were further filtered using double layered Whatman No 5 filter papers. The filtrates were concentrated under reduced pressure using a rotary evaporator ( ). The concentrated extracts were dried at 50^{o}C for 40-50 minutes on a water bath. The cooled dried extracts were stored in 100 ml amber coloured bottles for use for the analysis.

2.5 Preparation of water extracts of cooked spice-treated foods

The method described by Geonaras et al. [27]. Freshly cut thigh muscles of beef and pork were boned manually with the aid of a kitchen knife. The free muscles were chopped into cubes (about 1cm x 1cm x 1cm) and then milled into semi solid mass using a hand-operated colloid mill. Vegetable mix (1:1 ratio of African spinach to fluted pumpkin leaves, w/w) were shredded into small pieces with the aid of a kitchen knife. Parboiled rice sample (500 g) was

ground into coarse powder. Then 150 g of each of the prepared meat, vegetable and rice samples was mixed with1.5 L of boiling water (at 1:10, w/w basis), allowed to cook for 10 minutes and extracted to yield at least 10%, w/v) suspension. The suspension while hot was homogenized in a kitchen blender for 60 S and then passed twice through a single layer of cheese cloth to get crude food extracts. From each of the food extracts, 100 ml was transferred to sterile 200 ml amber-colour glass bottles. This was done in triplicates for each of the food extracts, and the bottles labelled as R1, R2, R3 and R4 for rice extracts; P1, P2, P3 and P4 for pork extracts and V1, V2, V3 and V4 for vegetable extracts.

2.6 Preparation of cooked spice-treated food extracts

Freshly cut thigh muscles of beef and pork (1.5 kg each) were boned manually with the aid of a kitchen knife. The free muscles were chopped into cubes (about 1 cm x 1 cm x 1 cm) and then minced into semi-solid mass with the aid of a hand-held mechanical meat mincer. Vegetable mix (1:1 ratio of African spinach to fluted pumpkin leaves) (700 g) were shredded into small piece with the aid of a kitchen knife. The parboiled rice (500 g) was milled into coarse powder. The minced beef and pork, chopped vegetable mix and rice powder were suspended in a boiling water at 1:10 w/v basis and maintained in the boiling water for 10 minutes. Each food suspension was then homogenized with the aid of a kitchen blender for 2 minutes to yield at least 10% (w/w) suspension. These were decanted immediately and the supernatants passed twice through a single layer of cheese cloth. These yielded crude extracts of the food sample. The method described by Geonaras et al. [27].

Then, 0.5 gand 1.5 gof each of the previously prepared spices (*Ocimum viride*, *Monodora myristica*, *Monodora tenuifolia* and *Tetrapleura tetraptera*) were separately mixed with 50 ml of each of the cooked spice extracts in 100 ml amber glass bottles. These concentrations were based on the use levels of these spices in local cuisines in soups by rural women. Also, 50 ml of each of the food extract were also

transferred to 100 ml amber glass bottles. These were corked and thoroughly mixed to yield 10 mg/100 ml (10%), 30 mg/100 ml (30%) and 0 mg/100ml (0%), respectively. These were autoclaved at 121^{o}C for 15 minutes, cooled to ambient temperature and then stored in the refrigerator (4^{o}C) till used the next day for phytochemical and antioxidant screening. The 0 ml spice extract acted as the negative control.

2.7 Determination of alkaloid content

The alkaloid content was determined using the gravimetric method by Harborne [28]. Ground sample of each spice or food (5.0 g) was dispersed in 50 ml of each solvent in 250 ml volumetric flask, shaken vigorously and allowed to rest for 4h before being filtered through what man no. 5 filter paper. Filtrates were then evaporated to one quarter (1/4) of original volumes and concentrated ammonium hydroxide (NH_4OH) added drop-wise to precipitate alkaloids. The mixtures were then filtered through weighed filter paper and washed with 1% ammonium hydroxide solution. The filter paper and residue (alkaloids) were oven-dried at 60^{O}C for 30 min. and alkaloids contents determined by weighing.

