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EFFECTS OF*MYRISTICA FRAGRANS, MURRAYA KOENIGI* AND *AFRAMOMUM MELEGUETA* LEAVE ON SOME BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS OF ALBINO RATS

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ABSTRACT

The study was carried out to investigate the effect of the aqueous extracts of *Myristicafragrans*(Nutmeg), *Murrayakoenigi*(curry leaf) *and Aframomummelegueta*(Guinea pepper) *on Some* Biochemical and haematologicalParameters. Sixteen (16) wister strain rats weighing between 130 – 180g were divided into four (4) groups of four (4) rats each and for 21 days fed the following diets: Group A – normal diet + *myristicafragrans* (Nutmeg) aqueous extract, Group B – normal diet + *murrayakoenigi* (curry leaf) aqueous extract, Group C – normal diet + *aframomummelegueta* (Guinea pepper) aqueous extract, Group D – normal diet (control). After a period of 21 days the rats were sacrificed and the serum was taken for the following estimations: total protein, albumin, total bilirubin, direct bilirubin, aspartate transaminase, alanine transaminase, alkaline phosphatase, total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol and glucose. The whole blood was taken for packed cell volume and white blood cell count. The results indicated that oral administration of *myristicafragrans*, *murrayakoenigi* and *aframomummelegueta* to rat's exhibit remarkable hypolipidaemic activity and lowering glucose concentration. The oral administration of these three spices exhibit protein increasing activities compared with the control rats. The packed cell volume and white cell values of all the rats decreased after feeding with experimental diet (aqueous extract) compare with the control rats. It is clear from this study that*Myristicafragrans(*Nutmeg*), Murrayakoenigi*(curry leaf) and*Aframomummelegueta* (Guinea pepper) contain significant amounts of phytochemicals and exhibit hypolipidaemic activity when consumed.

Keywords:*Myristicafragrans, Murrayakoenigi, AframomummeleguetaPhytochemical, hypolipidaemic.*

INTRODUCTION

Spices such as nutmeg, curry leaf and guinea peppers are group of esoteric food adjuncts that have been in use for thousands of years to enhance the sensory quality of food. These spice ingredients impact characteristic flavor, aroma or piquancy and color of foods. Some can also modify the texture of food. Spices consumed by humans are nutritional, therapeutic or toxic effects on the human body but due to low level of spice consumption, their impact on nutrient makeup may not be as dramatic as that of other food ingredients [1]. The components of spices responsible for the quality attributes have been designated as active principles and in many instances, they are also responsible for the beneficial physiological effects of spices. These beneficial physiological effects also have the potential of possible therapeutic application in variety of disease conditions [2].

Nutmeg (*Myristicafragrans)* is the actual seed of the nutmeg tree, roughly egg-shaped and about 20 to 30mm (0.8 to 1 inch) long and 15 to 18mm (0.6 to 0.7 inch) wide and weighed between 5 and 10g (0.2 and 0.4oz) dried [3]. Nutmeg is always used in ground or grated form, and is best grated fresh. Nutmeg is used for flavouring many dishes in all countries where it is available. It is often used as spice in many sweet as well as savoury dishes, also used in processed meat products, soups, sauces and baked goods [1]. Nutmeg is rich in potassium, calcium, phosphorus and magnesium. It has good amount of sodium and small amount of iron, zinc, copper, manganese and selenium. Nutmeg has good amount of vitamin A, C and choline. It also has small amount of thiamine, riboflavin, niacin, vitamin B6 and folate. 100g of nutmeg has 525calories [4]. Nutmeg cures stomach ache, diarrhoea and helps to detoxify the body, reduces blood pressure and increases blood circulation. It is also good for digestion, reduces acidity, relieving vomiting and flatulence and as a medicine for respiratory problems. The active principles in nutmeg have therapeutic applications in many traditional medicines as anti-depressant, aphrodisiac, anti-fungal and carminative functions [5].