2.8 Determination of phytate content

Phytate content was determined according to the method of AOAC [29]. Four grams (4.0 g) of the spice sample was soaked in 100 ml of the appropriate solvents for 3 h and then filtered through what man no. 2 filter paper. The filtrate (25ml) was pipetted into 50ml conical flask, and 5ml of 0.3% ammonium thiocyanate solution and 53.5 ml of distilled water were added. The mixture was titrated against standard Iron (iii) Chloride solution (containing 0.00195 g Fe^{3+}/ml) until a brownish yellow colour persists for 5min. Phytate content was expressed as percentage (%) phytate in the spice sample.

2.9 Determination of oxalate content

Oxalate content was determined as described by Oke [16]. A blend of each ground spice or food sample (1.0 g) was mixed with190 ml of appropriate solvent and 10 ml of 6M hydrochloric acid (HCl). This was digested at 90^{O}C for 4 hours, and then centrifuged at 2000 rpm for 5 min. The supernatant was diluted to 250 ml with distilled water and titrated with concentrated ammonium hydroxide solution until the pink colouration changed to endpoint faint yellow colour, using methyl orange indicator. This was heated (90^{O}C, 20min.) and 10ml of 5% calcium chloride ($CaCl_2$) solution added to precipitate calcium oxalate. This was rested overnight, centrifuged and decanted. The residue was oven-dried at 60^{O}C for 48 h, cooled and then weighed. This was done in triplicates and the mean weight expressed as percentage oxalate content using the expression,

% Oxalate content = weight of oxalate / weight of spice sample x 100 /1. …**Equation 1**

2.10 Determination of saponin content

Ground sample (1 g) of each spice or food sample was macerated in 10 ml of each solvent system and the extract decanted into a 50ml beaker. The residue was re-extracted with another 10ml of solvent, allowed to rest and then decanted into the formal beaker. The pooled extracts were evaporated to dryness and the residue re-dissolved in 6ml of ethanol and allowed to stand for 30min for colour development. Absorbance of the ethanol extract was read at 550 nm and used to extrapolate saponin content from a standard curve.

2.11 Statistical analysis

Data generated from all analysis were subjected to analysis of variance and means where significant ($p < 0.05$) were separated with Fisher's least significant difference using Statistical Package for Social Sciences (SPSS) version 13.0.

3 RESULTS AND DISCUSSION

3.1 Yield of spice extracts as affected by different solvents

Table 1 shows percentage yields of crude extracts from spices as affected by different extracting solvents. Very small amounts (0.32 - 0.96%) of the spices were extracted by the extracting solvents. Yields (%) varied widely among spices and also among different solvent extract of the same spices. Methanol (95%) was the most suitable yield of 0.96% with *M. myristica* and *T. tetraptera*. Also methanol in combination with hexane and acetone maintained relatively good yields (0.52 - 0.88%) of extracts among spices. Water and acetone/water/acetic acid solvents maintained close range of yields among the spices. Yields of extracts with water were relatively low (0.32 - 0.68%) compared to yields of extracts (0.73 - 0.80%) with acetone/water/acetic acid.

The solvents are all food grade and safe. Absolute water, aqueous mixture of ethanol, methanol, hexane and acetone, absolute methanol, methanol (80% and 70%), ethanol (70%, 80% and 95%), and acetone (50%, 70% and 80%) were used to extract antioxidants from fruits, vegetables, legumes and cereals [30, 31, 32].

Yields of extraction have not always coincided with antioxidant and antimicrobial activities of extracts. This is because yields of extractions and composition of yields correlate independently on the types of solvents with varying polarities and pH, extraction time and temperature. Under the same condition of extraction, time and temperature, the solvent used and chemical properties of the food samples remain the two most important factors [33, 12]. Thus, high yields of extracts may not always imply high phytochemical content, antioxidant and antioxidant activities of extracts.

Table 1: Yield (%) of crude extracts of spices as affected by different extracting solvents

Spices	Yields (%) of spice extracts as affected by different extracting solvents					
	ER	OL	AW	ONE	AN	MEAN
Monodora myristica	0.32	0.96	0.80	0.49	0.88	0.69
Monodora tenuifolia	0.32	0.56	0.80	0.60	0.56	0.57
Ocimum viride	0.68	0.32	0.76	0.76	0.52	0.61
Tetrapleura tetraptera	0.32	0.96	0.73	0.32	0.60	0.58

ER = distilled water, OL = 95% methanol, ONE = acetone/hexane (1:1; v/v), AN = hexane/methanol/acetone (2:1:1; v/v/v/v), AW = acetone/water/acetic acid (70:29.5:0.5; v/v/v)

3.2 Effects of different extraction solvents on non-phenolic phytochemical profiles of the spices

3.2.1 Alkaloid

Alkaloid content of five different solvent extracts from four Nigerian spices is presented in Table 2. Spice extracts from different extraction solvents differed significantly (*P*= .05) in their alkaloid content. The alkaloid content of *Monodora myristica* from different extraction solvents ranged from 2.07 to 5.76mg/100g, *Monodora tenuifolia* from 0.80 to 6.54mg/100g, *Ocimum viride* from 3.64 to 5.42mg/100g, and *Tetrapleura tetraptera* from 2.85 to 5.08 mg/100g.

Generally, alkaloid content of the four spices were significantly (*P*= .05) affected by the different solvents used. The alkaloid contents of the spices as affected by the extracting solvents were in the following order from high to low: acetone/hexane (1:1; v/v) >acetone/water/acetic acid (70:29.5:0.5; v/v/v) > hexane/methanol/acetone (2:1:1; v/v/v) > 95% methanol > distilled water for *monodora myristica*; acetone/water/acetic acid > acetone/hexane > 95% methanol >

hexane/methanol/acetone > distilled water for *Monodora tenuifolia*; hexane/methanol/acetone > distilled water > acetone/hexane > 95% methanol, and acetone/water/acetic acid for *Ocimum viride*; and acetone/water/acetic acid (70:29.5:0.5; v/v/v) > acetone/hexane > 95% methanol > hexane/methanol/acetone > distilled water for *Tretapleura tetraptera*. These results suggest that types of spices being extracted and types of solvent used influence the quantity of alkaloid extracted. It was also evident that the solvents work better when in combination than when used singly for alkaloid extraction from the spices. Distilled water was the weakest extraction solvent while acetone in combination with the other solvents, including hexane, methanol, acetic acid and distilled water was the best extraction solvent.

3.2.2 Oxalate

In order to estimate the potential of oxalate as a bioactive ingredient of selected spices in foods and other biological materials, oxalate content of various solvent extracts of the spices was analyzed, and the results presented in Table 2. Different extraction solvents of the spices differed significantly ($P < 0.05$) in their oxalate contents. The oxalate content of *M. myristica* ranged from 2.0 to 3.5mg/100g; *M. tenuifolia* from 3.5 to 7.0mg/100g; *O. virdie* from 3.0 to 4.0mg/100g; and *T. tetraptera* from 3.5 to 5.6mg/100g.. The oxalate content yields by the extraction solvents were in the following order from high to low: Acetone/hexane > acetone/water/acetic acid > Distilled water, and hexane/methanol/acetone > 95% methanol for *M. myristica*; distilled water > acetone/water/acetic acid > acetone/hexane and hexane/methanol/acetone > 95% methanol for *M. myristica*;

Acetone/water/acetic acid > 95% methanol, and hexane/methanol/acetone > distilled water, and acetone/hexane for *O. viride*; and distilled water > hexane/methanol/acetone > 95% methanol> Acetone/water/acetic acid, and acetone/hexane for *T. tetraptera*. These results suggest that distilled water was the best among the five extraction solvents for extracting oxalate from *M. tenuifolia* and *T. tetraptera* while acetone/water/acetic acid was the best solvent for *O. viride*, and acetone/hexane was the best for *M. myristica*. Thus, distilled water or distilled water in combination with other solvents seemed to be the best for extracting oxalate from spices. Also *M. tenuifolia* was highest in oxalate content, followed by *T. tetraptera, O. viride* and then *M. myristica*.