Curry leaf (*Murrayakoenigi)* is a leaf of a small tree, growing 4-6m tall with a trunk up to 40cm diameter. The leaves are pinnate with 11-21 leaflet, 2-4cm long and 1-2cm broad. They are highly aromatic. The flowers are small, white and fragrant. The small back shiny berries are edible, but their seeds are poisonous [6]. Curry leaf is used in preparations of dry vegetables dishes, coconut milk based curries, meat and chicken preparations and cooling drinks made with yoghurt. In their fresh form, they have a short shelf life and they don't keep well in the refrigerator. They are also available dried, though the aroma is largely inferior [7].Vitamin like carotene, thiamine, riboflavin, niacin, folic acid and vitamin C are also present in curry leaves. Minerals and trace elements present in curry leaf are magnesium, copper, manganese, zinc, chromium, chlorine, oxalic acid and phytin phosphorus [8]. Curry leaves are used as herb in Ayurvedic medicine. Their properties include much value as an anti- diabetic, antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, anti-hypercholesterolemic etc. It is also known to be good for hair, for keeping it healthy and long. Curry leaf possess the qualities of an herbal tonic, they strengthen the functions of stomach and promote its action [9].

Guinea pepper *(Aframomummelegueta)* has a somewhat palm-like appearance and forms dense

clumps that grow to a height of 1.2m to 1.5m trumpet shaped, purple flowers develop into pods measuring 5cm to 7cm and contain many small reddish brown seeds. In powdered form, they become pale grey [10]. Guinea peppers are spicy and hot with a bitter note. They go wonderful with vegetables and fish plates. It can also be used to make beer [10]. Guinea pepper contains calcium (0.000437g), Iron (0.00002886g) and magnesium (0.000194g) as well as vitamins A, B, C, D and E [11]. The seed of guinea pepper are used in the treatment of measles and leprosy. It can be taken to reduce excessive lactation and postpartum haemorrages. It is also used as purgative, anthelmintic and haemostatic agent. The fruit apparently also has aphrodisiac properties. It is also a promising remedy for the S hemoglobin [12]. The objectives of this study are to determine the phytochemical composition of these spices and to evaluate the effects of their aqueous extracts on some biochemical parameters.

MATERIALS AND METHODS

Plant Material:

The plants;*Myristicafragrans*(Nutmeg)*, Murrayakoenigi*(curry leaf) *and Aframomummelegueta*(Guinea pepper) used for this study were purchased from Terminus market Jos, Plateau state Nigeria, andidentified at the Department of Botany, University of Jos, Jos, Nigeria.

Preparation of Extracts:

The plant materials were dried under shade and grinded to powdered form using a blender. 150 g of the powdered forms were separately weighed and dissolved in 300 ml of distilled water and boiled for 45 minutes, allowed to cool and then filtered using Whatman Filter paper Grade No. 42. The filtrate was then allowed to dry in a hot air oven (40^{o}C). The extract was stored in an air tight container and was later reconstituted in distilled water to give the required dose of 400 mg kg-1bwt.which was administered.

Experimental animals:

Sixteen (16) wister strain rats of weighing 130 - 180g were obtained from the small animal holding unit, Department of Biochemistry, University of Jos. They were randomly assigned into four groups, of four rats each, and acclimatized for 7 days. The animals were maintained under standard conditions, had free access to food (Grand Cereal, Oils and Mills Products, Jos, Nigeria) and water *ad libitum*.

Experimentalgrouping:

The rats were divided into four groups of four rats each and placed in standard rats cages. The four groups and the diets (spices) are as follows.

The grouping and administration of the extracts is as follows:

Group	**Diet**
A	Normal diet + *myristicafragrans*extracts
B	Normal diet + *murrayakoenigi*extracts
C	Normal diet + *Aframomummeleguetae*xtracts
D	Normal diet (Control)

The aqueous extracts were administered accordingly by intubation at a dose of 400mg every day for twenty one (21) days.

Sample collection:

The rats were anesthetised with diethyl ether at 22nd day, the neck area was quickly cleared of fur and skin to expose the jugular veins. Blood samples were collected from the animals in batches. 2 animals' blood samples were separately collected into a clean, dry tube and allowed to clot for 45 minutes and spun at 3000 rpm for 5 minutes before the serum was collected for biochemical assay. Blood sample from the last 2 were separately collected into an anti-coagulant (EDTA) bottle and were used for Haematological assay.

Phytochemical Screening:

The phytochemical screening of the plant extracts was carried out using standard qualitative procedures as described by [13][14][15].

Biochemical parameters:

The following parameters were investigated using appropriate methods.

Blood glucose level was determined by the enzyme method using the fortress diagnostic kit product [16].

Serum total protein level was determined by the enzyme method using the fortress diagnostic kit product [16].

Serum albumin level was determined by the enzyme method using the fortress diagnostic kit product [16].

Serum total cholesterol level was determined by the enzyme method using kit product of

randoxdiagnostics [17].