3.2.3 Saponin

The effects of various solvent extraction systems on recovery of saponin from the selected spices are presented in Table 2. Spice extracts from different extraction solvents differed significantly (P= .05) in their saponin content. Saponin contents of *M. myristica* ranged from 0.01 to 0.74mg/100g; *M. tenuifolia* from 0.01 to 0.29 mg/100g; *O. viride* from 0.14 to 0.62mg/100g; and *T. tetraptera* from 0.16 to 0.60mg/100g. Generally, saponin content in the four spices was relatively low when compared with the compositions of other non-phenolic phytochemicals. The saponin yields by the extracting solvents were in the following order from high to low: 95% methanol > acetone/hexane (1:1; v/v) > hexane/methanol/acetone (2:1:1; v/v/v) > acetone/water/acetic acid (70:29.5:0.5; v/v/v) > distilled water for *M. myristica*; acetone/water/acetic acid > acetone/hexane > distilled water > 95% methanol > hexane/methanol/acetone for *M. tenuifolia*; hexane/methanol/acetone >

acetone/water/acetic acid > acetone/hexane >95% methanol > distilled water for *O. viride;* and distilled water > 95% methanol > acetone/water/acetic acid, and acetone/water > hexane methanol/acetone for *T. tetraptera*. The results showed that saponin contents of the spices was low and differed ($p < 0.05$) significantly among the spices. The spice *T. tetraptera* had the highest saponin content among the four spices.

3.2.4 Phytate

Phytate contents of solvent extracts of the four spices are presented in Table 2. The phytate contents of the different solvent extracts ranged from 2.14 to 2.38mg/100g in *M. myristica*; from 3.02 to 5.50mg/100g in *M. tenuifolia*; from 2.32 to 2.62mg/100g in *O. viride*; and from 3.24 to 3.80mg/100g in *T. tetraptera*. Spice extracts from different extraction solvents differed significantly ($P = .05$) in phytate contents. Meanwhile, each spice had a close range of values of phytate content among its different extraction solvents. The phytate content of the different extraction solvents were in the following order from high to low: acetone/water/acetic acid (70:29.5:0.5; v/v/v), and acetone/hexane (1:1; v/v) > hexane/methanol/acetone (2:1:1; v/v/v) > distilled water, and 95% methanol for *M. myristica*; distilled water > acetone/water/acetic acid , acetone/hexane and hexane/methanol/acetone > 95% methanol for *M. tenuifolia*; distilled water, 95% methanol and acetone/water/acetic acid > acetone/hexane, and hexane/methanol/acetone for *O. viride*; and acetone/hexane > hexane/methanol/acetone > distilled water > 95% methanol > acetone/water/acetic acid for *T. tetraptera*. These results suggest that the extractability of phytate by the extracting solvents varied with the type of spice being

extracted; and that distilled water was the best extracting solvent for *M. tenuifolia* and *O. viride* while acetone/hexane was the best for *T. tetraptera*. The spices could serve as good sources of phytate in food and food related systems due to the high content of phytate in them.

Table 2: Effects of different extraction solvents on non-phenolic phytochemicals of spices

Spices/solvents	Alkaloid (mg/100g)	Oxalate (mg/100g)	Saponin (mg/100g)	Phytate (mg/100g
Mondora myristica				
Distilled water	2.07^{c}+0.02	2.5^{c}+0.01	0.016^{d}+0.00	2.14^{b}+0.00
95% Methanol	4.88^{b}+0.04	2.0^{d}+0.02	0.736^{a}+0.02	2.15^{b}+0.00
Acetone/water/aceticacid	5.22^{ab}+0.06	3.0^{b}+0.01	0.503^{c}+0.01	2.37^{a}+0.02
Acetone/hexane	5.76^{a}+0.02	3.5^{a}+0.00	0.719^{a}+0.01	2.38^{a}+0.02
Hexane/methanol/Acetone	5.04^{b}+0.01	2.5^{c}+0.00	0.60^{b}+0.01	2.32^{a}+0.02
Monodora tenuifolia				
Distilled water	0.8^{e}+0.01	7.0^{a}+0.04	0.16^{bc}+0.00	5.50^{a}+0.02
95% Methanol	3.66^{c}+0.04	3.5^{c}+0.00	0.10^{c}+0.00	3.02^{c}+0.00
Acetone/water/acetic acid	6.54^{a}+0.03	4.0^{b}+0.01	0.29^{a}+0.01	3.88^{b}+0.10
Acetone/hexane	4.54^{b}+0.05	4.0^{b}+0.01	0.23^{a}+0.00	3.84^{b}+0.04
Hexane/methanol/Acetone	3.05^{d}+0.02	4.0^{b}+0.01	0.06^{a}+0.00	3.84^{bc}+0.02
Ocimum viride				
Distilled water	5.08^{a}+0.03	3.0^{c}+0.01	0.14^{b}+0.00	2.62^{a}+0.02
95% Methanol	3.64^{c}+0.02	3.5^{b}+0.01	0.19^{b}+0.01	2.63^{a}+0.02
Acetone/water/acetic acid	3.73^{c}+0.02	4.0^{a}+0.02	0.4^{a}+0.02	2.61^{a}+ 0.01
Acetone/hexane	4.67^{b}+0.02	3.0^{c}+0.1	0.38^{a}+0.02	2.34^{b}+0.00
Hexane/methanol/Acetone	5.42^{a}+0.01	3.5^{b}+0.02	0.06^{c}+0.05	2.32^{b}+0.04
Tetrapleura tetraptera				
Distilled water	2.85^{c}+0.01	5.55^{a}+0.04	0.60^{a}+0.02	3.38^{ab}+0.02
95% Methanol	3.96^{b}+0.02	4.0^{b}+0.02	0.44^{b}+0.01	3.24^{bc}+0.02
Acetone/water/acetic acid	5.08^{a}+0.01	3.50^{c}+0.01	0.33^{c}+0.01	2.79^{c}+0.01
Acetone/hexane	4.32^{b}+0.01	3.50^{c}+0.02	0.03^{e}+0.01	3.80^{a}+0.04
Hexane/methanol/Acetone	3.25^{c}+0.08	4.50^{ab}+0.03	0.16^{d}+0.02	3.43^{ab}+0.00

Data are means ± standard deviations (n = 3); values within each type of spice marked by the same letter within the same column are not significantly different ($p < 0.05$).

3.3 Phytochemical composition of cooked spice-treated food extracts

Table 3 shows non - phenolic photochemical composition of cooked food extracts [10 % (w/w)] treated with 2.5% (10 mg/ml) and 7.5% (30 mg/ml) of the spice samples. Phytate, Alkaloid, oxalate and saponin earlier recorded (Tables 2) in theses spices were also present in the cooked spice-treated food extracts and even in the controls (without spice). The food extracts are from plant materials. Phytate (mg/100g) ranged from 0.064 in control pork to 2.94 in rice treated with 7.5% of *O. viride*, alkaloid (mg/100g) from 0.003 in control pork to 9.01 in pork treated with 7.5% of *M. myristica*, oxalate (mg/100g) from 0.023 in control pork to 9.21 in beef treated with 7.5% of *O. viride*, and saponin (mg/100g) from 0.04 in control beef to 2.31 in vegetable treated with 7.5% *M. tenuifolia.* These phytochemicals were lower in these food extracts than in the spices. Vegetable extract had highest content of these chemicals, followed by rice, beef and pork. Thus, food types also influenced the photochemical contents. The control (without spices) beef and pork sample had significantly (P= .05) low contents of these chemicals. In most of the food extracts, phytochemical contents increased slightly with high increase, (from 10mg/ml to 30mg/ml spice concentration, 75% increase), of spices.

Table 3: Non-phenolic phytochemical profiles of water extracts of spice-treated cooked food samples