Serum triglyceride level was determined by the method of fossti and principle using randox kit product [17].

Serum high density lipoprotein (HDL-cholesterol) level was determined by the method of Assemann using QCA kit product [18].

Low density lipoprotein (LDL-cholesterol) level was determined by the method of Assemann using a commercial kit product [19].

Serum Bilirubin level was determined by the method of Jendrassic and Grof using commercial kit product [20].

Serum alkaline aminotransferase (ALT) activity was determined according to the method of Reitman and Frankel (1957) using fortress diagnostic kit products [21].

Serum aspartate aminotransferase (AST) activitywas determined by the method of Reitman and Frankei using fortress diagnostic kit product [21].

Serum alanine phosphatase (ALP) activity was determined **by** the method of Reitman and Frankei (1957) using fortress diagnostic kit product [21].

Haematological parameters:

Haematological parameters were determined using Mindray Haematology Analyser (Mindray BC-2300, Guangzhou Shihai Medical Equipment Co., Ltd, China).

Statistical Analysis:

The result was expressed as mean ± standard error of mean (s.e.m) for four rats in each group and all data were statically analysed using the student's t- test.

RESULTS

Phytochemicals	*myristicafragrans*	*murrayakoenigi*	*aframomummelegueta*
Akaloids	+	+	+
Flavonoids	+	+	+
Tannins	-	-	-
Saponins	-	-	+
Balsams	-	-	-
Cardiac glycosides	+	+	+
Terpene and steroids	+	+	+
Resins	+	+	+

Table 1: Result of the phytochemicals screening of *myristicafragrans*, *murrayakoenigi* and *aframomummelegueta*.

Keys + = present, - = not present.

Group	Total Protien (g/l)	Albumin (g/l)	Total Bilirubin (µmol/l)	Direct Bilirubin (µmol/l)
A	68.03 ± 0.12	26.15 ± 0.01	3.89 ± 0.17	1.722 ± 0.03
B	72.77 ± 0.70	28.56 ± 1.45	6.48 ± 0.05	1.968 ± 0.30
C	67.01 ± 0.40	28.85 ± 0.87	6.48 ± 0.02	3.440 ± 0.05
D	63.27 ± 0.23	32.31 ± 1.58	4.26 ± 0.22	1.968 ± 0.16

Table 2: Total Protein, Albumin, Total Bilirubin and Direct Bilirubin levels of the rats at the end of feeding with experimental rats.

Values are mean ± standard deviation (S.D)

Group	ALT (µ/l)	AST (µ/l)	ALP (µ/l)
A	12 ± 0.00	16 ± 1.73	29.15 ± 1.55
B	37 ± 1.73	89 ± 2.0	16.90 ± 0.98
C	27 ± 1.00	53 ± 1.73	32.54 ± 1.89
D	40 ± 1.00	125 ± 1.73	33.94 ± 0.70

Table 3: Alanine Transaminase (ALT), Aspartate Transaminase (AST), and Alkaline phosphatase (ALP) level of rats at the end of feeding with experimental diets.

Values are mean ± standard deviation (S.D)

Group	Total cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL cholesterol (mmol/L)	LDL cholesterol (mmol/L)
A	3.78 ± 0.12	1.50 ± 0.08	0.50 ± 0.02	0.74 ± 0.02
B	2.08 ± 0.27	1.22 ± 0.05	0.55 ± 0.04	0.46 ± 0.04
C	3.81 ± 0.10	1.07 ± 0.06	0.75 ± 0.02	0.93 ± 0.01
D	4.31 ± 0.07	1.56 ± 0.03	0.87 ± 022	1.87 ± 0.02

Table 4: Lipid profile of rats at the end of feeding with experimental diets.

Values are mean ± standard deviation (S.D)

Group	Glucose (mmol/L)	PCV (%)	WBC (mm^3)
A	4.20 ± 0.14	50 ± 1.00	4100 ± 173.2
B	3.62 ± 0.02	48 ± 1.00	3700 ± 173.2
C	4.60 ± 0.10	48 ± 1.00	3800 ± 100.0
D	4.71 ± 0.02	51 ± 1.00	4300 ± 0.00

Table 5: Glucose, Packed Cell Volume (PCV) and White Blood Cell (WBC) level of rats at the end of feeding with experimental diets.