Food/spices	Spice treatment mg/100g	Alkaloid (mg/100g)	Oxalate (mg/100g)	Saponin (Mg/100g)	Phytate (Mg/100g)
BEEF					
O. viride	10	0.943^{d} ±0.02	4.093^{b}±0.07	0.544^{b}±0.01	0.639^{b}±0.00
	30	1.763^{c} ±0.02	9.218^{a}±0.12	0.772a$^{\pm}$0.03	0.773^{ab}±0.03
M. myristica	10	1.937^{c}±0.08	2.274^{c}±0.06	0.257^{c}±0.03	0.076^{e}±0.00
	30	1.981^{c}±0.20	3.880^{b}±0.04	0.514^{b}±0.0	0.460^{c}±0.00
T. tetraptera	10	0.813^{d}±0.08	1.490^{d}±0.10	0.257^{c}±0.01	0.774^{ab}±0.02
	30	3.775^{a}±0.03	1.495^{d}±0.01	0.514^{b}±0.02	0.940^{a}±0.03
M. tenuifolia	10	1.485^{c}±0.31	3.558^{b}±0.02	0.257^{c}±0.00	1.165^{a}±0.02
	30	2.252^{b}±0.02	0.779^{e}±0.01	0.772^{a}±0.01	0.064^{d}±0.01
Control	0.00	0.037^{e}±0.00	N.D.	0.043^{d}±0.00	0.070^{d}±0.00
VEGETABLE					
M. myristica	10	2.710^{d}±0.07	5.830^{b}±0.13	0.544^{c}±0.02	1.439^{b}±0.04
	30	3.252^{c}±0.02	8.974^{a}±0.11	0.772^{b}±0.03	2.242^{a}±0.06
O. viride	10	2.195^{e}±0.06	5.976^{b}±0.08	0.557^{c}±0.01	2.711^{a}±0.05
	30	7.2711^{a}±0.30	8.926^{a}±0.12	0.772^{b}±0.01	2.711^{a}±0.10
T. tetraptera	10	2.375^{e}±0.01	5.386^{b}±0.06	0.772^{b}±0.01	2.038^{a}±0.03
	30	2.710^{d}±0.05	5.716^{b}±0.02	0.772^{b}±0.00	2.465^{a}±0.02
M. tenuifolia	10	2.671^{de}±0.05	5.813^{b}±0.04	0.772^{b}±0.00	1.186^{b}±0.01
	30	4.065^{b}±0.02	8.029^{a}±0.10	2.315^{a}±0.01	2.390^{a}±0.02
Control	0.00	0.489^{e}±0.01	1.773^{c}±0.05	0.579^{c}±0.01	1.106^{b}±0.04
PORK					
M. myristica	10	1.438^{d}±0.01	5.376^{c}±0.09	0.257^{d}±0.00	1.186^{b}±0.05
	30	9.014^{a}±0.02	7.429^{a}±0.11	0.772^{b}±0.02	1.076^{c}±0.01
O. viride	10	0.884^{e}±0.03	4.236^{cd}±0.10	0.243^{d}±0.03	0.768^{d}±0.01
	30	2.239^{c}±0.10	4.856^{cd}±0.03	0.772^{b}±0.01	0.774^{d}±0.03
T. tetraptera	10	1.42^{d}±0.02	3.557^{d}±0.07	0.257^{d}±0.01	1.838^{a}±0.06
	30	4.136^{b}±0.10	5.635^{c}±0.02	1.543^{a}±0.01	1.816^{a}±0.00
M. tenuifola	10	1.914^{d}c±0.01	1.5824^{e}±0.01	0.772^{b}±0.02	0.170^{b}±0.01
	30	4.673^{b}±0.05	6.155^{b}±0.04	0.514^{c}±0.02	1.140^{b}±0.01
Control	0.00	0.003 ^{f}±0.00	0.023^{f}±0.00	0.514±0.03	0.064e±0.00
RICE					
M. myristica	10	1.084^{b}±0.00	2.593^{b}±0.00	0.510^{c}±0.02	1.897^{c}±0.11
	30	1.562^{b}±0.02	7.015^{a}±0.03	0.772^{b}±0.04	2.224^{b}±0.10
O. viride	10	1.691^{b}±0.10	1.558^{c}±0.01	0.257^{d}±0.01	1.962^{c}±0.04
	30	2.168^{b}±0.11	5.456^{a}±0.31	0.772^{b}±0.07	2.937^{a}±0.01
T. tetraptera	10	10.295^{a}±0.20	2.598^{b}±0.07	0.772^{b}±0.03	2.156^{b}±0.05
	30	12.194^{a}±0.31	6.353^{a}±0.21	1.713^{a}±0.08	2.560^{b}±0.03
M. tenuifolia	10	1.350b±0.02	1.132c±0.08	0.257d±0.02	1.988c±0.10
	30	2.943b±0.02	3.377b±0.10	1.543a±0.03	2.384b±0.30
Control	0.00	0.466c±0.04	1.015c±0.00	0.529c±0.01	1.330d±0.07

Values are means of three determinations ± standard deviations, N.D. = Not detected.