Values are mean ± standard deviation (S.D)

DISCUSSION

The result shows that spices are rich in phytochemicals; nutmeg contains all the phytochemicals tested but tannins, saponins and balsams were not detected. Result of the phytochemical studies of curry leaf shows that curry leaf contains alkaloids, flavonoids, cardiac glycosides, terpene and steroids and resins were detected in water extract. Guinea pepper also has a high phytochemical content tested but tannins and balsams were not detected. From the result of phytochemical screening, it indicates that guinea pepper has high phytochemical content and could be used more medicinally.Others such as nutmeg and curry leaf have low phytochemical content and as such may not have much medicinal effect on the body as with guinea pepper.

The results of the effect of the spice extract shows that feeding nutmeg, curry leaf and guinea pepper increases their total protein level with curry leaf having the most significant effect as compared to control. A reduced value in serum albumin concentration was observed in rats fed with all the extracts as compared to their control. For the effect on bilirubin, there is a decrease in total bilirubin level in rats placed on normal diet + nutmeg and an increase in total bilirubin level in rats fed with normal diets + either of curry leaf or guinea pepper as compared to their control. There is a significant increase in direct bilirubin in rats placed on normal diet + guinea pepper and a significant decrease in rats fed with normal diet + nutmeg as compared to

their controls and rats fed with normal diet + curry leaves.

There is a decrease in alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) in rats placed on the extract as compared to the control.

The serum total cholesterol, triglyceride, and high density lipoprotein (HDL) cholesterol concentration decreased in rats placed on normal diet + either of nutmeg, curry leaf or guinea pepper as compared to the control. For the serum total cholesterol lowering effect, several hypotheses have been advanced, and the stimulation of the oxidation of cholesterol to bile acids as it is also in the case of polyunsaturated fatty acids. The observations are consistent with the fact that high lipid diets low in micro-nutrients and refined dietary sugars lack minerals and vitamins and are often called ´empty calories´ because the draw upon the body nutrients to be metabolised into the system and when these nutrients are depleted, metabolism of cholesterol and fatty acids is impeded, contributing to higher cholesterol levels and promoting obesity due to higher levels of fatty acid on the organs and tissues [22].

The mechanism for the decrease in serum triglyceride concentration is possible that stress (from factors beyond this experimental control) and partaking in a few large meals (rather than the more continuous feeding) or intermittent starvation or hunger strike in a bid by the rats to adjust to the experimental diets, producing these results [22]. The mechanism for the reduction of serum HDL- cholesterol concentration is not very clear but may be due to reduced synthesis of apo A – 1 (ligand for HDL receptors) and changes in activity of cholesteryl ester transfer protein (CETP, which facilitates transfer of cholesteryl ester from HDL to other lipoproteins).

There is a significant decrease in low density lipoprotein (LDL) cholesterol concentration in rats fed with normal diets + either of nutmeg, curry leaf and a significant increase in rats fed with only normal diet + guinea pepper as compared to their control. The decrease in serum LDL – cholesterol concentration could be due to stimulation of the LDL receptors (apo B – 100E), causing increased uptake of LDL by the liver and extra hepatic tissues with consequent lowering of the plasma cholesterol. The decreased serum LDL- cholesterol concentration could also be as a result of increased LDL – cholesterol catabolism via receptor – independent pathways (like the scavenger pathway which is not regulated) which probucol, a hypolipidaemic drug appears to promote [22].

The glucose level for rats placed on normal diets + either of nutmeg or guinea pepper was increased and glucose level of rats placed on normal diets + curry leaf decreased as compared to their control. The packed cell volume (PCV) values for rats placed on normal diet + either of nutmeg, thyme, curry leaf or guinea pepper was decreased compared to their control. The reason for these is not clear. The comparism between rats fed the extracts (normal + either nutmeg, curry leaf or guinea pepper) and their control showed a decrease in the white blood cell (WBC) count. The mechanism for these effects is also clear.

CONCLUSION

. This study shows that spices contains significant amount of phytochemicals. It is believed that phytochemicals are effective in combating or preventing diseases due to their antioxidant effect. It is the combination of all the phytochemicals in the spices that actually makes the antioxidant effective. Furthermore because of their hypolipidaemic effects these plants can have therapeutic values as anti-atherosclerotic, anti-coronary heart disease and anti- type II diabetic agents. It has been proposed that the additive and synergistic effect of phytochemicals in spices are responsible for their potent antioxidant and anticancer activities and that the benefits of diet rich in spices is attributed to the complex mixture of natural phytochemicals present in spices in achieving benefits.

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