4. CONCLUSION

The four Nigerian spices possess varying levels of phytochemicals. Spices and solvent extracts of spices differed significantly ($p < 0.05$) in phytochemical contents and the amounts of phytochemicals extracted were influenced by the solvent systems used. Methanol or methanol in combination with other solvents seems was the best extracting solvent for these phytochemicals. However, none of the solvent systems was consistently best in extracting a particular phytochemicals from the spices; suggesting that spice morphology and composition might have influenced extracting capacity of the solvents. Amount of phytate extracted was not significantly ($p > 0.05$) affected by solvent types used. *M. tenuifolia* had the highest alkaloid (6.54 mg/100g), oxalate (7.0 mg/100g) and phytate (5.5 mg/100g) contents, and *M. myristica* the highest saponin (0.719mg/100g) content. The individual phytochemicals should be isolated and characterized, using the best extracting solvents, for biochemical activities.

REFERENCES

[1] Birt DA. Phytochemicals and cancer prevention: from epidemiology to mechanism of action. *Journal of the American Dietetic Association.* 2006. 106: 20 – 24.

[2] Cowan MM. Plant products as antimicrobial agents. *Clinical Microbiological Review*. 1999. 12(4): 564 – 582.

[3] Ugwuona FU, Ani JC, Onwuzuruike UA, Ndife J, Ukom AN. Antioxidant Activities of Four Commonly Consumed Indigenous Spices of Nigeria as Affected by Extracting Solvents. *International Journal of Innovative Science and Research Technology.* 2019. 4 (7): 121 – 128.

[4] Nweze EL, Okafor JI, Njoku O. Methanolic extract of *Treme guineense* (Schum and Thon) and *Moringa lucida* Benth used in Nigerian herbal medicinal practices. *Biological Research.* 2004. 21: 39-48.

[5] Mishra AK, Mishra A, Kehri HK, Sharma B, Pendey AK. Inhibitory activity of Indian spice plant *Cinnamomum zeylanicum* extracts against *Alternaria solani*and *Curvvularia lunata*, the pathogenic dematioceous mould. Doi: 10.1186/1476-0711-8-*Annual Clinical Microbiology and Antimicrobiology.* 2009. 8: 9-16.

[6] Shahidi E. Natural antioxidants chemistry, health effects and applications. Champmaign 111; AOAC Press; 1996. p 241.

[7] Rowland I. Optimal nutrition: fibre and phytochemicals. *Proceedings of Nutrition Society.* 1999. 58: 415-419.

[8] Milner JA. Functional foods and health: a US perspective. *British Journal of Nutrition.* 2002. 88: S151 – S158.

[9] Lesschaeve I, Noble AC. Polyphenols: factors influencing their sensory properties and their effect on food and beverage preference. *American Journal of Clinical Nutrition.* 2005. 81: 3305 – 3355.

[10] Hayes DP. The protective role of fruits and vegetables against radiation – induced cancer. *Nutrition Review*. 2005. 63: 303 – 322.

[11] Okigbo RN, Igwe RN. The antimicrobial effects of *Piper guineense* (Uziza) and *Phyllantus amarus* (Ebe-beninzo) on *Candida albicans* and *Staphylococcus faecalis*. (*Acta Microbiologicaet Immunologica Hungarica.* 2007. 544: 353-366.

[12] Owoyele BY, Olayele SB, Elegba RA. Anti-inflammatory and analgesic activities of leave extracts of *Landolphia oweriensis*. *Biological Research.* 2002. 4 (3): 131 – 133.

[13] Ujowundu CO, Okafor OE, Agha CN, Nwaogu LA, Igwe KO, Igwe, CU. Phytochemical and chemical composition of C*ombretum zenkeri* leaves. *Journal of Medicinal Plant Research.* 2010. 10(4): 965-968.

[14] Sofowora LA. Medicinal Plants and Traditional Medicine in Africa. Spectrum Book Ltd, Ibadan; 1993. pp. 55 – 71.

[15] Abo KA, Ogunleye VO, Ashidi JS. Antimicrobial potential of *Spondias mombin*, *Croton zambasicus* and *Zygotritonia crocea*. *Phytotherapy Research.* 1999. 13: 494 – 497.

[16] Oke OL. Chemical studies on the more commonly used vegetables in Nigeria. *Journal of West African Science Association*; 1966. 11: 42-48.

[17] Sridhar KR, Bhat R. Agrobotanical, nutritional and bioactive potential of unconventional legumes-mucuna livestock. *Research on Rural Development*. 2007. 19(9): 125-130.

[18] Reddy NR, Person MD, Sathe SK, Salunkhe DK. Occurrence, distribution, content and dietary intake of Phytate. In: Phytates in Cereals and Legumes, CRC press, Boca Raton 11; 1989. pp. 19-56.

[19] Oboh G. Antioxidant properties of some commonly consumed and underutilized tropical legumes. *European Food Research Technology.* 2006. 224:61-65.

[20] Graf E, Eaton TW. Antioxidant functions of phytic acid. Free Radical Biology and Medicine. 1990. 8: 61 – 69.

[21] Niseteo T, Kornes D, Belscak-Cvitanovic A, Horzic D, Budec M. Bioactive composition and antioxidant potential of different commonly consumed coffee brews as affected by their preparation technique and milk addition. *Food Chemistry.* 2012. 134: 1870-1877.

[22] Rhodes N. Physiologically active compounds in plant foods; an overview. *Proceedings of Nutrition Society.* 1996. 55: 371 – 384.

[23] Essien EU, Izunwanne BC, Aremu CY, Eka OU. Significance for human of the nutrient contents of the dry fruits of *Tetrapleura tetraptera*. *Plant Food for Human Nutrition.* 1994. 45(1): 47 – 51.

[24] Burubai M, Amula WE, Dawonye P, Swoman T, Nimame P. Proximate composition and some technological properties of African nutmeg *(Monodora myristica)* seeds. *Journal of Agricultural Research.* 2000. 45(1): 85 -92.

[25] Burkill HM. The Useful plants of West Tropical Africa, Vol. 1, New England Scientific Publications Department, Royal Botanic Garden, England; 1985. p 236.

[26] Effraim KD, Salami HA, Osewa TS. The effect of aqueous leaf extracts of *Ocimum gratissium* on haemological and biochemical parameters on rabbits. *African Journal of Biochemical Research*. 2000. Vol 3 :175-179.

[27] Geonaras I, Yoon Y, Belk KE, Smith JC, Sofos JN. Antimicrobial Activity of E€-Polylysine against *Escherichia coli* 0157: H7, *Salmonella typhinurium,* and *Listeria monocytogenes* in various food extracts. *Journal of Food Science*. 2007. 70(8): M330-M334.

[28] Harborne JB. Phytochemical Method: A Guide to Modern Techniques of Plant Analysis, 1st Edn. Chapman and Hall, London; 1973. p 288.

[29] Association of Official Analytical Chemists (AOAC), Washington D.C. Official Method of Analysis; 2000. 16th Edition.

[30] Shahidi F, Naczk M. Antioxidant properties of food phenolics. In: Shahidi, F., and Naczk, M., (Editors). Phenolics in Food and Nutraceuticals. Boca Raton, Fla.: CRC Press; 2004. pp. 1, 403.

[31] Xu BJ, Chang SKC. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *Journal of Food Science.* 2007. 72 (2): S159-66.

[32] Christe RC, Freitatas M, Mercadante AZ, Fermandes E. The potential of extracts of Caryocarvillosum pulp to scaveng reactive oxygen and nitrogen species. *Food Chemistry*; 2012. 135: 1740-1749.

[33] Xu BJ, Yuan SH, Chang SKC. Comparative studies on the antioxidant activities of nine common legumes against copper-induced human low-density lipoprotein oxidation in vitro. *Botanical Bulletin of Academia Sinica*. 2007. 46: 99 – 106.